RELATING THE REPRODUCTIVE PERFORMANCE OF WESTSLOPE CUTTHROAT TROUT TO TRAIT SPECIALIZATION TO SCREEN FOR ARTIFICIAL SELECTION VULNERABILITY IN A CONSERVATION HATCHERY

by

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GLOSSARY

BIP: The between-individual portion of trait variation. This portion accounts for variation that exists between individuals in the same population for a given trait. This is calculated by finding the sum of the medians for all individuals for a given trait.

CC: Complete Case; A replicate measures dataset comprised of the data from all fish that were not missing any sampling data.

CVA: Canonical variate analysis is similar to PCA but uses priori group assignment to analyze group structure in a multivariate dataset.

Duplicate: A replicate measures dataset comprised of the data from all fish that had data collected at the first two iterations for each respective trait or variable.

DCL: The daily change in length was calculated as the total change in length (mm) between two timepoints, divided by the number of days between those two timepoints. This provided a standardized assessment of growth in length that was comparable for all time intervals throughout the duration of the study.

DCW: The daily change in weight was calculated as the total change in weight (g) between two timepoints, divided by the number of days between those two timepoints. This provided a standardized assessment of growth in weight that was comparable for all time intervals throughout the duration of the study.

i_n: A mathematical concept used to represent the median for the n^{th} individual for a given trait to calculate the between-individual component of variation.

IS: Individual specialization is the proportion of total population variation explained by within-individual proportion and is calculated by dividing the within-individual variation by the total response range.

j_n: A mathematical concept used to represent the interquartile range for the n^{th} individual for a given trait to calculate the within-individual component of variation.

K_n: Relative condition is the ratio of observed weight of a fish at a given length to the predicted weight of a fish at the same length as determined from a population-specific length weight regression.

PC [1…8]: The first eight principal components of the duplicate body shape analysis maximized the variation explained by a given component through a combination of the variation of multiple body shape landmarks. The first eight components described 74.0% of the variation in the body shape dataset.

PCA: Principal component analysis reduces the dimensionality of large datasets while also breaking the dataset down into “principal components” that maximize the variation described by the original dataset.

POL: An abbreviation for “pace of life”, an emergent theory that describes relationships of traits and life history strategies that organisms may exhibit as they attempt to maximize performance in energy limited systems.

TNW: Total niche width is the total breadth of variation in a trait exhibited by individuals making up a population comprised of two portions of variation: the within-individual portion (WIP) and the between-individual portion (BIP).

TRR: The total response range for a given trait. This value describes all of the variation that exists within a population for a given trait and can also be described as the sum of the variation due to between-individual and the within-individual portions.

VHA: The visual health assessment was a standardized visual assessment of eight different areas of anatomy that were hypothesized to have implications for performance if condition was reduced.

VHI: The visual health index was a standardized assessment of visual indicators of health on all fish.

WCT: An abbreviation for westslope cutthroat trout (*Oncorhynchus clarkii lewisi),* a subspecies of the cutthroat trout and the study organism of this research.

WIP: The within-individual portion of trait variation. This portion accounts for variation that exists within the same individual over time for a given trait. This is calculated by finding the sum of the interquartile ranges for all individuals for a given trait.

WVHI: The weighted visual health index assigned weights to the several categories in the VHI based on hypothesized biological importance.

ABSTRACT

To mitigate westslope cutthroat trout (WCT; *Oncorhynchus clarkii lewisi*) declines, Montana Fish, Wildlife, & Parks carries out large scale restorations, including wild-origin stocking efforts from conservation hatcheries. Although hatcheries have made progress in limiting the effects of artificial selection on stocked populations, concerns remain that rearing practices inadvertently reduce the diversity propagated into the wild.

The objective of this research was to identify traits of WCT driving poor survival or reproduction in a hatchery, allowing managers to reduce artificial selection by screening for fish requiring alternative rearing. In Chapter 1, I repeatedly measured 18 behavior, morphology, and health traits from hatchery intake (July 2019) to spawn (June 2021). I identified traits with low within- relative to between-individual variation as traits likely to be indicative of specialization. As specialists tend to maximize performance under a narrow range of conditions, they may be vulnerable to artificial selection within hatcheries. In Chapter 2, I tested whether the specialized traits identified in Chapter 1, growth rate, or age at hatchery intake of individual WCT could predict survival or reproduction.

In Chapter 1, I identified nine specialized (relative condition, weighted health index, health index, body shape, energetic reserves, latency, and upper jaw, lower jaw, and anal fin residual length) traits. I hypothesized these traits would predict later survival or reproductive performance. In Chapter 2, I identified October 2019 daily growth rate in weight and every replicate length measurement after October 2019 to strongly predict total ovulated eggs and total number of hatch embryos produced by females. However, among individual variation in the median percent hatch embryos was not explained by maternal size. Male gamete quality and fertilization success was consistently high, and I found no biologically significant predictors of reproductive performance for males. I also found no predictors of survival for males or females.

Despite high total ovulated eggs and total hatch embryo success for females, variable female median percent hatch embryos suggests that quality of ovulated eggs is driving current limitations to WCT hatchery reproduction. I recommend further investigation into impacts of rearing stressors and post-ovulatory aging on female WCT and their reproductive performance.

TRAIT VARIATION WITHIN AND AMONG INDIVIDUAL WILD-ORIGIN WESTSLOPE CUTTHROAT TROUT OVER TIME WITHIN A CONSERVATION HATCHERY AND IMPLICATIONS FOR MANAGEMENT

Traits may vary within or among individuals making up a population (Bolnick *et al.*, 2003; Burton *et al.*, 2011; Metcalfe *et al.*, 2016). Previous studies have linked interindividual variation in morphology and behavior to specialized life history strategies, including feeding style, age at maturity, fecundity, social aggressiveness, migration pattern, and many other community-level trends in fish populations (Bennett *et al.*, 2014; Homel *et al.*, 2015). Variation in life history strategies improves population resilience (Kovach *et al.*, 2019; Harder *et al.*, 2019) within heterogenous and stochastic natural ecosystems. Within fish hatcheries that produce populations for conservation stocking, life history specialization of some wild-origin brood stock may be incompatible with success under typical rearing conditions resulting in low reproductive performance or survival pre- and post-stocking. Loss of important population demographics through hatchery artificial selection is thought to impair resilience of stocked fish populations (Christie *et al.*, 2012; 2014; 2016). Therefore, in this chapter, I identify traits that vary among individuals while remaining relatively stable over time within individuals as promising indicators of specialized life history strategies associated with poor survival and reproductive success under typical hatchery rearing regimes.

Two common approaches to identify different life history strategies within populations are pace-of-life theory (POL) and morphometrics. The POL describes life history strategies on a spectrum from fast to slow (Réale *et al.*, 2010; Ricklefs and Wikelski, 2002; Martin *et al.*, 2006). Traits thought to vary with an individual’s position within the POL fast to slow continuum include, but are not limited to, social aggression, stress response, metabolic rate, and reproductive habits (Polverino *et al.*, 2018; Chavarie *et al.*, 2016b; Arostegui *et al.*, 2019). Fast POL individuals are expected to exhibit fast growth, social aggression, low stress responsiveness, early age at sexual maturity, high fecundity, and high metabolic rate, whereas slow individuals are expected to exhibit the opposite (Wolf *et al.* 2007; Réale *et al.* 2010). Individuals with a slow pace of life strategy and associated high stress responsiveness may fail to flourish under novel hatchery rearing conditions due to negative impacts of chronic elevated primary stress response pathways, which have inhibitive effects on growth, immune, and reproductive processes (Pickering, 1993; Schreck, 2010; Baschant and Tuckermann, 2010; Campbell *et al.*, 1992). Behavioral traits have been strongly linked to stress responsiveness within the POL continuum (Adriaenssens and Johnsson, 2009; Réale *et al.*, 2010). Studies on domesticated rainbow trout (*Oncorhynchus mykiss*) have found that individuals with lower stress responsiveness were frequent dominators in size matched pairs (Pottinger and Carrick, 2001), were more aggressive following a standardized confinement stress (Øverli *et al.*, 2004), and had a more rapid resumption of feed intake after exposure to a novel environment (Øverli *et al.*, 2002). Additionally, studies have found behavioral boldness to be positively correlated with growth (Westerberg *et al.*, 2004; Frost *et al.*, 2007) and body condition (Pellegrini *et al.*, 2010). Thus, behavior is correlated with many traits that have high interindividual variation within populations and may be represented of organismal life history strategy.

Morphological traits, especially related to exploitation of a specific diet niche or habitat, have also been highly correlated with life history variation within populations (Gillespie and Fox, 2003; Mittelbach, 1984; Parsons and Robinson, 2006; 2007). Within-population variation in morphological traits is considered to reflect natural selection for phenotypic plasticity in body shape allowing for specialization to specific diet items or flow habitats in fishes (Keeley *et al.*, 2005). For example, morphological traits such as jaw, fin and overall body shape, size, and orientation have high fidelity to shifts in feeding and swimming behavior. Thus, as diet or habitat niches shift, so do these aspects of morphology in predictable trajectories (Chavarie *et al.*, 2013; 2016a; 2016b; Mittelbach *et al.*, 1999; Imre *et al.*, 2002; Walker, 1997; Keeley *et al.*, 2007).

Life history type, like position within the POL continuum or morphology matching diet or habitat specialization, can be relatively stable or varying for individuals over time, depending on ecological context. While the between-individual portion of variation explained by POL or morphology remains consistent relative to the population as external factors change (Brandl *et al.*, 2015), there is evidence that individuals may vary over time due to density dependent and environmental factors (Skúlason *et al.*, 2019). Few studies have tracked trait variation over time within individuals (exceptions: Vander Zanden *et al.*, 2000; Musseau *et al.*, 2020), and the impacts of individual plasticity on temporal variation in traits remains poorly understood (Fraser and Bernatchez, 2005; Seebacher and Franklin, 2012; Luis- Miguel and Hoffmann, 2017).

When individuals benefit from a narrow range of variation for a given trait over time (Chavarie *et al.*, 2021; Jirka and Kraft, 2017), natural selection can reinforce low within-individual variation creating specialists. Within-individual variation refers to the variation of a given trait for one individual over time whereas between-individual variation refers to the variation that exists between individuals in a population for a given trait (Chesson, 1991). Low within-individual variation indicates that an individual does not vary in a trait over time and is relatively predictable, whereas high within-individual variation indicates the opposite relationship. Low between-individual variation indicates that individuals do not vary from each other in a given trait and that the population tends to be generalist, whereas high between-individual variation indicates the opposite (Bolnick *et al.*, 2003; Toscano *et al.*, 2016). Evolutionary niche theory defines individual specialization (IS) as the proportion of total population variation explained by within-individual proportion (Bolnick *et al.*, 2003; Araújo *et al.*, 2011). Resilience of populations living in the heterogenous and stochastic conditions occurring within natural ecosystems is dependent on IS and phenotypic plasticity (Seebacher *et al.*, 2015). However, highly specialized individuals may be unable to adjust to the relatively novel, consistent, and homogeneous conditions of a conservation hatchery.

Salmonids are an excellent example of a taxa that exhibits high degrees of interindividual variation and specialization within populations of the same species (Behnke, 2002; Schlichting and Pigliucci, 1998; Killen *et al.*, 2016). Not only do salmonids within the same population exhibit high diversity in trophic niches, but they also display high diversity in migratory ability, feeding strategy, growth rates, and reproductive strategies. Within the same population of *O. mykiss*, it was found that genetically indistinguishable individuals could exhibit both anadromous and resident ecotypes, with associated tradeoffs in growth rate and fecundity (Riva-Rossi *et al.*, 2007). Additionally, one population of westslope cutthroat trout (WCT; *O. clarkii lewisi*) may contain up to three distinct life-history forms that are distinguished by their reproductive strategy: fluvial, adfluvial, and resident (Averett and MacPhee, 1971; Rieman and Apperson, 1989).

Salmonids can be extremely plastic in response to situations such as aging through life stages and fluctuations in density dependent factors (Chavarie *et al.*, 2020), which are commonly experienced during rearing in conservation hatcheries. Salmonid phenotypic plasticity can make trait-based predictions of future individual performance difficult even with a strong understanding of the present life history strategies (Hutchings, 1996). Depending on the timing or strength of cues, individuals may be affected enough to produce novel traits, expanding within-individual variation for the population (Dingemanse and Wolf, 2013; Westneat *et al.*, 2015). An understanding of these demographics is key to understand which individuals may be at higher risk of artificial selection within a hatchery facility.

Hatchery stocking programs have emerged as a tool to restore extirpated and vulnerable conservation populations, but there are concerns that artificial selection within stocking programs inadvertently reduce the within and among population variation that make salmonid communities resilient (Schindler *et al.*, 2010). Reduced performance of hatchery origin fish in the wild has been documented in several salmonid species (Christie *et al.*, 2014) and is strongly associated with domestication effects from hatchery rearing (Christie *et al.*, 2012; 2016). Additionally, hatchery rearing has been found to result in genetic bottlenecks, which may increase the effects of genetic drift and homozygosity in subsequent generations (Ryman and Laikre, 1991; Christie *et al.*, 2012). Genetic drift and artificial selection can reduce genetic variation on traits vulnerable to selection in the wild and can thus reduce the fitness of individuals and the entire population after release into the wild (Araki *et al.,* 2007b; Willoughby and Christie, 2019). Modern conservation hatcheries have made efforts to reduce artificial selection in response to concerns over hatchery-related effects on wild populations influenced with stocking efforts (Araki and Schmid, 2010).

Modern salmonid conservation hatcheries differ from traditional hatcheries as they typically capture local-origin juvenile salmonids in the wild and rear them in the hatchery before stocking the first-generation offspring. Conservation hatcheries reduce the time offspring spend in the facility, avoid breeding individuals with captive ancestry, and minimize the spawning events of individuals over time, as this has been found to mitigate reductions in population size and genetic diversity over time (Harder and Christie, 2019; Willoughby *et al.*, 2018; Willoughby and Christie, 2019; Araki *et al.*, 2007a; 2007b; 2008). This approach focuses on preserving the genomic and phenotypic diversity by implementing alternative rearing strategies to avoid artificial selection during rearing and spawning that could negatively influence stocked populations and the wild populations they interact with (Christie *et al.*, 2014; Berejikian *et al.*, 2008; 2012). However, concerns remain that some population demographics may still be selected against in a hatchery environment. Tools to predict later hatchery performance at the individual fish level could equip hatchery managers with strategies to tailor rearing practices to specialized population demographics that do not thrive under historical hatchery rearing practices.

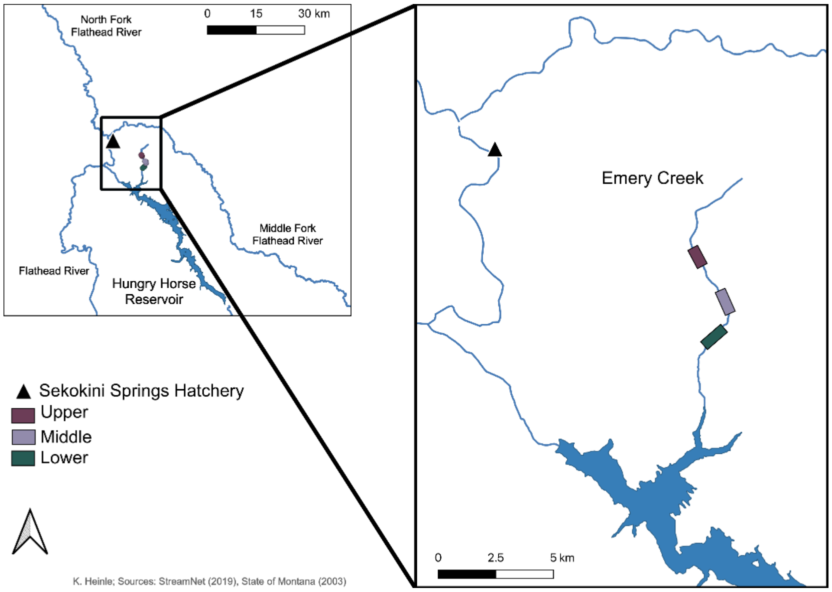
While the current toolset available to hatchery managers to identify specialized population demographics that do not thrive under historical hatchery is limited, traits which are relevant to life history strategies, such as behavior and morphology, have high potential for application in this context. Identification of traits related to poor in-hatchery reproductive performance or survival would allow screening for vulnerable fish demographics that would benefit from alternative rearing strategies aimed at improving in-hatchery survival and reproduction. However, a better understanding of within- and among-individual variation in traits over time within specific populations is required. If traits lack consistency over time within individuals, it may be difficult to link a trait to eventual performance as it is unknown how that individual may shift in trait over time. Additionally, traits with high between-individual variation, indicating a higher potential for specialization within the population, are most likely to be successfully linked to reproductive performance.

In this study, I tracked the intra- and inter-variation in traits related to behavior, morphology, and health over time within WCT individuals at Sekokini Springs Hatchery. Using these relationships, I sought to identify promising traits that had the highest likelihood of predicting population demographics at risk of artificial selection within hatcheries in Chapter 2 of my thesis. I hypothesized that body shape and morphology would be two of the most stable traits studied and behavior would be moderately stable within the population regardless of environmental changes or density dependent factors. I hypothesized that health, whole-body energy, and relative condition would be moderately consistent within individuals relative to the population.

Methods

Study System and Specimens

Juvenile WCT were collected from Emery Creek, Montana in late June and early July 2019. Fish were captured via backpack electroshocking (LR-24 Electrofisher, Smith-Root, Inc., Vancouver, WA, USA) in three separate reaches of Emery Creek (Figure 1.1). A systematic random sampling method (Singh and Masuku, 2014) was used to maximize accurate representation of the genetic diversity of the wild population. Sampling was conducted over 3 days (June 29- July 1) with an objective of collecting ~300 juvenile fish for hatchery rearing. During collection, fish were stored in an in-stream mesh live car until the end of the workday and then were anesthetized with a 10% dilution of AQUI-S (AQUI-S, New Zealand Ltd, Lower Hutt, NZ) and their total length was measured. All fish with a total length less than 119 mm were released back into the stream as they were likely less than two years of age based upon previous size at age approximations for wild WCT cohorts captured by Montana Fish Wildlife and Parks Region 1 staff (Relyea, personal communication, 2019). Fish with a total length greater than 119 mm were kept in the live car until the end of the day, when they were placed in well-aerated (>98% dissolved oxygen (DO)) transport bags filled with cool stream water (13 °C (Range ±1°C)) and transported to Sekokini Springs Hatchery.



K. Heinle; Sources: StreamNet (2019), State of Montana (2003)

Figure 1.1. Map of the collection site, Emery Creek, relative to Hungry Horse Reservoir and the Flathead River.

Once at the hatchery, fish transport bags were placed into a designated 2000L fiberglass tank (0.85 x 4.88 x 0.51m) to allow gradual adjustment to the tank water temperature before releasing fish into the tank. The DO (>98%) and water temperature (range: 12.0-14.0°C) within the transport bags were monitored using the Pro20i DO Instrument (Xylem Inc., Yellow Springs, OH, USA). Transport bags were left in the tank for one hour or until the water temperature inside the bag was within 1°C of the tank water temperature (11.2°C). Once the transport bag water temperature was equilibrated to the tank temperature, the fish were encouraged to swim out of the bag and into the water of the tank. Electrofishing and transport to the hatchery were repeated over three days, at which point, 256 fish of the target size range had been captured and transported to the hatchery.

Hatchery Intake Inventory Sampling

After all fish had been collected, an initial inventory was performed to individually mark fish, establish a baseline of body condition, and collect tissue to genetically test for hybridization. Batches of six to ten fish were anaesthetized and then individually marked by injecting a 12mm FDX passive integrated transponder (PIT) tag (Biomark Inc., Boise, ID, USA) into the dorsal musculature on the left side. After PIT tag injection, the left pelvic fin was clipped and collected for genetic analysis. Fish were then measured for total length (±1.00 mm) and weight (± 0.01 g) and allocated to size-matched rearing tanks of similar stocking density.

Based on PIT tag number, all fish were randomly assigned into an experimental (n = 128 fish) or a control (n = 127 fish) group. The control group was handled identically to standard hatchery operating procedures for production (Relyea, personal communication, 2019) whereas the experimental group experienced additional handling to track temporal and inter-individual phenotypic variation during hatchery rearing. These two groups allowed for exploration of the impacts of additional handling on the performance of the experimental group for a separate study (see Chapter 2). The fish assigned to the experimental group also had two additional measurements taken for assessment of health: microwave energy meter readings for estimates of whole-body energetic reserves and a standardized visual health assessment (see Experimental Sampling: Health).

Genetic Testing and Reduction in Sample Size

Genetic testing for hybridization with rainbow trout (*O. mykiss*) or Yellowstone cutthroat trout (*O. c. bouvieri*) was performed on fin clips with suspect hybrids removed from the hatchery population in July 2019. Genotyping was performed by the Montana Conservation Genetics Lab (University of Montana, MT, USA) via the RAD-capture sequencing method (Ali *et al.* 2016) and revealed hybridization in 40.2% of the population using Yellowstone cutthroat and rainbow trout markers. All definite and likely hybrids were culled, leaving only non-hybridized WCT in the population. Following hybrid culling at the beginning of the study, there were 77 experimental and 79 control fish. Due to mortalities and loss of PIT tags, by the conclusion of the study there were 67 experimental and 74 control fish.

Rearing Conditions

Day-to-day rearing of fish was consistent between the experimental and control fish with standard operating procedures at Sekokini Springs Hatchery (Relyea, personal communication, 2019). After 10 days of fasting, daily feeding was initiated (BioTrout artificial feed; BioOregon, Longview, WA, USA). Feed rates were calculated using total biomass for each tank (kg/L) to achieve a feed rate of 2% biomass daily. Fish were fed once daily for the duration of the study with random dispersion via hand feeding on the surface of the water. Stocking density was always kept below 1.6 kg/L. The photoperiod was 10L: 14D with artificial lights in the fall, winter, and spring, and, during the summer, photoperiod was 16L: 8D with a combination of artificial lights and natural light. Water temperature fluctuated seasonally (range: 5.6 –15.5°C) throughout the study, as the rearing tanks were supplied with water from one of four springs that mimic the temperature profile of a natural waterbody. A HOBO Pendant temperature logger (HOBO Pendant MX Water Temperature Logger, Onset Computer Corporation, Bourne, MA, USA) monitored the temperature of water influx from the water supply spring at hourly intervals (Figure 1.2). Flows were set at 7.5L/min for each tank. Rearing tanks were either 2120L raceways (0.8534 x 4.877 x 0.51m) or 4800L circular tanks (1.83 x 0.457m) depending on tank availability in the hatchery throughout the rearing period. Experimental and control fish were mixed in the tanks throughout hatchery rearing and were treated identically by hatchery staff.

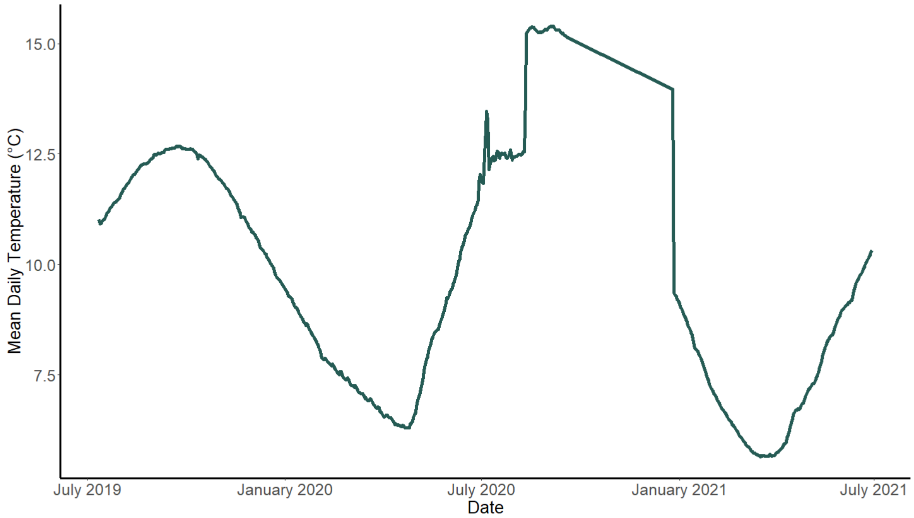


Figure 1.2. Mean daily temperature calculated from hourly measurements using a HOBO Pendant MX Water Temperature Logger placed at the inlet of the source spring for the duration of the fish rearing in Sekokini Spring Hatchery (July 2019 - July 2021). The lowest daily mean temperature was 5.6°C and the highest was 15.5°C.

Sampling Regimes

There were three types of sampling inventories used in this study: a one-time Hatchery Intake Inventory (described above), 13 Standard Inventories, and four Experimental Inventories. The Standard Inventory methods followed hatchery standard operating procedures to measure fish weight and length to track growth, adjust tank allocations and feed rations. The Experimental Inventory methods involved handling above and beyond standard hatchery procedures to track a suite of phenotypic characteristics, including behavior, health, and morphological traits.

Standard Inventories were carried out 13 times throughout the study (July 2019 - May 2021). At nine Standard Inventories (October 2019, December 2019, March 2020, May 2020, July 2020, October 2020, December 2020, March 2021, May 2021), all fish were only sampled following the Standard Inventory methods. At four inventories (August 2019, January 2020, September 2020, and January 2021), control fish were sampled following the Standard Inventory methods, whereas more extensive phenotypic characterization of experimental fish was carried out through the Experimental Inventory methods. The Hatchery Intake Inventory (July 2019) combined the methods from the Standard Inventory with an abbreviated Experimental Inventory eliminating behavior measurements to minimize additional stress to fish on top of capture and hatchery transfer.

Figure 1.3. Schedule of inventories, color coded for inventory type, throughout the duration of the study. In total, there were 14 Inventories, Standard Inventories were performed at all 14 inventories, Experimental Inventories were performed at four, and the modified experimental Hatchery Intake Inventory was performed at one. Each type of inventory had different sampling protocols.

Standard Inventory

The Standard Inventories (Figure 1.3) included standard hatchery handling and measurements performed to track fish growth over time. In batches of six to ten, fish were directly netted out of tanks into anesthetic, scanned for PIT tag identification, and measured for length and weight. Fish were then redistributed into rearing tanks according to growth performance since the previous inventory, with one tank of fish that had increased in weight, one tank of fish that had maintained weight, one tank of fish that lost up to ~5% body weight, and one tank of fish that had lost more than 5% body weight. This process was referred to as growth grading. The growth grading incorporated both experimental and control fish, and the type of tank used (circular or raceway) was determined based on tank availability at each Standard Inventory and did not account for how the different tank types may affect the fish.

Experimental Inventory

The Experimental Inventories (Figure 1.3) involved three main categories of trait assessments not included in the Standard Inventories: morphology, behavior, and health traits. Each Experimental Inventory was performed over four to eight days. Each tank was processed over the course of two days, resulting in a total duration of sampling determined by the number of tanks of fish the hatchery population was distributed through at the specific inventory time point (i.e., if fish were held in four total tanks, sampling would take eight days). On the first day, all fish in a tank were netted singly and the PIT was read to determine if the fish was an experimental or control fish. Control fish were placed back in the tank for later weight and length measurements, and experimental fish were distributed into four holding baskets for later behavior, health, morphology, weight, and length measurements. Holding baskets were mesh baskets submerged in a designated tank and up to nine fish were distributed into each basket. The control fish were sampled according to the Standard Inventory protocol. Experimental fish were held overnight in the mesh holding baskets (18-22 hrs.) until initiation of behavior (see ‘Experimental Inventory: Behavior’ section below), followed by health (see ‘Experimental Inventory: Health section below), weight, length, and morphometric (see ‘Experimental Inventory: Morphology section below) measurements the next day. By holding basket, all fish within a single holding basket were assessed for behavior (see ‘Experimental Inventory: Behavior’ section below) before being returned to their initial basket. Once fish had undergone the behavioral assessment, fish were anaesthetized for measurements of length and weight and assessment of health and morphology. Experimental fish were then growth graded into tanks with the control fish. This procedure was repeated until all experimental fish had been assessed.

Experimental Inventory: Behavior

As an assessment of stress responsiveness, we recorded and later quantified behavior of individual experimental fish in custom-built behavior arenas via video at each of the four experimental inventories. After the overnight period in holding baskets, one basket at a time, fish were individually transferred from the basket into one of nine identical approximately 1.0 m x 0.5 m behavior arenas divided into equal sized home and novel sides separated by a central barrier (See Appendix A for specifics of arena construction). Within the barrier was a passage hole with a closable gate to control access between the home and novel sides. The hole size was consistent throughout the study and did not account for changes in fish size that may have caused changes to fish perception of the hole. The arena was covered to avoid disturbances that could influence fish behavior and a night vision video camera (Q-SEE International Limited, Hong Kong, China) that recorded fish movements was positioned under the cover.

Fish were placed into the home side of the arena without access to the novel side for a habituation period before behavior tracking began. After a 30-minute habituation in the home side, the barrier gate was raised using a pulley system activated from outside of the arena to provide free access to both sides of the arena. Then, volitional movements between the home and novel side of the arena were video recorded for a 60-minute behavior trial for later quantification of behavior. After this period, fish were returned to their initial holding basket, and the next basket of fish was processed until all fish had been assessed for behavior. Behavioral measurements on all experimental fish within an individual rearing tank were completed from 0600 -1400 on the same day. Once behavior recordings were completed for all fish from a single tank, the fish were anaesthetized for measurements of length and weight and quantification of morphology and health traits.

Video recordings of individual fish were later observed for estimation of behavior, quantified as latency and total number of crosses. Latency was defined as the time lag between the time the gate was opened and the first time a fish fully crossed from the home side to the novel side. If a fish did not cross to the novel side during the entire 60-minute behavior trial, latency was recorded as 60 minutes (White *et al.*, 2013). Total number of crosses was determined as the number of times the fish passed between the home and novel side, regardless of passage direction.

Experimental Inventory: Health

Health of experimental fish was assessed using two quantifications of accumulated energetic reserves and a score quantifying visual signs of injury, disease, or malformation during all four Experimental Inventories and the Hatchery Intake Inventory. As energetic reserves are primarily accumulated as lipid stores in fishes, which require lethal sampling for direct quantification, two indirect quantification approaches were used to track energetic reserves over time. Relative condition (K_n) was calculated as it is an index that is commonly used by fisheries biologists as a proxy for health and condition of fish (Pope and Kruse, 2007; Al-Chokhachy *et al.*, 2019; Cooper, 1961), although with inconsistent reliability in predicting actual nutritional or energetic status (Schloesser and Fabrizio, 2017; Kaufman *et al.*, 2006; Sutton *et al.*, 2000; Hartman and Margraf, 2006; 2008). To account for this inconsistency, microwave energy meter (Fatmeter, Distell, West Lothian, Scotland, UK) measurements were used as a non-invasive proxy of whole-body energetic reserves (Bangs and Nagler, 2014). The third health metric was a standardized Visual Health Assessment (VHA) that was based on previous studies that have assessed visual observations as indicators of overall individual health (Novotny and Beeman, 1990; Hoyle *et al.*, 2007, Strzyżewska-Worotyńska *et al.*, 2017; Martinelli *et al.*, 2020; Blazer *et al.*, 2018; Tierney and Farrel, 2004).

Fatmeter measurements to quantify whole-body energetic reserves were performed only on experimental fish at the Hatchery Intake Inventory and the four Experimental Inventories and cautiously applied as a proxy for juvenile WCT whole-body energetic reserves (Appendix C). The Fatmeter was set to the uncalibrated SR-Research setting and programmed to output the average of two replicate measurements. Anaesthetized fish were placed with their left side up, and mucus and excess water were gently wiped off the Fatmeter measurement site over the lateral line under the dorsal fin of the fish (Figure 1.4). This position is recommended by Distell to achieve reliable readings on juvenile salmonids of the size range used in this study (David Fulton, personal communication, 2019). The Fatmeter was held to the measurement site until the reading stabilized.

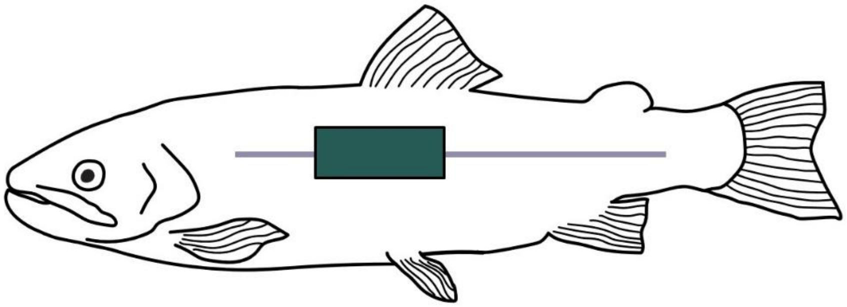


Figure 1.4. Measurement location for Fatmeter measurements as a proxy for whole-body energetic reserves of westslope cutthroat trout. The rectangle indicates the Fatmeter sensor placement over the lateral line (horizontal line) under the dorsal fin on the left side of the fish.

The K_n of each individual was calculated as the ratio of observed weight to expected weight at length for experimental WCT using a population specific formula. To derive the population specific formula to estimate expected weight at length, a linear regression using the $\log_{10}$ weight and $\log_{10}$ length from a subset of the experimental fish replicate measurements was conducted and the formula for this weight to length relationship derived (Appendix D). The K_n of each individual fish observation was calculated as the expected ratio of the observed weight to the predicted weight based on the observed length using this formula (Blackwell *et al.* 2000).

The VHA was conducted on each experimental fish to quantify eight indicators of health status at each Experimental Inventory (Appendix B). The concept of a VHA to relate external health indicators to overall individual health is not novel (Novotny and Beeman, 1990; Hoyle *et al.*, 2007, Strzyżewska-Worotyńska *et al.*, 2017; Martinelli *et al.*, 2020; Blazer *et al.*, 2018; Tierney and Farrel, 2004). To conduct the VHA each fish was visually assessed for damage or apparent loss of condition in the eight indicators while anaesthetized. Five of these indicators (eyes, fins, gills, wounds, and color) were quantified on a scale of 1 to 5, with a score of 1 indicating lowest possible condition (e.g., both eyes missing; fish is fully blind) and a score of 5 indicating best possible condition (e.g., no eye damage or apparent vision effect) (See Appendix B for further details on scoring for each indicator). The remaining three indicators (jaw, spine, and operculum) were quantified on a scale of 1 to 3, with a score of 1 indicating lowest possible condition (e.g., severe jaw deformation; feeding likely severely impaired), and a score of 3 indicating highest possible condition (e.g., normal jaw formation; feeding unlikely impaired). I scored jaw, spine, and operculum with a smaller scale range than eyes, fins, gills, wounds, and color based on preliminary observations that suggested the latter group of indicators could be consistently scored with greater resolution.

Two indexes of the VHA were calculated for each fish, an unweighted VHA Index (VHI) and a weighted VHA index (WVHI). For both WVHI and VHI, the scores for each indicator were separately converted to ratios of the maximum possible score. For VHI, this raw ratio was summed across all indicators using the below formula:

$$VHI = \sum \left(\frac{Indicator\ Actual\ Score}{Indicator\ Maximum\ Potential\ Score} \right)$$

where the indicator actual score is the score of as given indicator for an individual, indicator maximum potential score is the maximum possible score for a given indicator. This resulted in each fish having a final VHI score out of 8. For WVHI, an indicator-specific weighting was applied to each indicator ratio based on presumed biological importance based upon prior studies on salmonids (McKinstry *et al.*, 2007; Halvorsen *et al.*, 2012). These studies found injuries to the gills, operculum, and body flesh to be heavily weighted whereas body coloration and fin appearance were lightly weighted. I am unaware of any studies that have investigated the relative importance of indicators to future performance under hatchery rearing conditions, therefore, weights were assigned based on the previously reported weighting of barotrauma-related injuries as well as hypothesized contextual importance of each respective indicator in a hatchery setting. The assigned weights were color 0.25, fins 0.25, eyes 0.25, spine 0.25, jaw 0.10, wounds 0.10, operculum 0.15, and gills 0.55. WVHI was calculated using the below formula:

$$\text{WVHI} = \sum\left(\frac{Indicator\ Actual\ Score}{Indicator\ Maximum\ Potential\ Score}\text{x Weight of Indicator}\right) X\ 100$$

where the indicator actual score is the score of as given indicator for an individual, indicator maximum potential score is the maximum possible score for a given indicator for an individual, weight of indicator is the assigned weight for the specific indicator, and the WVHI is the sum of all of the standardized indicators for a given individual. This resulted in each fish having a final WVHI score out of 100.

Experimental Inventory: Morphology

Morphology of individual experimental fish was quantified using two different approaches: assessments of the relative size of individual body parts via linear measurements and assessments of overall body shape via geometric morphometrics (Zelditch *et al.*, 2004; Appendix E). For both approaches, anatomical landmarks (hereby “landmarks”) (Figure 1.5) were measured on digital images acquired at each of the four experimental inventories (Seiler and

Keeley, 2009; Chavarie *et al.*, 2012). There were 28 total landmarks assessed, 21 were used for geometric morphometrics analyses and 18 were used for linear measurements.

The linear measurements were conducted manually on each image in TPSDig2 (Rohlf, 2017) by measuring the distance between predetermined pairs of the landmarks. To adjust linear measurements for size variation among individuals and inventories, all linear measurements were $\log_{10}$ transformed and regressed against standard length using the lm () function in R. Residuals from these regressions were then used in subsequent analyses to minimize the effects of among and within fish variation in size on the linear measurements (Reist, 1985).

Fish body shape was quantified using geometric morphometrics analyses on 21 of the landmarks recorded on images in TPSDig2. A multivariate coordinate matrix of Procrustes standardized coordinates (hereby "Procrustes coordinates") was generated to serve as standardized superimposition data with effects of size, position, and orientation on variation in body shape removed for subsequent analysis.

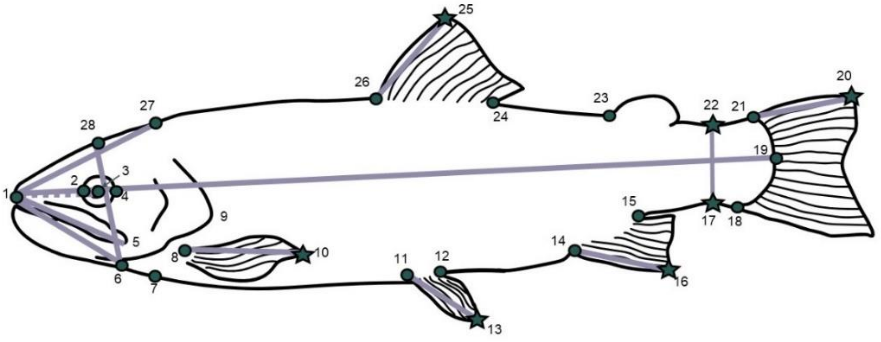


Figure 1.5. The position of 28 landmarks recorded on digital images of experimental westslope cutthroat trout and used to perform linear measurements and geometric morphometrics analysis of body shape. Circles denote landmarks used for geometric morphometrics and linear measurements. Stars denote landmarks used only to determine linear measurements and not for geometric morphometrics. The 12 linear measurements (lines) quantified are as follows, with associated landmarks in parentheses: upper jaw residual length (1-5), lower jaw residual length (1-6), residual head depth (28-6), residual snout-eye length (dashed line; 1-3), residual head length (1-20), residual dorsal fin length (25-26), residual caudal fin length (21-20), residual caudal peduncle depth (22-17), residual anal fin length (14-15), residual pelvic fin length (11-13), residual pectoral fin length (8-10), standard length (1-19).

Data Analysis

Data were analyzed with the objective of identifying behavior, health, and morphological traits with low within-individual variation over time relative to among individual variation for WCT during hatchery rearing. I quantified within-individual variation in relation to the total range of variation within the population for all behavior, health, and morphology traits tracked in experimental fish. Evolutionary niche theory partitions the total niche width (TNW), which is the total breadth of variation in a trait exhibited by individuals making up a population, into two portions: the within-individual portion (WIP) and the between-individual portion (BIP) (Roughgarden, 1972). The degree of individual specialization (IS) within a population is quantified as the proportion of TNW explained by within-individual variation (Bolnick *et al.*, 2003; Araújo *et al.*, 2011). An IS ratio that approaches 1 suggests that all individuals within a population exhibited the full range of responses present within that population with little to no degree of specialization (Roughgarden, 1972; 1974; 1978). However, I employed the IS analytical approach as a quantitative tool to identify behavior, health, and morphology traits with relatively low within-individual variation over time for WCT during hatchery rearing. To avoid confusion with the evolutionary applications of TNW analyses, here I refer to total response range (TRR) in the place of TNW. I quantified IS as a ratio for the 18 univariate traits (Table 1.2). Patterns of variation within the multivariate body shape datasets separately were explored based on the total variance using Principal Component Analysis (PCA) and the variance standardized to replicate measurements of individual fish using Canonical Variation Analysis (CVA) (Renaud *et al.*, 2015).

Due to mortalities and human errors, it was necessary to choose between maximizing sample size or replication of both analyses of the 18 univariate trait and the multivariate body shape datasets. In order to explore both maximal sample size and maximal replication, I performed analyses on two separate datasets for each of the univariate and multivariate traits. ‘Duplicate’ analyses, which allowed for maximization of sample size, were performed on data collected from all fish at the first two inventories. ‘Complete case’ analyses, allowed maximization of replication, while sacrificing sample size, were performed on data collected from only fish with data available for every inventory. As missing data points varied among the 18 univariate traits, the sample size for complete case and duplicate analyses varied among these traits (Table 1.2).

For the 18 univariate traits, the TRR, WIP, BIP, and IS were calculated separately for each duplicate and complete case analyses. The WIP of a trait was the sum of the median values of the trait for each individual fish, calculated using the below formula:

$$WIP = \sum (i_n)$$

where *i* is the median value for the nth individual for a given trait, calculated using the median() function in Base R (R Core, 2021).

BIP of a given trait was the sum of the interquartile range (IQR) of replicate measurements of the trait for each individual fish. BIP was calculated using the below formula:

$$BIP = \sum (j_n)$$

where *j* is the IQR for the nth individual for a given trait, calculated using the IQR() function in Base R (R Core, 2021)

TRR of a trait was calculated as the sum of WIP and BIC. Finally, IS was calculated separately for each trait to quantify the ratio of the TRR occupied by the variation occurring within one individual (WIP). Analyses of IS were conducted on the 18 univariate traits assessing behavior, morphology, and health. For the purposes of this study, an IS ratio of 0.25 or less was regarded as highly specialized, an IS of 0.25-0.50 was moderately specialized, and an IS greater than 0.50 was a generalist. These cutoffs were based on published interpretations of IS values among populations (Bolnick *et al.*, 2003).

For the multivariate body shape dataset, I initally analyzed the data to determine if body shape was affected by fish growth in size over time, as replicate body shape comparisons among and within individuals are often complicated by allometric affects (Heino, 2014; Simonsen *et al.*, 2017). I applied randomized residual permutation analysis of variance (ANOVA) using the procd.lm. () function from the "geomorph" package followed by the anova () function of the "geomorph" package (Adams *et al.*, 2019) to test for effects of body size, quantified as centroid size, and inventory date on the Procrustes coordinates for duplicate and complete case datasets separately. This revealed substantial shifts in body shape due to size, but date did not affect this relationship, indicating that size had a consistent effect on morphology at each date (Table 1.1).

Table 1.1. Residual permutation ANOVA results comparing the effects of inventory date and centroid size on the Procrustes coordinates for individual fish over the duration of the study (July 2019- June 2021).

	df	**f value**	**P value**	**R^2**
Complete Case				
Inventory Date	1	1.07	0.36	0.02
Centroid Size	1	16.0	0.01×10^{-4}	0.12
Duplicate Case				
Inventory Date	1	1.21	0.25	0.01
Centroid Size	1	6.81	0.01×10^{-4}	0.06

In order to correct for these size effects on body shape, I regressed log of centroid size against the Procrustes coordinates using the procd.lm. () function from the "geomorph" package (Zelditch *et al.*, 2004; Adams *et al.*, 2019). The residuals from this regression (hereby "Procrustes residuals") were subsequently used for all further geometric morphometric analysis. I explored patterns in variation of Procrustes residuals within and between individuals through multiple analytical approaches. I applied analysis of variance (ANOVA) using the procd. lm () function with a 1,000 -iteration residual randomization permutation procedure (Collyer *et al.*, 2014) followed by the anova () function of the "geomorph" package (Adams *et al.*, 2019) to test whether Procrustes residuals differed between inventory dates or between individual fish. Inventory date was used as a proxy for the WIP as differences over time within individuals would be represented by a change in shape between dates. Individual fish identity as determined by PIT tag number (hereby “individual”) was used as a proxy for the BIP as differences in shape between individuals would be represented by this grouping. The R^2 outputs of this ANOVA allowed me to compare the variation in Procrustes residuals explained within individuals and between individuals. Additionally, I visually explored patterns in Procrustes residuals variation through PCA of the duplicate and complete case datasets of Procrustes residuals separately using the gm. prcomp () function in R from the “geomorph” package and CVA using the cva () function from the “Morpho” package (Schlager *et al.*, 2017).

Table 1.2. Sample size (n) for each trait assessed on all experimental fish from July 2019 to June 2021, segregated by the duplicate and complete case datasets (WVHI: Weighted Health Index, VHI: Health Index; K_n: Relative Condition). The duplicate analysis includes fish that either eventually died or were missing data from the September 2020 or January 2021 Experimental Inventories. The complete case analysis only includes individuals that had data collected for each respective trait at every Experimental Inventory. The sample sizes for each complete case trait may vary as some individuals would have a complete dataset for one trait, but not another trait, due to human error.

Trait	**Duplicate (n)**	**Complete Case (n)**
Univariate Traits		
Latency	45	35
Crosses	45	35
WVHI	70	59
VHI	70	59
Fatmeter	70	59
K_n	72	61
Upper Jaw Residual Length	60	53
Lower Jaw Residual Length	60	53
Snout-Eye Residual Length	60	53
Head Residual Length	60	53
Head Residual Depth	60	52
Caudal Peduncle Residual Depth	60	53
Pectoral Fin Residual Length	60	53
Pelvic Fin Residual Length	60	52
Anal Fin Residual Length	60	53
Caudal Fin Residual Length	60	53
Dorsal Fin Residual Length	60	53
Multivariate Trait		
Body Shape	83	30

Results

There was a wide range of IS values among the 18 univariate traits for both duplicate and complete case analyses (Table 1.3-1.4). The IS ratio for the duplicate analyses ranged from 0.02 for K_n to 0.50 for Snout-Eye length residual and the IS ratio for the complete case analyses ranged from 0.02 for K_n to 0.65 for Snout- Eye length residual. For the duplicate analyses, fourteen of the univariate traits were moderately specialized and four were highly specialized (K_n, WVHI, VHI, and energetic reserves). For the complete case analyses, 14 of the univariate traits had a generalist degree of specialization and four were highly specialized (K_n, WVHI, VHI, and energetic reserves). The differences among traits with a high degree of specialization and a generalist degree of specialization are represented in Figure 1.6.

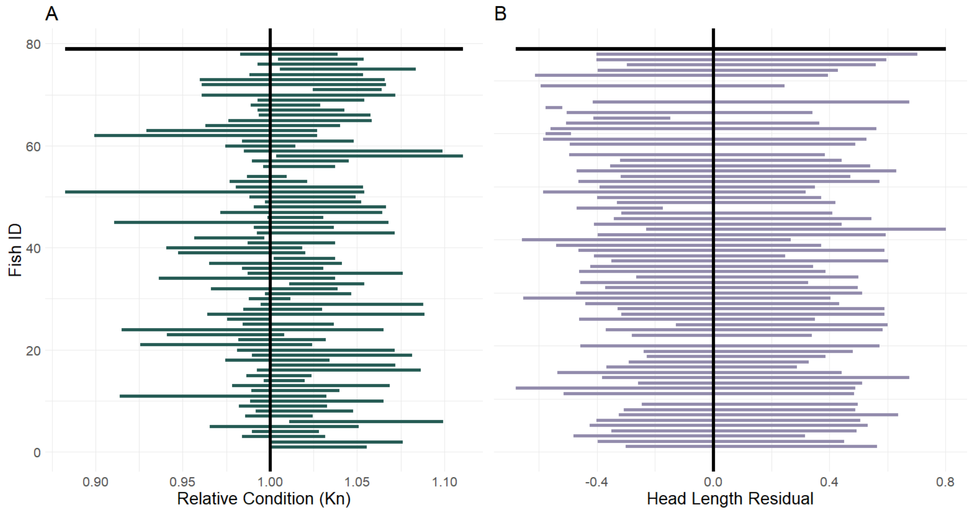


Figure 1.6. The ranges of relative condition (K_n), a highly specialized trait, and head residual length, a generalized trait, for replicate observations of individual fish (colored lines) and the population (i.e., total response range; black line). The vertical black line represents the average for each respective trait.

Table 1.3. The individual specialization (IS) and categorical degree of specialization for each of the univariate traits used in the duplicate analyses (WVHI: Weighted Health Index, VHI: Health Index; K_n: Relative Condition). Latency was defined as the time lag between the time the gate was opened and the first time a fish fully crossed from the home side to the novel side The duplicate analysis includes fish that either eventually died or were missing data from the September 2020 or January 2021 Experimental Inventories. For the purposes of this study, an IS ratio of 0.25 or less was regarded as highly specialized, an IS of 0.25-0.50 was moderately specialized, and an IS greater than 0.50 was a generalist.

Trait	**WIP**	**BIP**	**IS (Duplicate)**	**Degree of Specialization**
VHI	54	2232	0.02	Highly Specialized
K_n	1.49	73.8	0.02	Highly Specialized
WVHI	257	6468	0.04	Highly Specialized
Energetic Reserves	291	2184	0.12	Highly Specialized
Upper Jaw Residual	2.95	5.41	0.32	Moderately Specialized
Latency	140	196	0.36	Moderately Specialized
Lower Jaw Residual	2.53	4.36	0.37	Moderately Specialized
Anal Fin Residual	2.90	4.63	0.38	Moderately Specialized
Dorsal Fin Residual	2.33	3.38	0.41	Moderately Specialized
Crosses	132	103	0.42	Moderately Specialized
Caudal Peduncle Residual	4.10	4.75	0.46	Moderately Specialized
Head Depth Residual	9.66	10.3	0.48	Moderately Specialized
Pectoral Fin Residual	14.3	14.7	0.49	Moderately Specialized
Pelvic Fin Residual	20.7	21.4	0.49	Moderately Specialized
Caudal Fin Residual	11.0	11.3	0.49	Moderately Specialized
Snout-Eye Residual	15.3	15.4	0.50	Moderately Specialized
Head Length Residual	35.5	19.2	0.50	Moderately Specialized

Table 1.4. The individual specialization (IS), the within-individual portion (WIP), the between-individual portion (BIP), and categorical degree of specialization for each of the univariate traits used in the complete case analyses (CC) (WVHI: Weighted Health Index, VHI: Health Index; K_n: Relative Condition). Latency was defined as the time lag between the time the gate was opened and the first time a fish fully crossed from the home side to the novel side The complete case analysis only includes individuals that had data collected for each respective trait at every Experimental Inventory. The sample sizes for each complete case trait may vary as some individuals would have a complete dataset for one trait, but not another due to human error. For the purposes of this study, an IS ratio of 0.25 or less was regarded as highly specialized, an IS of 0.25-0.50 was moderately specialized, and an IS greater than 0.50 was a generalist.

Trait	**WIP**	**BIP**	**IS (CC)**	**Degree of Specialization**
K_n	1.10	61.6	0.02	Highly Specialized
WVHI	204	5242	0.04	Highly Specialized
VHI	81	1832	0.04	Highly Specialized
Energetic Reserves	358.3	1858	0.16	Highly Specialized
Upper Jaw Residual	5.99	5.80	0.51	Generalist
Lower Jaw Residual	4.95	4.51	0.52	Generalist
Caudal Fin Residual	9.13	7.67	0.54	Generalist
Head Depth Residual	9.81	7.75	0.56	Generalist
Pelvic Fin Residual	17.7	14.1	0.56	Generalist
Anal Fin Residual	4.86	3.80	0.56	Generalist
Dorsal Fin Residual	3.91	3.05	0.56	Generalist
Crosses	132	103	0.56	Generalist
Pectoral Fin Residual	14.3	10.3	0.58	Generalist
Caudal Peduncle Residual	5.38	3.92	0.58	Generalist
Latency	665	487	0.58	Generalist
Snout-Eye Residual	24.4	14.0	0.65	Generalist
Head Length Residual	15.3	15.40	0.65	Generalist

The duplicate and complete case multivariate body shape datasets were significantly affected by individual, but only the duplicate analysis had a significant effect of inventory date (ANOVA, Table 1.5). The differences among individuals accounted for 31% and 57% of the variation in body shape for complete and duplicate cases, respectively, whereas inventory date did not have a significant effect on body shape and accounted for less than 1% of the variation in body shape (ANOVA, Table 1.5). These findings suggest that more of the total variation for body shape was accounted for between individuals than within individuals, as individual accounted for more variation than the inventory date.

Table 1.5. ANOVA results comparing the effects of inventory date and centroid size on the Procrustes residuals for individual fish over the duration of the study (July 2019 - June 2021), segregated by duplicate or complete case analyses. The duplicate analysis includes fish that either eventually died or were missing data from the September 2020 or January 2021 Experimental Inventories. The complete case analysis only includes individuals that had data collected for each respective trait at every Experimental Inventory.

	df	**f value**	**P value**	**R^2**
Complete Case				
Inventory Date	3	0.54	0.99	0.01
Individual	29	1.27	0.01	0.31
Duplicate				
Inventory Date	1	0.02	0.01	2.0×10^{-4}
Individual	50	1.32	2.0×10^{-4}	0.57

The PCA and CVA plots correspond with the ANOVA findings of greater between individual than within-individual variation for duplicate and complete case datasets. For the complete and duplicate case analyses, respectively, the first two principal components explained 42.5% and 35.9 % of the variation in the WCT experimental population body shape over the study duration. Plots of PCA axis 1 and 2 revealed a qualitatively large amount of between-individual variation, but also a large amount of overlap among individuals for duplicate and complete case datasets (Figure 1.7). Plots of CVA axis 1 and 2 qualitatively revealed a greater degree of between individual variation relative to within-individual variation than the PCA plots (Figure 1.8). PCA differs from CVA as there is no assignation or prior information regarding groupings of interest within the dataset (Zelditch *et al.*, 2004). PCA determines principal components accounting for the majority of variation in a dataset, whereas CVA minimizes the within-group variance while maximizing the between-group variance within a given dataset and given a prior grouping factor. Thus, PCA can provide “uncalibrated” scores that can then be used to examine clustering or variation among factor levels, whereas CVA is specific to the priori group assignment.

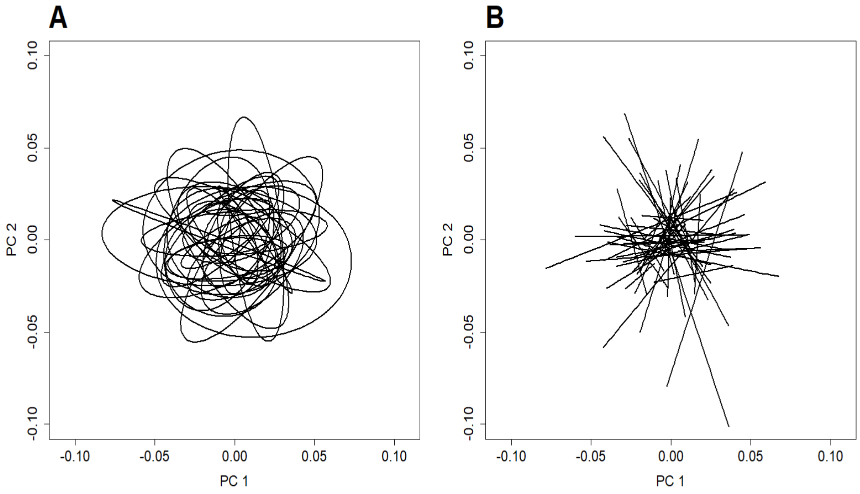


Figure 1.7. PCA plots with 95% confidence ellipses surrounding PCA axes 1 and 2 of individual westslope cutthroat trout body shape for the complete case (A) and duplicate case (B) datasets. PC1 explained 25.0% and 20.9% of the variation in complete and duplicate cases, respectively. PC2 explained 17.5% and 15.0% of the variation in complete and duplicate cases, respectively.

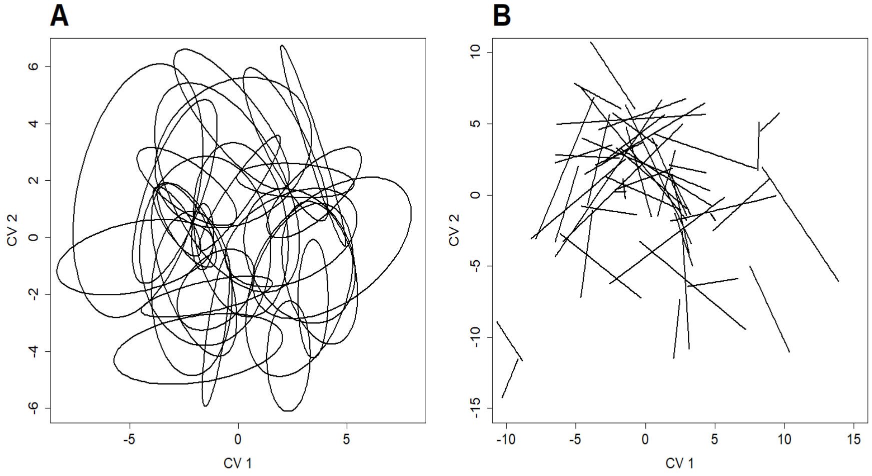


Figure 1.8. CVA plots with 95% confidence ellipses surrounding CVA axes 1 and 2 of individual westslope cutthroat trout body shape for the complete case (A) and duplicate case (B) datasets. CV1 explained 24.9% and 22.6% of the variation in complete and duplicate cases, respectively. CV2 explained 16.7 % and 19.2% of the variation in complete and duplicate cases, respectively.

Discussion

In this study, I examined a suite of behavioral, morphological, and health-related traits of wild-origin WCT in a hatchery setting to identify traits showing relatively consistent IS over time as candidate traits for predicting future in hatchery performance in Chapter 2 of my thesis. Although the majority of traits investigated were relatively inconsistent for individuals over the two-year hatchery rearing period, a small number demonstrated a high level of specialization over time. Based on these findings, I make recommendations for traits to include in Chapter 2 analyses of correlations between traits and reproductive performance of wild-origin WCT in a hatchery setting.

Although I had hypothesized that morphological traits would be among the most stable traits within individual WCT, the morphological traits assessed tended to be moderately specialized for individuals over a short period of time but unspecialized when replicate measurements over the two-year hatchery rearing period were considered. For all linear measurements, the fish were classified as moderately specialized for the duplicate dataset, but generalists for the complete case dataset. Within this suite of 21 phenotypic traits, Upper Jaw, Lower Jaw, and Anal Fin residual length were the most specialized with IS values ranging from 0.32-0.38 for the duplicate dataset and 0.51-0.56 for the complete case dataset. For the multivariate body shape, R^2 outputs from ANOVA for effects of inventory date and individual on Procrustes residuals suggest that individuals are moderately specialized, with 31 to 56% of the variation in body shape, depending on complete or duplicate datasets, explained by fish identity. Although CVA plots qualitatively illustrated a greater degree of between-individual variation relative to within-individual variation than the PCA plots, I recommend reducing the duplicate multivariate shape data to the principal components accounting for 70% of the variation, as CVA uses priori group assignment to minimize within group relative to between group variation, as opposed to maximizing the variation explained by each respective component as in PCA. Although morphological traits were less specialized than I had hypothesized. I recommend the duplicate dataset of upper jaw residual, lower jaw, and anal fin residual length, and body shape for Chapter 2 analyses of correlations between individual traits and reproductive performance of wild-origin WCT in a hatchery setting.

Morphological traits may have been less specialized than I had hypothesized due to variation in individual body shape introduced with unpredictable phenotypic plasticity, ontogenetic shifts, or growth rates. This hypothesis did not account for life-stage specific ontogenetic shifts later in life, which are common in fishes, particularly those that display differentiation in life history in the adult stage (Meyer, 1990; Snorrason *et al*. 1994; Mittelbach *et al*. 1999; Moles *et al*. 2010). My original hypothesis also did not account for the capacity of individual fish to plastically respond to a range of environmental conditions unpredictably (Keeley *et al*., 2007). Additionally, disparities in age and growth rate could influence the relative size of each respective part of the anatomy while maintaining a consistent body shape (Reist *et al*., 2013). Subsequent research should examine the relationships among age, growth rates, and morphology to determine if age or growth rate has a significant effect on linear measurements or body shape.

Again, while I had hypothesized that behavioral traits would be relatively consistent within individuals regardless of environmental changes, WCT were moderately specialized or generalist for the behavioral traits assessed. As behavioral latency for the duplicate dataset classified fish as moderate specialists, I recommend this trait for Chapter 2 analyses of correlations between individual traits and reproductive performance of wild-origin WCT in a hatchery setting. Behavior can be highly contextual and the shift from wild habitat to hatchery conditions and subsequent micro-shifts in feed and tanks may have been sufficient to increase the within-individual variability in crosses and latency (Ruiz-Gomez *et al.*, 2011). Behavior could be useful as a tool to predict trout life history strategy, but there could be considerable variability within fish that could make certainty of predictions difficult.

My hypothesis that that health traits would be moderately consistent within individuals over time was supported by my findings. However, health traits (energetic reserves, WVHI, VHI, and K_n) had the highest degree IS of the traits measured in this study, with K_n for the complete case group being the most specialized at 0.02 %, which surpassed my predictions. This indicates that almost the majority of the variation exhibited in K_n was due to the BIC. Additionally, this indicates that if a fish enters the hatchery in one condition (whether it be good, moderate, or bad) it will remain in that state consistently throughout the duration in the hatchery. There was no significant difference between WVHI and VHI in the IS, so in this context, biologically weighting the VHI did not significantly affect the IS. Thus, if health status could be related to other performance metrics, it could be possible to predict performance based on early health status in the hatchery.

Inter-individual variation in phenotypic and life history traits are key to the perpetual persistence of salmonids such as WCT (Reist *et al.*, 2013). However, traditional hatchery practices may alter phenotypic diversity via artificial selection. The study of IS and its components offers a novel method to identify traits with high potential for specialization; ergo, the individuals most vulnerable to artificial selection pressure. While the majority of the traits examined within this study do not have a high potential to be used to identify vulnerable fish due to the high degree of within-individual variation, I was able to identify several very promising phenotypic traits. WVHI, VHI, K_n, energetic reserves, and the body shape measurements were all very consistent over time for individuals. Latency and the upper jaw, lower jaw, anal fin residual lengths, had intermediate levels of specialization, indicating potential for Chapter 2 analyses of correlations between individual traits and reproductive performance of wild-origin WCT in a hatchery setting. However, the analyses conducted in this study did not assess the population range for each phenotypic trait, so it is unknown if there is a high degree of variability among individuals. This study can only state that the variability present for these traits is primarily due to the variation between individuals. Subsequent research will need to determine what the population range for these traits actually is, and thus, if the variation between individuals for each given traits disparate enough to be related significantly to performance.

RELATING THE REPRODUCTIVE PERFORMANCE OF WESTSLOPE CUTTHROAT TROUT TO SPECIALIST TRAITS FOR FUTURE APPLICATION AS AN ARTIFICIAL SELECTION SCREENING TOOL IN A CONSERVATION HATCHERY

Due to widespread declines and extirpations of freshwater aquatic organisms, conservation hatcheries have risen in prominence to replace lost populations of a variety of taxa, specifically focusing on addressing declines of cutthroat trout (Su *et al.*, 2021). While there have been successes with this approach, there are still aspects that remain problematic: removal of wild fish from ecosystems and evidence of a reduction of genetic and phenotypic variation within the out planted offspring relative to historic wild populations (Christie *et al.*, 2012; 2014; 2016). Although conservation hatchery practices have improved in recent years, maximizing reproductive contributions from all wild-captured individuals continues to be an important development goal that is not always achieved by these facilities. As the initial causes for fish declines are unlikely to resolve with any urgency, hatcheries will continue to be a necessary component of fisheries conservation and must ensure maintenance of genotypic and trait diversity while maximizing the efficiency of wild-origin salmonid rearing.

Impacts of habitat and hydrological modifications on native salmonids have been exacerbated by introductions of non-native salmonids, such as rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), brook trout (*Salvelinus fontinalis*), and lake trout (*S. namaycush*), which threaten native salmonids with hybridization, predation, and competition for resources (Drinian *et al.*, 2015; Ostberg *et al.*, 2011; Muhlfeld *et al.*, 2009; 2014; Koel *et al.*, 2005; Hitt *et al.*, 2017; Al-Chokhachy and Sepulveda, 2019; Lawrence *et al.*, 2014; Stapp and Hayward, 2002). The majority of species in the genus *Oncorhynchus* have faced serious declines, with some estimates stating that *Oncorhynchus* populations in the Pacific Northwest are at less than 10% of their pre-1850 population estimates (Lackey, 2008) and nearly all current populations are performing poorly with dramatically reduced productivity (Dorner *et al.*, 2017; Ohlberger *et al.*, 2016). Of the 16 distinct recognized *Oncorhynchus* species and myriad subspecies, some are extinct, some are federally listed as threatened, and nearly all are protected at the state level (Kruse *et al.*, 1997; Shepard *et al.*, 1997; Horan *et al.*, 2000; Behnke, 2002). Trout and salmon conservation methods are diverse, typically including a combination of habitat restoration, removal of non-native competitors, partition of habitat to prevent non-native competition, and stocking efforts via remote site incubator or hatchery produced fishes (Novinger and Rahel, 2003; Andrews *et al.*, 2016; Shepard *et al.*, 2019; 2021; Ertel *et al.*, 2017; Kovach *et al.*, 2018).

Hatcheries have traditionally been used for commercial and sport stocking across the country, and at the peak of hatchery utilization from the early-1800s to the mid-1900s, they were primarily used to mass-produce fishes to promote easy exploitation of the fisheries crop for recreational and subsistence opportunities (Behnke, 2002; Quist *et al.*, 2004). Commercial hatcheries are most frequently employed to produce mass quantities of fishes for commercial harvest and sport hatcheries are used to produce mass amounts of a variety of fish species for recreational harvest opportunities. There has been some controversy regarding the use of hatcheries in trout conservation, with evidence suggesting that in some contexts, hatchery-reared fish have lower fitness in the wild than wild-origin trout, and that this lower performance can perpetuate into wild populations as the two groups breed over time (Christie *et al.*, 2012; 2014; 2016; McClure *et al.*, 2008; Hilborn *et al.* 2003). The relatively stable conditions experienced within hatcheries can result in the lower performance of hatchery-origin salmonids in the wild, where conditions tend to be variable and stochastic (Fleming and Einum, 1997; Baskett and Waples, 2013). Additionally, the initial causes for salmonid declines will only continue to intensify, so hatcheries will continue to be necessary tools to mitigate the perpetual population declines associated with habitat loss and degradation (Liknes and Graham, 1988), overharvest (Behnke, 2002), introductions of non-native competitors (Kovach *et al.*, 2018; Campbell *et al.*, 2002; Allendorf and Leary, 1988) and climate change (Young and Harig, 2001; Harig and Fausch, 2002; Kovach *et al.*, 2016) that are unlikely to resolve in the immediate future (Roberts *et al.*, 2017; Williams *et al.*, 2009; Kovach *et al.*, 2016). Thus, an alternative hatchery rearing system was developed to ameliorate the historical issues associated with hatchery rearing of salmonids, the conservation hatchery, for such systems where preservation of wild traits is the goal, rather than harvest (Mobrand *et al.*, 2005).

Conservation hatcheries are used to produce small quantities of high-quality fish for species that have been identified as a conservation concern. A key distinction of conservation hatcheries is that the fish produced are not intended for immediate consumption by a human entity, but rather, to meet long-term fish management goals for a given species often in conjunction with other land restoration goals. Conservation hatcheries have made concerted efforts to mitigate the problems associated with artificial propagation identified by earlier research. These issues can be generally grouped into four main categories of action based upon the available literature: 1) reduce the artificial selection of traits within the hatchery environment; 2) improve the wild performance of hatchery-origin salmonids; 3) decrease or eliminate stocking of hatchery-origin salmonids into waterbodies with wild-origin populations;

and 4) restore/maintain the historic level of genotypic and phenotypic diversity found in the wild (Flagg, 2015; Flagg *et al.*, 1999; Mobrand *et al.*, 2005; Waples, 1991; Al-Chokhachy *et al.*, 2020; Puchany, 2021). To reach the goal of category 1, conservation hatcheries have implemented the use of alternative rearing strategies such as natural tank materials, tank cover, and wild-like thermal regimes to reduce the stress of the fish in the hatchery (Maynard *et al.*, 1995; 1996; Harbicht *et al.*, 2020; Rosengren *et al.*, 2017). By reducing stress, more fish are likely to survive and breed in the novel artificial setting. For category 2, conservation hatcheries have begun using methods that prevent fish from becoming domesticated by using atypical feeding schedules or feed types, stress tests with novel stimuli to promote threat avoidance, and experimenting with tank structures to promote wild-like behavior (Vidergar *et al.*, 2003). To meet the goal of category 3, conservation hatcheries have reduced or eliminated stocking hatchery-origin fish in sympatry with wild-origin fish, and have instead switched to "reintroduction" stocking, or planting of fish in an area where the stock has not been previously found or has been extirpated (Grisaak and Marotz, 2003). By focusing on these alternative stocking patterns, hatcheries can potentially avoid historical issues that have emerged when hatchery fish are stocked in sympatry with wild fish. Salmonid reintroduction stocking efforts using these rearing methods have helped reestablish many thriving populations of a variety of species, including but not limited to: Lahontan cutthroat trout (*O. clarkii henshawi*), chinook salmon (*O. tshawytscha*), and westslope cutthroat trout (*O. c. lewisi*) (Al-Chokhachy *et al.* 2020; Hilderbrand, 2002; Anderson *et al.* 2013). Many conservation hatcheries are preoccupied with meeting goals 1, 2, and 3, and do not directly address category 4. However, certain facilities have made the maintenance of wild diversity in stocked populations the absolute priority and address all categories of action through the lens of category 4.

Sekokini Springs Hatchery is a small-scale facility in northwest Montana focusing on producing small cohorts of high quality westslope cutthroat trout (WCT) for reintroduction into the Flathead River drainage as part of a larger restoration effort. Sekokini Springs uses a novel conservation approach completely focused on refining traditional hatchery rearing practices that may threaten to alter the diversity of the stocked offspring via artificial selection. The facility only implements nearest-neighbor stocking (category 4; Grisaak and Marotz, 2003), by matching donor strains to target water bodies under the assumption that adjacent populations have experienced similar historic selective pressures and, thus, evolved more similar genotypes and local adaptations than distant populations (Hitt *et al.*, 2003). Annually, juvenile WCT are captured in the wild and reared in the hatchery until they reproduce, and then the first-generation offspring are stocked into restored habitat within the same year (category 1 and 2, Leary *et al.*, 1995). Sekokini Springs differs from many similar facilities as it does not have a permanent broodstock, and wild-origin fish are brought to the facility every year. Additionally, Sekokini Springs only implements reintroduction stocking, so there is no risk of potential deleterious effects on wild-origin salmonids (category 3; Harig *et al.*, 2000). However, the primary characteristic that distinguishes Sekokini Springs from other conservation hatcheries is the focus on the health and performance of individual fish via regular monitoring to mitigate any potential loss of life or lack of reproduction that may lead to loss of diversity as defined by category 4. By using this approach, they are able to identify fish that may be potentially suffering in the hatchery as evidenced by low growth or poor visual health and can quickly move these fish to designated tanks and alter rearing conditions in an attempt to resolve these issues.

While this approach addresses all of the categories of action prioritized by conservation hatcheries, there are several inherent limitations. As the nearest neighbor stocking method is defined by using source streams that are representative of historical diversity, the hatchery is limited to deriving its stock from small headwater tributaries within the Flathead River drainage (Grisak and Marotz, 2003). Typically, these streams have low population numbers and density of resident WCT, so the hatchery is strictly limited in what it can remove from the stream without negatively affecting the wild population (Carim *et al.*, 2016). However, the hatchery needs to balance this limitation with the need to remove enough fish to have enough diversity in the offspring that will be produced. Thus, the need for each individual to perform well is critical, resulting in the high-intensity monitoring program at Sekokini Springs Hatchery.

However, the need to monitor fish comes with its own risks, mainly the effects that repeated handling stress may have on the successful sexual and gamete development of individual fish. One of the most indicative signs of stress in fishes is the production of glucocorticoids, namely cortisol (Midwood *et al.*, 2014; Barton, 2005; Sloman *et al.*, 2001; Alderman *et al.*, 2012). While the production of cortisol following an acute stress may benefit an individual, chronic or extreme stress can impair reproductive performance via disruption of normal development of the gonads or the gametes. In either case, the severity of the effect of a given stressor on gonadal or gamete development is largely determined by the time at which stressor occurs relative to the individual’s development as well as the duration and intensity of the stressor (Schreck, 2011; Pankhurst, 2011; Consten *et al.*, 2002; Pickering *et al.*, 1987; Lister *et al*., 2008). If fish undergo a severe stressor during spermatogenesis or vitellogenesis, it may cause follicular atresia, advance or delay oocyte maturation and ovulation, or affect egg size, fertilization success, spawning behavior and progeny quality (Clearwater and Pankhurst, 1997; Schreck *et al*., 2001; Okumura *et al*., 2002; Roy *et al*., 1990; Campbell *et al*., 1992). Thus, chronic stress can have severe consequences on the reproductive performance of individual fish, particularly if that individual has traits that predispose it to have a higher magnitude of stress following a standard stressor.

In Sekokini Springs Hatchery, there is evidence to suggest that there are different life histories present in prior cohorts that are vulnerable to different selection pressures. Anecdotal reports from hatchery managers indicate that some traits appear to correlate, with some fish exhibiting signs of social dominance, fast growth, and large size at sexual maturity while other fish display social submission, low growth, and small size at sexual maturity (Matt Boyer, personal communication, 2018). Hatchery staff also report that these traits appear to correlate to reproductive performance, although direct quantification has not been conducted (Relyea, personal communication, 2019). These reports have been further validated by analysis of historic hatchery records that revealed that in one cohort alone from the Young's Creek 2016 source stock, only 138 fish out of 176 spawned over two spawning events, and of the females that spawned, there was very high variation in the quantity of eggs produced (data not shown). Additionally, growth rates over time for this cohort was not related to survival or reproductive performance, indicating that current methods are insufficient to identify fish that will not reproduce successfully. While Sekokini Springs Hatchery generally has a majority of fish that survive and spawn successfully, there consistently is a certain proportion of fish that fail to spawn or have low percent hatch embryos for unknown reasons. As stress can have large effects on reproductive performance, it seems likely that the individuals that die or fail to spawn are disproportionately affected by the handling stress and chronic stress from being in an unfamiliar environment. Unfortunately, stress responsiveness is difficult to sample due to the rigor required, so if another trait such as behavior or morphology could be related to reproductive performance, implications to stress response or life history strategy could be made.

To identify traits that could predict performance within a conservation hatchery, I quantified the sources of variation for 18 different traits (Chapter 1). I found several traits that had a low proportion of variation comprised of the within-individual proportion relative to the total response range for each respective trait (≤ 40%). The traits that were found to be highly or moderately specialized in Chapter 1 were used in this chapter to determine if these they could be related to the hatchery performance of an individual fish and could thus be used as screening tool to identify vulnerable fish in future cohorts. Traits that had a higher proportion of variation comprised of the between individual proportion were not used in the study within this chapter. Within this chapter, I incorporated age at Hatchery Intake Inventory and sex of individuals to see how these factors might influence any relationships among traits and reproductive performance. I additionally related growth of individuals to reproductive performance. In this study, I aimed to discover traits that were significantly related to hatchery performance in order to identify traits that could be used to later predict population demographics at risk of artificial selection within hatcheries. I hypothesized that age at Hatchery Intake Inventory would be strongly related to the reproductive performance of individuals. I also hypothesized that health (relative condition (K_n), Weighted Visual Health Index (WVHI), Visual Health Index (VHI), and energetic reserves) would be highly predictive of eventual reproductive performance. Additionally, I hypothesized that several traits (upper jaw, lower jaw, anal fin residual length, latency, and body shape) at certain timepoints would be predictive of reproductive performance. The final hypothesis was used to test for the effect of the additional experimental handling on reproductive performance.

Methods

To investigate the relationships among traits of wild-origin WCT and hatchery reproductive performance, I tracked a suite of behavioral, morphological, and health traits from hatchery intake (July 2019) to reproduction (May-June 2021). In Chapter 1, I identified nine traits (K_n, WVHI, VHI, energetic reserves, latency, body shape, and upper jaw, lower jaw, and anal fin residual length) with relatively low within-individual variation over time relative to the between-individual variation within the population. In addition to these nine traits, here I investigate whether age at Hatchery Intake Inventory or growth can predict survival and reproductive performance of wild-origin WCT in a conservation hatchery and if these relationships differed by sex.

Study System, Specimen Collection, and Hatchery Rearing

WCT collection from Emery Creek, MT, transport and intake to Sekokini Springs Hatchery, hybrid identification and culling, and daily hatchery rearing practices were described in detail in Chapter 1. At the beginning of the study, there were a total of 156 WCT. In order to validate if experimental handling affected reproductive performance, WCT were split evenly into two groups at the Hatchery Intake Inventory described below: experimental and control. The control fish were not subject to any additional handling as part of the experimental study, whereas the experimental group underwent several additional assessments. At the beginning of the study, there were 77 experimental and 79 control fish and by the cessation of the study there were 67 experimental and 74 control fish due to mortality.

Throughout the study, WCT mortalities were grouped into two categories based on the most likely cause of death as determined by hatchery staff: incidental or mechanistic. Incidental was defined as fish that died due to random accident or human error and were thus not included in analyses relating mortality to traits or age. In contrast, mechanistic mortalities were individuals where the cause of death could not be related to human error. Individuals later identified as hybrids were removed from analysis.

Sampling Inventory Procedures

Throughout this study, there were multiple sampling regimes performed at various time points: a one-time Hatchery Intake Inventory, 14 Standard Inventories, four Experimental Inventories, and one Reproductive Inventory (Figure 2.1). The procedures for Standard Inventories, Experimental Inventories, and the Hatchery Intake Inventory are explained in detail in Chapter 1, but the Reproductive Inventory procedure is novel to Chapter 2.

Briefly, 14 Standard Inventories followed hatchery standard operating procedures to measure fish weight and length to track growth and adjust tank allocations and feed rations. The four Experimental Inventories involved handling above and beyond standard hatchery procedures to track a suite of phenotypic characteristics, including behavior, health, and morphological traits. The Hatchery Intake Inventory (July 2019) combined the methods from the Standard Inventory with an abbreviated Experimental Inventory eliminating behavior measurements to minimize additional stress to fish on top of capture and hatchery transfer. The data collected at each of these three different types of inventories was grouped into three phenotypic categories of interest: health, morphology, and behavior. The data collection methods for these categories are described in Chapter 1.

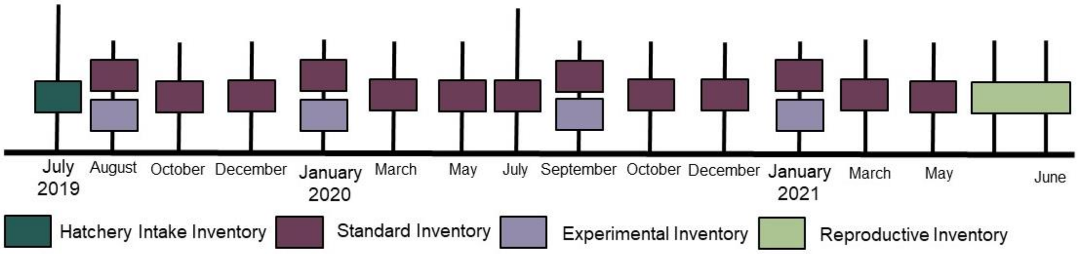


Figure 2.1. Schedule of inventories, color coded for inventory type, throughout the duration of the study. In total, there were 15 Inventories, the modified experimental Hatchery Intake Inventory was performed at one, Standard Inventories were performed at all 14 inventories, Experimental Inventories were performed at four, and the Reproductive Inventory was conducted from May-June 2021. Each type of inventory had different sampling protocols.

Reproductive Performance

The Reproductive Inventory entailed spawning of WCT from May-June 2021 (no individuals from the Emery cohort were successfully spawned in 2020), and subsequent quantification of gamete quality and embryonic survival at several key developmental points. Gamete quality has been linked to ultimate reproductive performance, so several assessments were made to determine if any reproductive failures could relate to the quality of eggs or sperm (Contreras-Sanchez *et al.*, 1998; Nagler *et al.*, 2000; Bobe and Labbe, 2010). Reproductive performance of wild-origin WCT was assessed using the number of total ovulated eggs, fertilization success determined by percent cleavage at 6 hours at 15°C, the median percent eyed embryos, the total hatch embryos, and the median percent hatch embryos (Bardon-Albaret and Saillant, 2017; Bascinar and Okmus, 2004). The hatchery collected data on total ovulated eggs, total eyed embryos, and total hatch embryos for each cross regardless of experimental group in accordance with their standard procedures. I used these data to calculate median percent eyed and hatch embryos for all fish. Thus, the reproductive performance of all crosses was assessed whether both parents were experimental, control, or a mixed group cross, to examine for an effect of experimental or standard handling on reproductive performance. However, gamete quality was only assessed on experimental fish.

In preparation for spawning, all fish were tested for ripeness biweekly beginning in mid-April 2020 and 2021. Ripeness was tested for with a gentle prod to the ventral side of each fish. Fish were deemed spawning ready when eggs or milt flowed freely from the duct with prodding. Non-spawning-ready fish were re-examined biweekly until mid-July, when any unspawned fish were either returned to rearing tanks (2020) or culled without spawning (2021). Immediate spawning of ripe females was a priority as egg viability can deteriorate quickly after a female becomes ripe (Rime *et al.*, 2004). Some males were spawned on multiple days if no unspawned males became ripe on a day when a female became ripe. Spawning was carried out over a 35-day period. Once all WCT brood stock had spawned or were deemed not to successfully spawn, all fish were culled, and otoliths were extracted for age determination.

In order to maximize genetic variation in offspring and replicate for genetic cross effects on embryo performance, I aimed for 2 female x 5 male crosses. However, due to variability in spawning readiness among the individuals, 2 x 5 crosses were not always achieved, and each fish was crossed with as few as one or as many as seven other fish. Prior to collection of eggs or milt, water and slime from the vent and tail area were dried with a towel to ensure that no water would prematurely activate the sperm or induce closure of the egg micropyle. Gametes were collected in a dry steel bowl by applying gentle pressure on the abdomen. Eggs from an individual female were subdivided into separate bowls for each planned cross, and the milt from the male associated with the planned cross was added. Once milt and eggs were mixed in a bowl, activator (Appendix F) was added, and the mixture was gently swirled to mix the milt and eggs. Water from a hatchery tank was then added to activate the sperm and eggs, and each bowl was gently swirled continuously for several minutes to ensure even distribution of the sperm and to prevent the eggs from settling. Additional water was added through the water hardening process and after several minutes, the fertilized embryos were transferred to cross-specific labelled cups in a Heath tray incubator. Each Heath tray had a capacity of nine cups, and each incubator held approximately 10 trays. All of the cups from a single day of spawning fit in one or two trays. Each Heath tray incubator was provided with a constant flow of spring water from the hatchery system (15.0-16.5°C; DO >98%). Throughout the incubation period, embryos were checked at regular intervals and dead embryos, characterized by a white and cloudy appearance, were discarded. The number of embryos discarded at each check was recorded by hatchery staff for later quantifications of reproductive performance for each individual by cross.

Samples for quantification of gamete quality were collected immediately after egg or milt expression. Egg quality was quantified as the diameter and weight of a group of ten unfertilized eggs from each female (Bromage *et al.*, 1990; Burrows, 1951). Diameter was assessed by placing all ten eggs in a Von Bayer trough and recording the total cumulative diameter of the eggs. These eggs were then placed onto a piece of pre-weighed wax paper and the weight (± 0.01 g) was recorded. Ovulatory success was quantified as the total number of successfully ovulated eggs produced by each separate experimental female, which was marked as zero if a female did not produce any ovulated eggs (Springate *et al.*, 1984). Sperm quality was quantified as triplicate spermatocrit measurements per experimental male immediately after expression (Ciereszko and Dabrowski, 1993). Triplicate micro hematocrit tubes (75 mm length, 1.1-1.2 internal diameter) were filled with milt from one male and centrifuged at 5000 RPM for 5 minutes in a microcentrifuge (Amuza, Inc, San Diego, CA, USA). The packed sperm volume as a percent of total fluid volume was immediately measured from the micro hematocrit tubes and calculated. The median of these three measurements were used in subsequent analyses.

To quantify reproductive performance, several metrics were assessed for both males and females and one assessment was quantified for only females. Total ovulated eggs, or the total number of eggs produced by each female, was assessed for only females. For both males and females, I assessed the percent fertilized embryos (fertilization success) in a subset of embryos from each experimental only cross, the total number of eyed embryos, the median percent eyed embryos, the total number of hatch embryos, and the median percent hatch embryos. The percent eyed and hatch embryos were assessed as the proportion of embryos to successfully reach the eyed stage or successfully hatch relative to the initial of embryos for each respective cross. For individuals involved in multiple crosses, I found the median for each of these metrics for use in subsequent analyses.

Fertilization success was quantified by randomly selecting ten embryos from each cross immediately following fertilization and placing them in a labelled cup. The total number of successfully fertilized embryos within the subsample was determined approximately 6 hours after fertilization (15°C; Danner, 2008) by counting the number of embryos that had successfully undergone cleavage and was ultimately expressed as a percent (fertilization success). Successful fertilization, e.g., cleavage, is the only stage where male gamete quality is thought to determine success more than egg quality (Granath *et al.*, 2004; Houde *et al.*, 2011). Every development stage following fertilization, while generally a combination of male and female gamete quality, can largely be equated with female gamete quality due to the overwhelming importance of egg quality and maternal effects upon a developing embryo (Green, 2008; Nagler *et al.*, 2000).

Total ovulated eggs was calculated by adding the number of dead embryos that were removed prior to the eyed stage to the total number of embryos that successfully reached the eyed stage. Total eyed embryos was quantified for each individual (male and female) by counting the number of embryos for each cross that reached the eyed stage, determined when two distinct black dots were apparent on each embryo. This metric has been linked to ultimate reproductive performance and can be useful in determining a more precise measurement of embryonic failure as opposed to just measuring hatch success (Ghaedi *et al.*, 2015). The median percent eyed embryos was calculated for each cross separately by dividing total eyed embryos by the initial number of fertilized embryos for each cross. Total hatch embryos was the total number of embryos from each cross that hatched. The percent hatch embryos was calculated for each cross separately by dividing total hatch embryos by total initial number of fertilized embryos for each cross, and then I calculated the median percent hatch embryos for all individuals. Total hatch embryos was used as an absolute representation of the genetic contribution of each individual to the next generation (Nagler *et al.*, 2000). The median percent hatch embryos was used as the genetic contribution of each individual to the next generation, relative to the number of total initial embryos in each cross. This distinction was important for this study, as I wanted to understand the relationship between the total ovulated eggs produced by each female and the median percent hatch embryos, as it was unknown if females that produced many eggs also had high egg quality, and thus had a higher proportion of median percent hatch embryos.

Approximately 27 days post fertilization (520 degree-days), all embryos had either hatched or died. The alevin were moved from the cups in the Heath tray incubator to a communal rearing tank, making any further cross-specific quantification of offspring performance impossible. Females that lived to spawn but did not produce eggs were included in subsequent comparisons of total ovulated eggs (n=0) and total hatch embryos (n=0), but not in comparisons of egg quality or embryo development. All males successfully spermiated and were thus included in all subsequent comparisons of sperm quality and embryo development.

Age

Otoliths were collected from all experimental fish assessed to determine age at intake to the hatchery via back calculation. Fish that died during the duration of the study were stored at -20°C at Sekokini Springs Hatchery until transport back to MSU for analysis. Hatchery staff recorded the PIT tag, date of death, and probable cause of death on a waterproof zippered bag before placing the carcass in the bag to store in a freezer. Fish that lived to spawn were culled after the cessation of the Reproductive Inventory, and otoliths were collected immediately after the fish died. For all fish, both sagittal otoliths were removed via a lateral cut through the top of the cranium, cleaned of excess viscera, and placed in a manilla envelope labelled with the date of death and PIT tag number. Otolith samples were set in epoxy and carefully ground down with fine grit sandpaper to obtain a cross section containing the nucleus and annuli. Otoliths were viewed under a binocular microscope (10-40x magnification) with transmitted light and aged by counting the number of hyaline rings outward from the otolith nucleus (Zymonas and McMahon, 2009). After age was determined, the date of hatchery intake was subtracted from the date of otolith collection to determine the age at Hatchery Intake Inventory, and this value was used in subsequent analyses.

Data Analysis

Based upon the results from Chapter 1, a limited set of traits with low within-individual variation were used in analysis in Chapter 2, including: K_n, WVHI, VHI, energetic reserves, body shape, latency, and upper jaw, lower jaw, and anal fin residual length. The multivariate body shape was reduced to the first eight principal components from the duplicate dataset Principal Components Analysis from Chapter 1. These eight principal components accounted for 73.4% of the variation in the duplicate body shape dataset. Additionally, I incorporated the residual daily growth rate (for weight and length) and age as potential predictors of performance.

Daily growth rate was calculated for each individual at each of the thirteen latter inventories as the change in weight or length per day between successive inventories. Residual daily growth rates were determined as the residuals around the best fit line determined by regressing all weight or length recorded at the first of a pair of inventories against the daily growth rate calculated between the pair of inventories using the lm () function in R. I used the Chi-squared test to determine if the best fit line was quadratic, linear, or piecewise linear (Table 2.1). Piecewise linear regressions are used when the independent variable, is segmented and the regression analysis is performed separately for these segments. Relationships were visually inspected to determine if any relationships appeared to have segments with significantly different slopes. Once these were identified, I found the values for each regression that indicated the transition point and created the piecewise. I used a trial-and-error approach to find the most precise knot value, and then compared this piecewise regression to a simple linear regression with the Chi-squared test to determine if the piecewise significantly improved the model. When the p-values of the linear or quadratic regressions exceeded 0.05, suggesting the slope between initial weight or length and daily growth rate was not significantly different from zero, the median daily growth rate was used to calculate residuals. The median was used instead of the mean as the median is less vulnerable to outliers skewing the data. As daily growth rates fluctuated with seasonal water temperature fluctuations, regressions for determination of residual daily growth rates were performed separately for each of the 13 pairs of inventories. Residual daily growth rates were used in all subsequent analyses.

Table 2.1. The best fit line equation forms used to determine residual daily growth rates for weight and length at each separate inventory pair. Linear equations were in the form: y~mx+b; Quadratic equations were in the form: $y \sim ax+mx^2+b$, the Linear piecewise equation took the form: y~mx+b from x= a to x=b and y~c for x≥c. When slopes did not significantly differ from zero, equations took the form y~median daily growth rate. Values in brackets after equation types are median daily growth rate value or Linear Piecewise transition value.

Inventory Dates	Time Interval	Weight Equation	Length Equation
July 2019-August 2019	56	Quadratic	Median (0.21)
August 2019- October 2019	47	Median (0.29)	Linear
October 2019 -December 2019	51	Linear	Linear
December 2019-January 2020	42	Linear	Linear
January 2020-March 2020	63	Linear	Median (0.18)
March 202-May 2020	53	Linear	Linear
May 2020- July 2020	63	Linear	Linear
July 2020- September 2020	67	Linear	Median (0.45)
September 2020-October 2020	42	Linear	Median (0.36)
October 2020- December 2020	41	Linear	Median (0.29)
December 2020- January 2021	43	Linear	Linear
January 2021- March 2021	44	Linear Piecewise (2)	Median (0.09)
March 2021- May 2021	61	Linear	Median (0.10)

To determine if age at Hatchery Intake Inventory influenced reproductive performance, I examined the relationships between reproductive performance (total ovulated eggs, fertilization success, median percent eyed embryos, total hatch embryos, and median percent hatch embryos) and age. I visually inspected overlap of the interquartile range and medians of the boxplot for each respective age to look for differences.

Experimental Handling Stress

To test for effects of the extra fish handing required to quantify traits in experimental fish, I compared embryo survival among full experimental, full control, and mixed experimental and control crosses. I categorized every cross into one of three groups: experimental male crossed with an experimental female, control male crossed with control female, or a cross where one parent was control and one was experimental. I then compared median percent hatch embryos and total hatch embryos among these three groups with a one-way type III analysis of variance using the anova (lm, 3) function in the “car” package in R (R Core Team, 2021).

I also investigated traits capable of identifying fish at risk of dying or failing to reproduce despite surviving under hatchery conditions. Fish were categorized into two groups to test for traits predictive of mortalities: mechanistic mortalities or survived to spawning. Separately, all female fish were categorized into two groups to test for traits predictive of failure to ovulate: failed to ovulate despite survival to spawn and survived to spawn and successfully ovulated. As all males that survived to spawn successfully produced milt, I was unable to test for traits predictive of failure to spawn for male WCT. I created a series of boxplots comparing the traits of mechanistic mortalities and survived spawning individuals and compared the medians and overlap of interquartile ranges to determine if there were any similarities between groups. I repeated this procedure to compare the females that failed to ovulate despite survival to spawn and the females that survived to spawn and successfully ovulated groups.

I investigated potential relationships between traits and reproductive performance through a series of correlation matrices. I created a series of correlation matrices, including regression plots, using the chart. Correlation () function from the “PerformanceAnalytics” package (Peterson *et al.*, 2014), and created matrices of R-values and P-values using the function cor () of the “stats” package within base-R (R Core Team, 2021) in R, relating every replicate quantification of each trait to reproductive performance for males and females separately. I examined the correlation matrices regression plots for patterns suggestive of relationships between traits and reproductive performance while also considering P-values to identify relationships to further investigate. For all pairs of traits and reproductive performance metrics identified for further investigation, I performed linear regression using the lm () function in R and plotted the data points around the best fit line. I visually examined each linear regression plot for a threshold point of the trait on the x-axis defining a transition between low and high values for the reproductive trait on the y-axis and used a trial-and-error process to identify the datapoint where the difference between the fish above and below that point was maximized. When a threshold point for the trait was apparent, I created box plots to compare the values of the reproductive trait for the two fish groups defined by the threshold values for the phenotypic trait. Data are presented as medians ± 1 standard deviation and the value for that standard deviation.

Results

General

Of the 67 experimental fish assessed here, 43 were males and 24 were females. In total, there were 2 incidental mortalities and 9 mechanistic mortalities. The female experimental fish ranged from two to four years old, and males ranged from 1-3 years old, with the majority of fish at age 2 at the Hatchery Intake Inventory, (Figure 2.1). At Hatchery Intake Inventory, females had a median weight of 29.0 g (SD=11.5) and a median length of 146.2 mm (SD= 18.7), whereas males had a median weight of 28.1 g (SD= 10.5) and a median length of 146.0mm (SD=17.1). Females alive at the final Standard Inventory in May 2021, had a median weight of 398.6 g (SD= 172.3) and a median length of 325.6 mm (SD= 46.4), whereas males during the May 2021 inventory had a median weight of 391.8 g (SD= 234.90 and a median length of 319 mm (SD= 60.0). Of the 56 fish that survived to spawning, there were 26 females and 30 males. All males that lived to spawn successfully spermiated whereas 5 females that lived to the Reproductive Inventory did not successfully ovulate.

Of the 73 control fish assessed here, there were 2 mortalities (categories of mortality were not assessed on control fish). At Hatchery Intake Inventory, the median weight for all fish was 23.0 g (SD=9.73) and median length was 136 mm (SD= 16.8) (sex data not available for controls at the first inventory. Females alive at the final Standard Inventory in May 2021, had a median weight of 495 g (SD= 214) and a median length of 365 mm (SD= 49.2), whereas males during the May 2021 inventory had a median weight of 276 g (SD=244) and a median length of 304 mm (SD= 52.7). Of the 71 fish that survived to spawning, there were 33 females, 39 males, and one fish of unknown sex.

The age distribution of this population differed for males and females. At hatchery intake, females ranged from 2-4 years old whereas males ranged from 1-3 years old. Of the 24 females that lived to reproduce, 16 were age-2, six were age-3, and two were age-4. The age distribution of the four females that failed to ovulate was two age-2, one age-3, and one age-4. Of the 43 males that lived to reproduce, seven were age-1, 32 were age-2, three were age-3, and one male did not have age data for unknown reasons. The 11 total mortalities were of an unknown sex, but 1 was age-1, 8 were age-2, 2 were age-3, and 0 were age-4. Age was not assessed on the control fish.

There were several assessments that had low representation of some groups that made statistical comparisons difficult. The small number of age-3 females, age-4 females, age-1 males, and age-3 males made comparisons of reproductive performance among these groups difficult to statistically test. Similarly, the low number of experimental mortalities made comparisons between fish that lived and fish that died difficult to statistically test. Additionally, the low number of females that failed to ovulate made comparisons between these fish and females that ovulated difficult. For all of these comparisons, only descriptive statistics and visual representations were used.

Of the 20 females that successfully ovulated eggs, the median egg diameter was 4.13 mm with a range of 3.4 - 4.6 mm, and the median egg weight was 0.56 g with a range of 0.44 - 0.83 g. The weight of females in May 2021 related significantly to the total ovulated eggs, total hatch embryos, and median percent hatch embryos (Figure 2.2, SLR; $R^2 = 0.70$, p-value = 1.40×10^{-4}, $R^2 = 0.78$, p-value = 6.80×10^{-6}, $R^2 = 0.49$, p-value = 0.03). As described above, there were a low number of females that failed to ovulate, but each of these females had a weight in May 2021 less than the median. Egg diameter was not significantly related to female weight at spawn, total ovulated eggs, total hatch embryos, or median percent hatch embryos (SLR; $R^2 = 0.18$, p-value = 0.45, $R^2 = -0.07$, p-value = 0.78, $R^2 = -0.22$, p-value = 0.36, $R^2 = 0.31$, p-value = 0.20). Egg weight was also not significantly related to female weight at spawn, total ovulated eggs, total hatch embryos, or median percent hatch embryos (SLR; $R^2 = 0.21$, p-value = 0.38, $R^2 = 0.21$, p-value = 0.39, $R^2 = 0.27$, p-value = 0.27, $R^2 = 0.1$, p-value = 0.68). Correlation matrices relating all gamete quality assessments for males and females respectively are described in Appendix H.

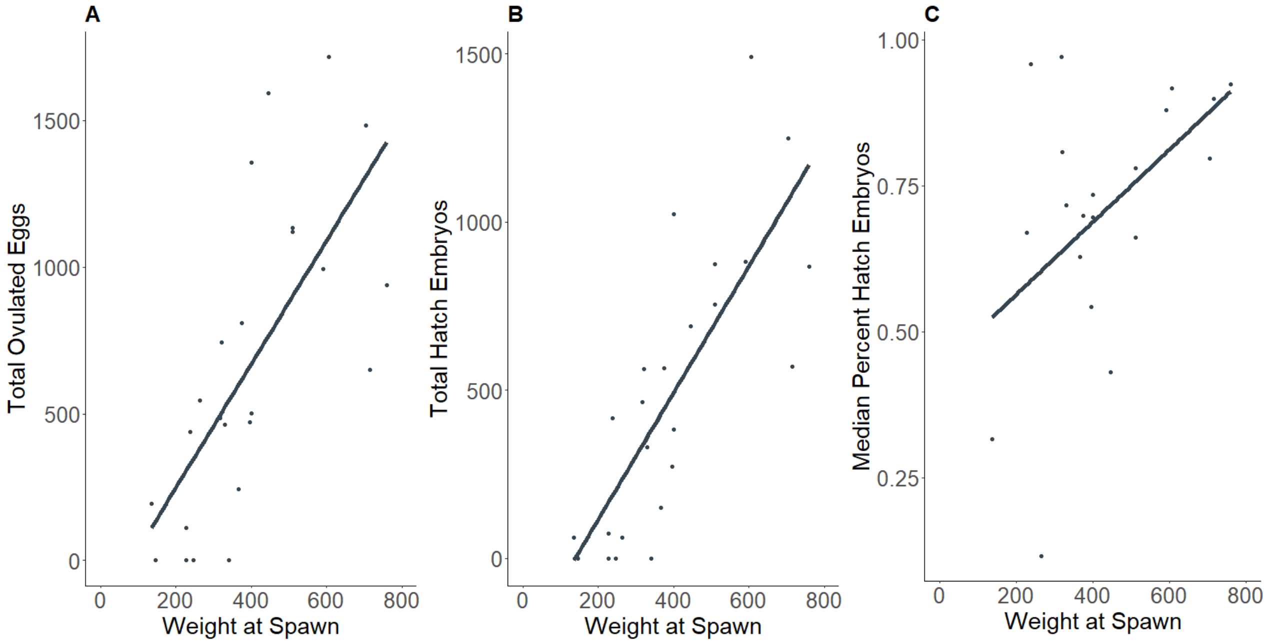


Figure 2.2. Linear regression between the weight at spawning and total ovulated eggs (A, SLR $R^2 = 0.70$, p-value = 1.40×10^{-4}), total hatch embryos (B, SLR, $R^2 = 0.78$, p-value = 6.80×10^{-6}), and the median percent hatch embryos (C, SLR, $R^2 = 0.49$, p-value = 0.03), of each female. Figure 2.2 A and B include females that failed to ovulate (n=24), but C only includes females that successfully ovulated (n=20).

Of the 43 males that lived to spawn, all of the males spermiated, spermatocrit was collected from 40 males, and 39 males were used in spawning. For the males that had spermatocrit data, the median spermatocrit was 20% and had a range of 10-60 %. The spermatocrit values for three males were not included in analyses due to concerns about urea contamination in the milt. The weight of males in May 2021 did not relate significantly to fertilization success, the total hatch embryos, and median percent hatch embryos (SLR; $R^2 = -0.19$, p-value = 0.31, $R^2 = -0.10$, p-value = 0.54, $R^2 = -0.15$, p-value = 0.42). Spermatocrit was not significantly related to male weight at spawn, total hatch embryos, or median percent eyed embryos (SLR; $R^2 = 0.07$, p-value = 0.68, $R^2 = 0.09$, p-value = 0.62, $R^2 = -0.05$, p-value = 0.81).

Overall, the reproductive performance of this population indicated high fertilization success for both males and females but lower eyed and hatch success, respectively (Table 2.2). These results are slightly complicated by the pseudo-replication within the female dataset, as one female would be crossed with up to 7 males, but males did not have replicate crosses. Thus, the median reproductive performance of each female was calculated from multiple values whereas males only had one singular value.

Table 2.2. Summary of reproductive performance measurements for crosses involving at least one experimental fish. "All Crosses" summarizes measurements by individual crosses. "Male" and "Female" summarize measurements by individual male or female fish, respectively. Measurements are summarized as median with minimum and maximum values within parentheses. The median was used to avoid skewing the data due to outliers. As males or crosses did not ovulate eggs, the total ovulated eggs cell for males and all crosses are denoted with an "NA". Fertilization success was not evaluated for all crosses, so this cell does not have a value and is denoted with "NA".

	Total Ovulated Eggs	Fertilization Success	Median Percent Eyed Embryos	Total Hatch Embryos	Median Percent Hatch Embryos
Males	NA	100% (50.0-100)	79.5% (16.0-95.0)	342 (80.0-1005)	75.9% (16-93.4)
Females	560 (0-1821)	96.7% (50.0-100)	76.0% (17.7-97.8)	565 (0.00 -1615)	71.4% (11.0-95.7)
All Crosses	NA	NA	86% (6.94-100)	142 (0.00 -722)	82% (0.00– 99)

Traits and Reproductive Performance

I found no evidence of traits that correlate with later mechanistic mortality or ovulatory failure of females. Boxplot inter quartile ranges and medians had high levels of overlap in traits between fish that lived to spawn and mechanistic mortalities (Figure 2.3). Additionally, boxplot inter quartile ranges and medians had high levels of overlap in traits between females that lived to spawn and successfully ovulated and females that lived to spawn but failed to ovulate (Figure 2.4).

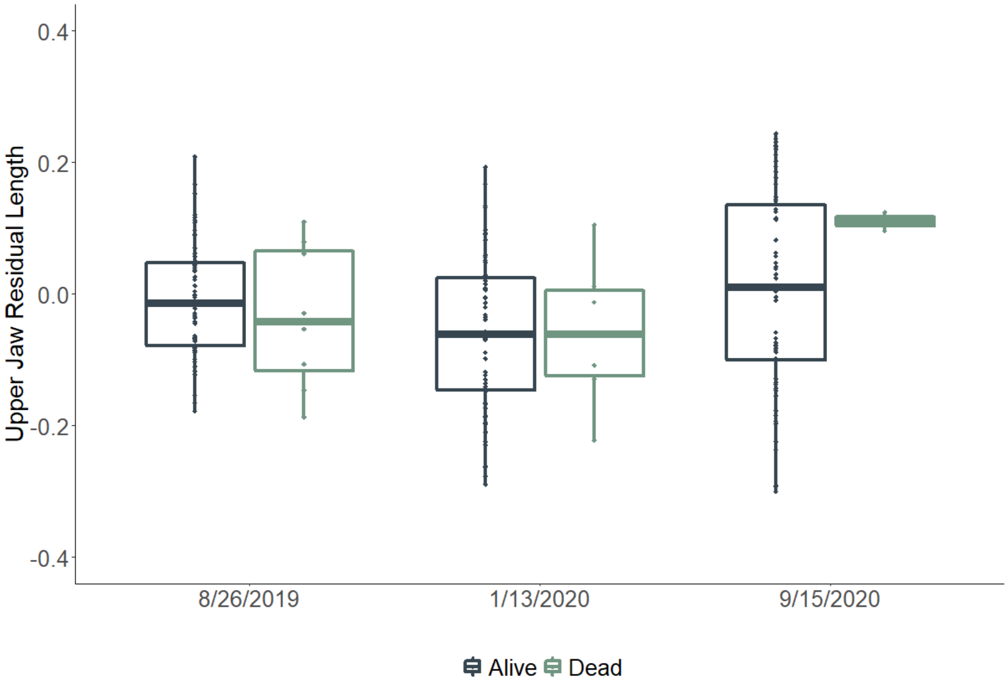


Figure 2.3. Boxplots depicting the upper jaw residual length at three separate Experimental Inventories between individuals that were mechanistic mortalities and fish that lived until the end of the study. A mechanistic mortality was categorized as any fish death that could not be attributed to human error or an accident. Only two mechanistic mortalities had data in 9/15/2020, as all other individuals died before that timepoint. All mechanistic mortalities died before the 1/19/2021 Experimental Inventory, so that date was omitted from this figure. The line in the middle of each box is the median, with the upper and lower margins of the box representing the 25th and 75th percentiles, respectively.

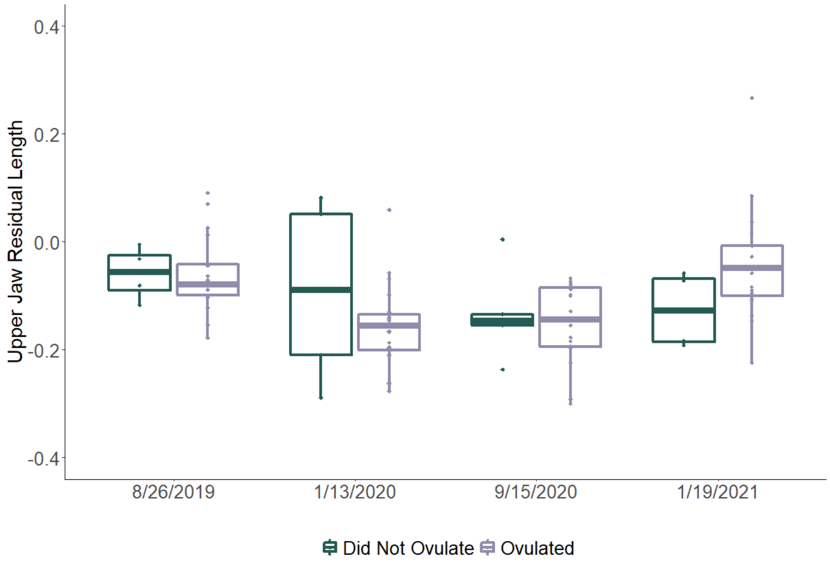


Figure 2.4. Boxplots depicting the upper jaw residual length at three separate Experimental Inventories between females that ovulated successfully (n=20) and females that did not (n=4). The line in the middle of each box is the median, with the upper and lower margins of the box representing the 25th and 75th percentiles, respectively.

Several traits significantly correlated with reproductive performance. These included total length of females at every inventory after October of 2019, with the highest correlation between the length of females in January 2020 and March 2020 and total hatch embryos, anal fin residual length of males in August 2019 and median percent hatch embryos, and residual daily growth in weight for females in October 2019 and total hatch embryos.

Total length of females in January 2020 (Figure 2.5.A, SLR, $R^2 = 0.65$, p-value = 2×10^{-3}) and March 2020 (Figure 2.5.B, SLR, $R^2 = 0.69$, p-value = 7×10^{-4}) correlated positively with the total number of hatched embryos. The trends in total hatch embryos versus total length for January 2020 and March 2020, suggested a breakpoint at 220 mm and 230 mm total length, respectively, where a threshold value of performance was apparent. Females at total length less than 220 mm in January 2020 produced a median of 152 total hatch embryos relative to a median of 811 hatched embryos produced by females at total greater or equal to than 215 mm in January 2020 (Figure 2.5.C). Females at total length less than 230 mm in March 2020 produced a median of 113 total hatch embryos relative to a median of 875 hatched embryos produced by females at total length greater or equal to than 225 mm in March 2020 (Figure 2.5. D). The relationship between length over time (after October 2019) and total hatch embryos was very consistent, with larger fish producing more total hatch embryos. However, the median percent hatch embryos did not have a significant relationship with length.

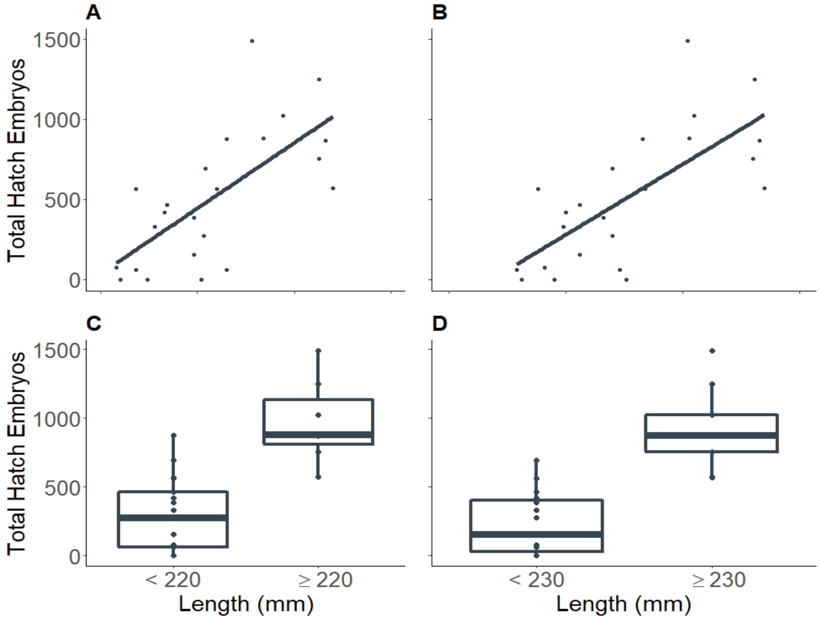


Figure 2.5. Linear regression between the total length of females in January 2020 vs. the total quantity of hatched embryos (A) and March 2020 vs. the total quantity of hatched embryos (B) produced by each respective female (January 2020, SLR, $R^2 = 0.65$, p-value = 2×10^{-3}: March 2020, SLR, $R^2 = 0.69$, p-value = 7×10^{-4}). Boxplots depicting the difference in total hatch embryos between females with a total length less than 215 mm and those with a total length greater than or equal to 215 mm in January 2020 (C). Boxplots depicting the difference in total hatch embryos between females with a total length less than 225 mm and those with a total length greater than or equal to 225 mm in March 2020 (D). The line in the middle of each box is the median, with the upper and lower margins of the box representing the 25th and 75th percentiles, respectively. The whiskers represent outliers outside of the 25th and 75th percentiles.

The anal fin residual length of males in August 2019 was correlated negatively with the median percent hatch embryos (Figure 2.6, SLR, $R^2 = -0.44$, p-value = 0.01), however, anal fin residual length explained less than 50% of the variation in median percent hatch embryos. The negative correlation appears to be driven strongly by a group of seven fish with relatively large residuals for length of the anal fin and ≤0.50 median percent hatch embryos.

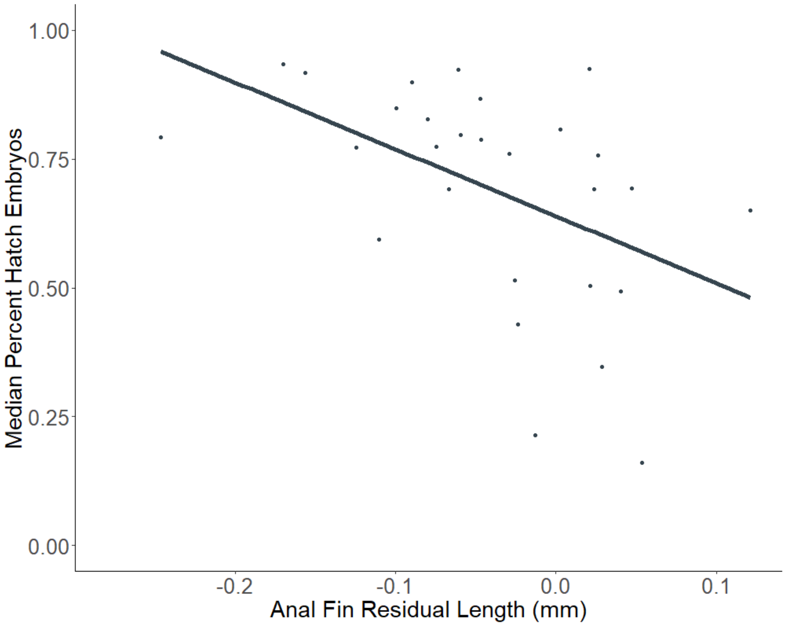


Figure 2.6. Linear regression between the anal fin residual length of males in August 2019 vs. the median percent hatch embryos (SLR, $R^2 = -0.44$, p-value = 0.01).

The residual daily growth in weight of females in October 2019 correlated positively with the total hatch embryos (Figure 2.7.A, SLR, $R^2 = 0.73$, p-value = 6.7×10^{-5}). The trends in total hatch embryos for residual daily growth in weight for October 2019, suggested a breakpoint at 0.125 residual daily growth in weight. Females with residual daily growth in weight less than -0.125 in October 2019 produced a median of 213 total hatch embryos relative to a median of 882 total hatch embryos produced by females with residual daily growth in weight greater or equal to 0.125 in October 2019 (Figure 2.7.B).

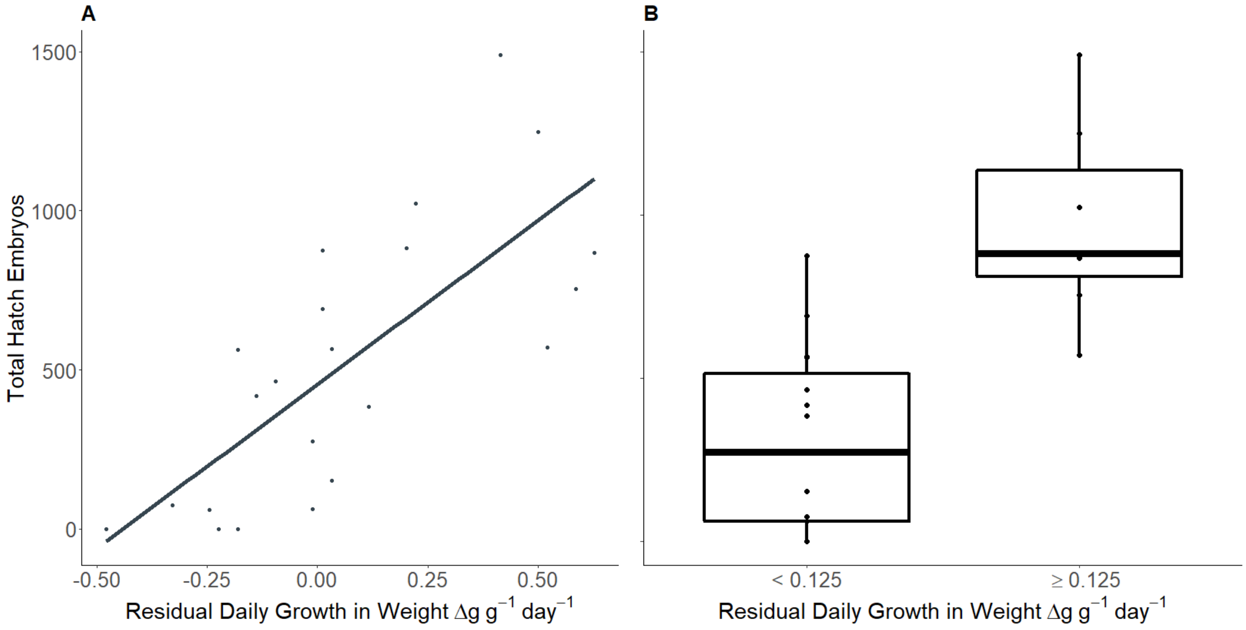


Figure 2.7. Linear regression between the residual daily growth in weight in October 2019 of females and the total hatch embryos produced by each respective female (A, SLR, $R^2 = 0.73$, p-value = 6.7×10^{-5}). Boxplots depicting the difference in total hatch embryos between females with an October 2019 residual daily growth in weight less than 0.125 and those with a residual daily growth in weight greater than or equal to 0.125 in October 2019 (B). The line in the middle of each box is the median, with the upper and lower margins of the box representing the 25th and 75th percentiles, respectively. The whiskers represent outliers outside of the 25th and 75th percentiles.

Health Status and Reproductive performance

I found no evidence of correlation of health status with later mechanistic mortality or ovulatory failure for females at any timepoint. Boxplot inter quartile ranges for health status of fish that lived to spawn and mechanistic mortalities overlapped (Figure 2.8). Additionally, boxplot inter quartile ranges and medians had high levels of overlap for health status between females that lived to spawn and successfully ovulated and females that lived to spawn but failed to ovulate (Figure 2.9).

There were no health traits that significantly predicted the reproductive performance of males or females (Appendix G).

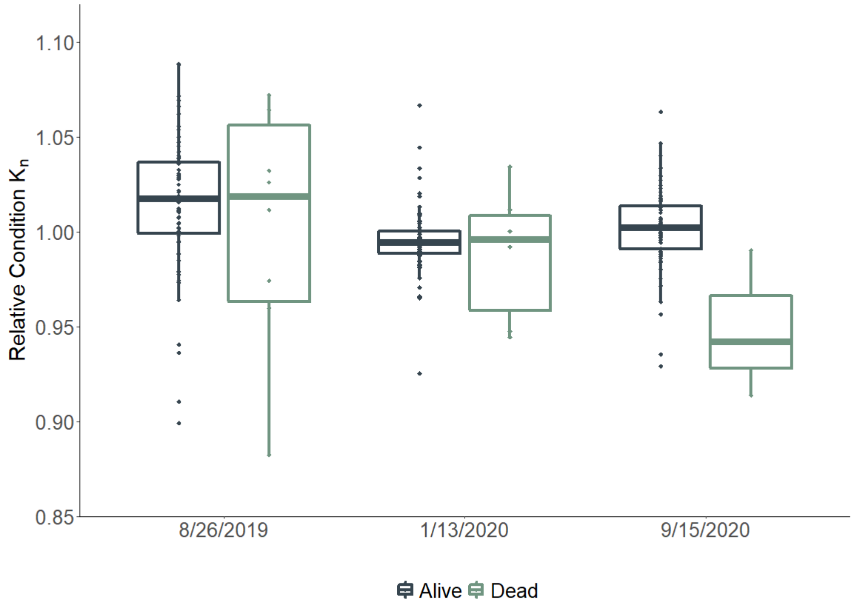


Figure 2.8. Boxplots depicting relative condition at three separate Experimental Inventories between individuals that were mechanistic mortalities and fish that lived until the end of the study. A mechanistic mortality was categorized as any fish death that could not be attributed to human error or an accident. Only two mechanistic mortalities had data in 9/15/2020, as all other individuals died before that timepoint. All mechanistic mortalities died before the 1/19/2021 Experimental Inventory, so that date was omitted from this figure. The line in the middle of each box is the median, with the upper and lower margins of the box representing the 25th and 75th percentiles, respectively. The whiskers represent outliers outside of the 25th and 75th percentiles.

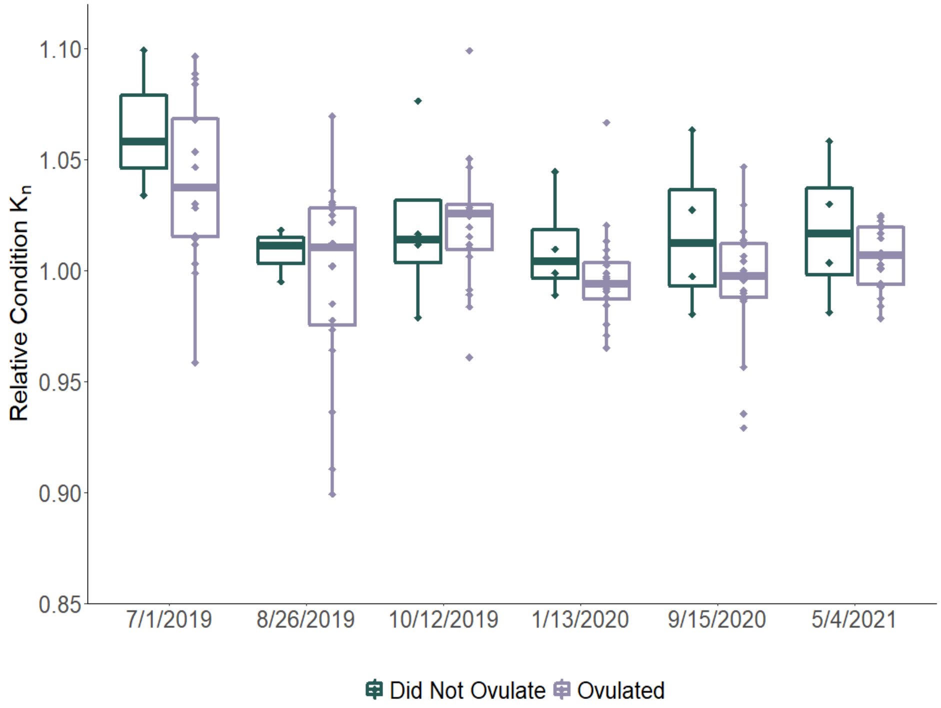


Figure 2.9. Boxplots depicting relative condition at three separate Experimental Inventories between females that ovulated successfully (n=20) and females that did not (n=4). The line in the middle of each box is the median, with the upper and lower margins of the box representing the 25th and 75th percentiles, respectively. The whiskers represent outliers outside of the 25th and 75th percentiles.

Age

I found no evidence of age at Hatchery Intake Inventory effects on survival or reproduction (Figure 2.10, 2.11, 2.12). Interquartile ranges of reproductive performance measurements split by males and females overlapped for most ages at the Hatchery Intake Inventory. One exception included low age-4 female median percent hatch embryos, but as the sample size was small, I was unable to compare interquartile ranges of this age class with other age classes (Figure 2.13).

Figure 2.10. The frequency of ages at the Hatchery Intake Inventory for females (A), males (B) and unknown (C) split by age at the Hatchery Intake Inventory. Females ranged from 2-4 years old, males ranged from 1-3 years old, and the unknown sex mortalities ranged from 1-3 years old.

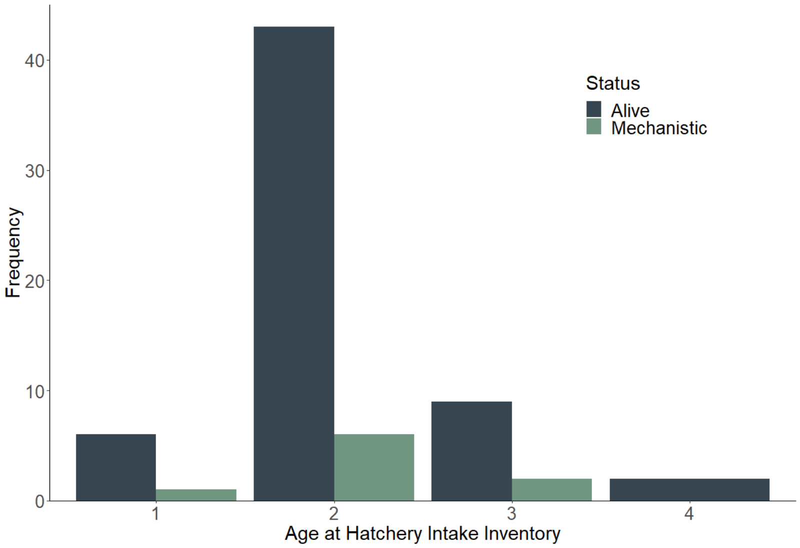


Figure 2.11. Bar plot comparing the age of all fish (range 1- 4 years old) within the population at Hatchery Intake Inventory and ultimate mortality status before the 2021 Reproduction Inventory. A mechanistic mortality was categorized as any fish death that could not be attributed to human error or an accident. The ages of incidental, or accidental, mortalities were not considered here.

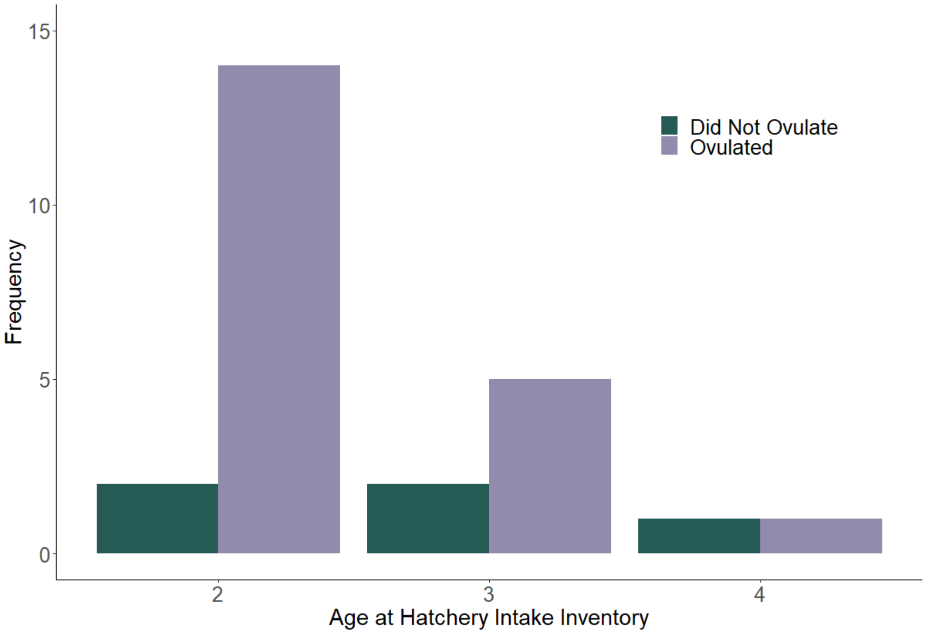


Figure 2.12. Bar plot comparing the age of females (range 1 - 4 years old) within the population at Hatchery Intake Inventory and ovulation status at the 2021 Reproduction Inventory.

Figure 2.13. Boxplots depicting the median percent hatch embryos for the various ages present for males and females, respectively. The line in the middle of each box is the median, with the upper and lower margins of the box representing the 25th and 75th percentiles, respectively. The whiskers represent outliers outside of the 25th and 75th percentiles.

Control vs Experimental Fish

Reproductive performance was similar for males and females involved in crosses including control or experimental fish, suggesting the extra handling required for Experimental Inventories did not affect WCT hatchery performance. The ANOVA comparing total hatch embryos and median percent hatch embryos among crosses of control males with control females, control males with experimental females, and mixed parent crosses did not distinguish any differences between these groups (Figure 2.14, one-way type III ANOVA, df=3, F= 0.87, p-value= 0.46).

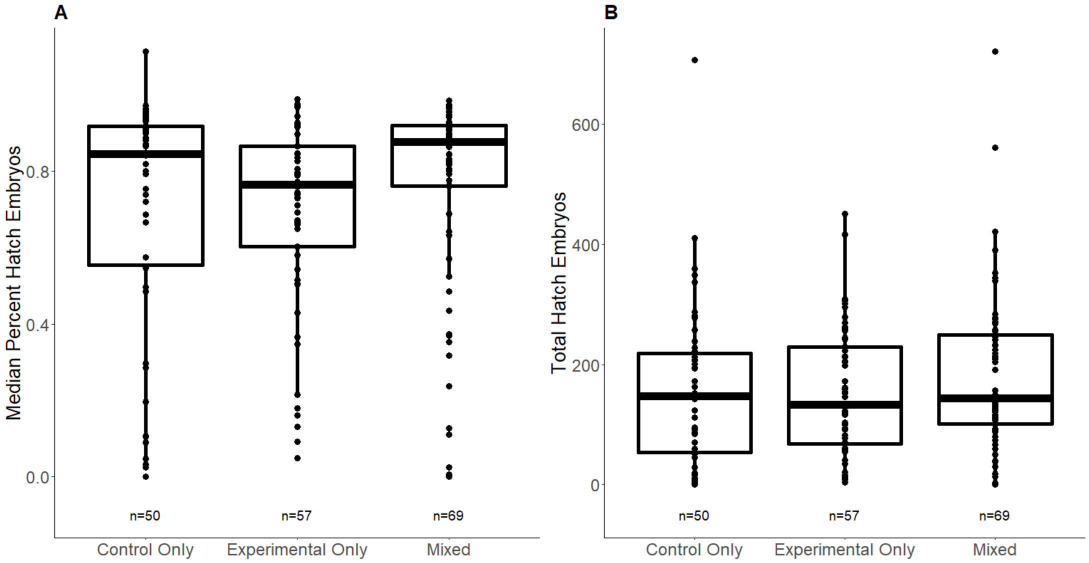


Figure 2.14. The median percent hatch embryos (A) and total hatch embryos (B) of every cross separated into groups by parental cross: control male vs. control female, control male vs. experimental female, mixed parent cross. There were no significant differences in relative median hatched embryo among these groups.

Discussion

The goal of this research was to determine if any traits of individual fish could be related to reproductive performance or poor survival. Traits predictive of later reproductive performance or survival could be applied to screen for individual fish for alternative rearing strategies to reduce artificial selection and better serve the hatcheries' goal of preserving the diversity of stocked populations. I identified traits at specific time points that predict reproductive performance in a working conservation fish hatchery. Overall, I did not find support for three of my hypotheses: age at Hatchery Intake Inventory influences the interactions between traits and reproductive performance, health of fish at hatchery intake strongly influences reproductive performance, and experimental handling influences reproductive performance. However, I found some support for my hypothesis that the traits of fish at certain timepoints will be related to survival and reproductive performance.

The age of fish can be strongly related to various traits depending on the life history strategy of the individual. However, I found no such correlations among age and reproductive performance in this study. Interestingly, females and males entered the hatchery at the same median size, but males had an age range of 1-3 years old, and females had an age range of 2-4 years old. It does not appear that the different ages for each respective sex had significant differences on reproductive performance, but the low representation of 2- and 4-year-old females and 1- and 3-year-old males made statistical comparisons impossible. While I am unsure of the sex and age structure of the larger population of Emery Creek, it seems that based on current size requirements for source stock capture, the hatchery could expect several different ages for both males and females. As I was unable to test for age at hatchery entry effects on in hatchery performance, this could be a direction for future research.

Health status of individuals over time did not appear to be related to reproductive performance. The results of Chapter 1 indicated that the health status assessments were the most specialized of all of the traits measured within this study, and yet there were no strong relationships among any of the health status measurements conducted here and reproductive performance. Additionally, health status was not different between mechanistic mortalities and fish that lived to spawn, or between females that failed to ovulate and those that successfully ovulated. This indicates that even if a fish enters the hatchery in low or high condition, this is not predictive of mortality or reproductive performance. The hatchery rearing conditions could be ameliorating any deleterious effects of poor health, and this warrants further investigation not included here.

A few of the traits significantly correlated with reproductive performance, namely, the total length of females after October 2019, with the strongest relationships in January 2020 and March 2020, and residual daily growth in weight for females in October 2019. The residual daily growth in weight in October 2019, and the length of females after October 2019 were strongly and significantly correlated with the total hatch embryos for females, including those that did not successfully ovulate. The relationship was strongest for length of females in January and March 2020. As the residual daily growth in weight increased, so did the total number of hatched embryos produce by that female. Interestingly, the breakpoint I identified was above 0, indicating that even females growing at the population average rate produce relatively low numbers of hatched embryos. Additionally, females with a higher than average length at inventories after October 2019 ultimately produce more successfully fertilized eggs and hatched embryos than females with a lower than average length at the same inventories. This suggests that crosses including a female able to maintain a higher than average total length after the first winter in the hatchery are likely to produce large numbers of total hatch embryos. This is not an unusual finding, as there is a well-established relationship between female size at spawn and the number of hatch embryos (Jonsson and Jonsson, 1999). Based on these results, hatchery managers could focus on females smaller than 215 mm in size after the first winter in the hatchery to identify fish that eventually produce fewer or no hatched embryos. Ultrasonography can identify maturing females (Carim *et al.*, 2021) with high accuracy, which could help managers identify these individuals more precisely, as there were no external measurements such as weight or length that differed significantly for males and females. Additionally, they could track growth closely in the first fall after hatchery intake and focus on individuals that are not growing faster than the average growth rate

Overall, no gamete quality characteristics of either males or females were related to their embryonic development. However, there was very low variation in egg diameter, egg weight, and spermatocrit that made analyses difficult. The number of median percent eyed embryos was moderately related to relative hatched embryos, but these did not correlate with fertilization success. Fertilization success was generally high for this population; the lowest values (50% and 70%) came from the same female with two different males. However, that female was involved in three other crosses with 95%-100% fertilization, indicating that the issue with fertilization was likely due to the male parent or post fertilization embryo handling. Every other cross had 90-100% fertilization success. This indicates that while spermatocrit was not linked to fertilization success, the fertilization rate of males in this study was very high, suggesting that any poor performance of a given cross is largely due to the female parent. Unfortunately, the egg quality metrics assessed here could not be linked to reproductive performance, indicating that there is some maternal effect that is not being accounted for. Many of the relationships between female traits and reproductive indices had seemingly moderate relationships, but with several individuals that had much lower reproductive performance than expected. For example, several females with a total length between the 25th and 50th percentile in January and March 2020 produced many fewer total hatch embryos than expected based on the predicted size vs. total hatch embryo relationship in Figure 2.5. A potential aspect of female reproduction that could have influenced these results and caused a female with high reproductive potential to have poor reproductive performance is female ovulation timing relative to when the female was actually spawned. Post-ovulatory aging of oocytes within the body cavity has been found to have adverse effects on ultimate egg quality and show a progressive reduction in embryonic viability as aging increases (Azuma *et al.*, 2003, Bromage *et al.*, 1992; Komrakova and Holtz, 2009; Sakai *et al.*, 1975). Depending on the individual characteristics of a female WCT, there may be decreases in performance of embryos if oocytes remain within the female after the moment of ovulation. The biweekly checks for ovulation conducted at the hatchery may be sufficiently capturing oocytes within a sufficient interval of post-ovulatory aging, but this could be a potential area of concern. Managers could investigate the use of sex-steroid sampling to assess ovulation timing more precisely, as these have been used with success in other salmonids (Scott and Baynes, 1982), but this would also need to be investigated further. Another factor that could have caused late-stage reproductive failure in the females is chronic stress.

Not only are the fish at Sekokini Springs in an entirely new environment with constant unfamiliar stimuli, but they are also being handled at regular intervals to monitor performance. Chronic stress has well documented deleterious effects on the immune, growth, and reproduction of salmonids, so this is a likely candidate to investigate to resolve the low reproductive performance of some females. Another piece of evidence that suggests chronic stress may be present in the population is the lack of significant differences among the reproductive performance of experimental only, control only, and mixed parent crosses. I had anticipated that the high intensity sampling I employed for my experimental methods would have some effect on the experimental fish, but this was not the case. This suggests that all fish in this population are already operating under a certain level of chronic stress, thus, the allostatic load of all individuals in the population is quite high, and reproductive performance may be determined by the ability of an individual to plastically adjust to these stressors via hormesis (Contreras-Sanchez *et al.*, 1998; Schreck, 2010). While I was unable to identify a specific trait other than female size that is related to reproductive performance, it seems likely that susceptibility to stress is a driver of poor performance. If there is not an external trait that can predict poor performance, the hatchery may need to consider adjusting the handling regularity or rearing stress on all fish collectively.

Conservation hatcheries are an essential tool for replacing lost populations of native WCT while also promoting a historic portfolio of phenotypic and genotypic diversity (Grisaak and Marotz, 2003. While these facilities have made great strides in reducing the artificial selection present throughout the rearing process, hatchery managers want to ensure that they are maximizing the potential output of each facility, especially since the brood used is wild origin. The study of the relationships between phenotypic traits, age, survival, and reproductive performance of wild-origin WCT in a conservation hatchery allows managers to better understand which traits may make fish more vulnerable to selection pressures and key timepoints throughout the rearing process where it appears these selection pressures are most extreme. Based on my results, it appears that selection pressures are consistent in the hatchery, as it appears that there is no strong effect of time on the relationship between traits and reproductive performance. While the majority of traits assessed during the study did not have strong and significant relationships with reproductive performance, the total length of females after October 2019, but most strongly in January and March 2020, and the residual daily growth in weight of females in October 2019 were significantly related to reproductive performance. Additionally, the significant relationship I found between anal fin residual length of males in August 2019 does not seem to be biologically significant and was not consistent over time. This fact, as well as the fact that all males successfully spermiated indicates that managers should not focus on male performance within this context. These traits at these times could be used as general guides for hatchery managers to have a better idea of which fish are most vulnerable. However, it is important to note that the scope of inference for this study is quite limited, having only used one cohort from one stream. For these results to be applied more generally, follow up studies would need to be conducted involving other source streams.

Overall, this study was able to determine that there were a few key traits that could be used to predict performance, but that it is difficult to predict performance with high certainty within the hatchery context. It seems that current hatchery practices are sufficient to maximize the potential reproductive output, but some areas could be investigated further. While larger females tended to produce more total ovulated eggs and total hatch embryos, the median percent hatch embryos was more variable, indicating a potential issue with egg quality for females. Hatchery managers could shift sampling effort to focus on the key timepoints identified here (early first spring after hatchery entry and first fall after hatchery entry), as these seem to be times when selection pressures slightly differ for females. However, as I was unable to find a trait that predicted reproductive performance, managers should focus on the late-stage reproductive failure of females as the area of action to best improve rearing efficiency and minimize reduction of phenotypic and genotypic diversity. Managers could focus on more efficiently monitoring for ovulation timing to reduce any post-ovulatory aging in females that could reduce egg quality or embryonic success via sex steroid sampling or more frequent monitoring during the Reproductive Inventory. Additionally, managers could consider reducing the sampling frequency of the monitoring program to reduce the effects of chronic stress on the female reproductive performance. By ameliorating these issues, the hatchery could potentially increase the numbers of females that are able to spawn successfully which could reduce artificial selection and ultimately increase the number of WCT that are able to be stocked each year.

APPENDICES

APPENDIX A

SCHEMATICS OF EXPERIMENTAL ARENA USED TO QUANTIFY BEHAVIOR OF WESTSLOPE CUTTHROAT TROUT

The behavior arenas measured 1.00 x 0.60 x 0.50 m and were constructed from white fiberglass reinforced plastic (FRP) and divided in half with a wall of FRP (0.60 x 0.50 m) (Figure A.1). Each arena was composed of two identical halves: a home side, which fish were initially introduced into and habituated to for 30 minutes; and a novel side, which fish were provided access to after habituation to the home side. The bottom of the tank was made from perforated aluminum sheeting (0.36 x 1.76m; hole size 50.0 mm) to allow for constant water flow and to maintain similar oxygen and temperature conditions as in the rearing tanks at each given sampling date (temperature varied, but oxygen consistent >97% DO). A 125 mm hole was cut into the center of the wall and fitted with an FRP gate that could be opened with a pulley system. The hole size was determined based on the ratio of the average fish length so that the hole size could remain consistent over time. A QSee night-vision security camera was centered above the gate of each behavior arena and hung 1.50 m above the surface of the water on a wooden scaffolding. This height provided a field of view that captured the entire behavior arena as well as the sides of the tank. Black sheeting was securely fastened over the entire tank to shield the fish from external stimuli that may have influenced behavior. The cameras were connected to live feed computer monitors so that scientists could remotely monitor the fish for distress, and videos were recorded to an external hard drive for later quantification of behavior.

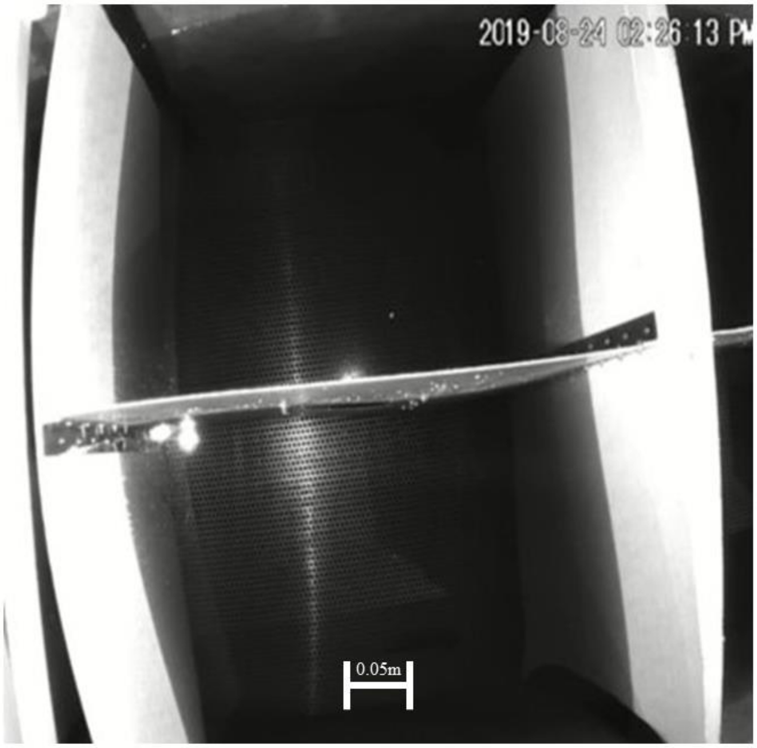


Figure A.1. Top view photograph of a behavior arena composed of two identical halves: a home side, which fish were initially introduced into and habituated to for 30 minutes; and a novel side, which fish were provided access to after habituation to the home side. The wall dividing the halves has a 125 mm hole in the center and was fitted with a fiberglass reinforced plastic gate that could be opened with a pulley system that allowed the fish free access to either side.

APPENDIX B

CATEGORIES AND SCORING METRICS FOR VISUAL HEALTH ASSESSMENT

Although I am unaware of studies testing for a relationship between a visual health assessment and later performance in a hatchery or in nature, studies have linked visual health indicators to performance in a variety of contexts. Intensity of anthropogenic sonic vibrations experienced by chinook salmon has been linked to eye damage, change in color, and wounds, which, depending on severity, were hypothesized to potentially cause reduced survival or performance (Halvorsen *et al.*, 2012). Wounds or damage to the jaw and operculum caused by predation has been weakly linked to reduced survival in chinook salmon and steelhead (Naughton *et al.*, 2011). Damage to fins, spine, and other anatomical wounds caused by fish capture and handling in a seine or fish passage at a dam has been linked to eventual reduced survival and reproduction (Cook *et al.*, 2018; Knott *et al.*, 2019; Helfrich *et al.*, 2011). Repeat swimming ability of individual sockeye salmon has been linked to wounds and injury to gills, operculum, eyes, and fins (Tierney and Farrell, 2004). Based on these previous studies, I included fins, gills, spine, external wounds, jaw, operculum, and eyes in the Visual Health Assessment. Additionally, I included color as an additional external indicator of health as I hypothesized reduced condition in color could have similar implications for performance in survival, growth, or reproduction (Table B.1).

Table B.1. Categories and definitions for scoring of each indicator of the Visual Health Assessment.

Trait	1	2	3	4	5
Jaw	Severe jaw deformation; feeding likely severely impaired	Intermediate jaw deformation; feeding potentially impaired	Normal jaw formation; feeding unlikely impaired		
Eyes	Both eyes missing; fish is fully blind	One eye missing and the other is damaged; vision may be severely affected	Both eyes present and moderately damaged; vision may be moderately affected	Both eyes present; one eye damaged; vision may be affected	No eye damage or apparent vision effect
Operculum	≥50% of both opercula missing; gill arches at least slightly exposed; gill protection may be severely affected	One or both opercula damaged; >50% of the gills are covered; gill may be moderately affected.	Both opercula intact; gills not exposed; gill protection not likely impaired		
Gills	100% of the gill filaments > 50% eroded; filaments are white	100% of gill filaments eroded 50% - 25%; filaments are pale pink	100% of gill filaments are <25% eroded; filaments are pale pink	100% of gill filaments are <25% eroded; filaments are dark red	0% of gill filaments are eroded; filaments are dark red

Table B.1. Continued

Fins	Severe damage on all small fins or severe damage on one big fin; 75% of fin is eroded or greater than 50% tatter depth at low density; swimming performance likely severely affected	Moderate to severe damage on all small fins or one big fin; 50-75% of fin is eroded or 25-50% tatter depths at low density; swimming performance likely moderately - severely affected	Severe fin damage on two small fins or moderate damage to one big fin; 25-50% of fin is eroded, or 25-50% tatter depth at low density or greater than 50% tatter depth at high density; swimming performance likely moderately affected	Any damage on any fin of lesser severity than in categories 1, 2, or 3; swimming performance likely slightly affected	No fin erosion; swimming performance unlikely affected
Wounds	Fish mortally wounded; not expected to survive	Deep lacerations in the tissue of fish; may not survive	Shallow gashes or tissue damage; will likely survive	Slight abrasions on the skin; will likely survive	No wounds present
Spine	The spine is crooked and broken; not expected to survive	Spine is crooked; swimming performance may be reduced	The spine is normal and unaffected		
Color	Lack of depth in color; light-silvery color; no coloration along the lateral line; Stage-specific: poor definition of parr marks and spotting	Little variation in color; light green body; no/little coloration along the lateral line; Parr marks scarcely visible	Drab green color, little coloration along the lateral line; Stage-specific: parr marks and spotting visible but do not stand out	Color is bright green; red hue along the lateral line; Stage-specific: parr marks and spotting evident	Dark green color; red coloration along the lateral line; Stage-specific: clear parr marks

APPENDIX C

SCHEMATICS RELATING FATMETER MEASUREMENTS TO WHOLE-BODY ENERGETIC RESERVES OF WESTSLOPE CUTTHROAT TROUT

To validate Fatmeter measurements to estimate whole-body energetic reserves of juvenile westslope cutthroat trout (*Oncorhynchus clarkii lewisi;* WCT), I took advantage of the well-established relationship between whole-body lipid content and whole-body moisture content (Brandes and Dietrich, 1953; Yeannes and Almandos, 2003; Breck, 2008; Hartman and Margraf, 2008; Stolarski *et al.*, 2014) and tested for a relationship between Fatmeter measurements and fish carcass moisture content. Twenty-five WCT were collected from Washoe Park Hatchery, Montana. The fish were euthanized via 250 mg/L buffered dose of Tricaine methanosulfate (MS-222) (Pentair Aquatic Eco-Systems, Inc., Apopka, FL, USA), total length (±1.00 mm) and weight (± 0.01 g) were recorded, and Fatmeter measurements were taken following the same methods and at the same location as described in the methods of Chapter 1 and Chapter 2. The size of fish samples ranged from 100-500 mm total length, which captured the range of experimental fish used for Fatmeter measurements in the Sekokini Springs study (123 – 444 mm total length). The carcasses were immediately frozen on dry ice and stored long term at -20°C until processing.

Percent moisture of each thawed carcass was determined in triplicate. Carcasses were thawed, cut into small pieces, and homogenized. Fish with a total length of greater than 150 mm were homogenized in an industrial meat grinder (Hobart Corporation, Troy, OH, USA). Fish with a total length of less than 150 mm were homogenized in a 12-cup food processor (Cuisinart, Stamford, CT, USA). The whole-carcass homogenized samples were stored in sealed 15 ml conical tubes in a -20°C freezer until they were subsampled to measure whole-body energy. Dry weight was determined following the desiccation technique described in Johnson *et al.* (2017), using a tabletop muffle furnace (Thermo Fisher Scientific Inc., Waltham, MA, USA). Triplicate samples with a wet weight of 5-7g were desiccated at 60°C for 2-3 days until weight stabilized. The percent moisture of each replicate sample was calculated using the below formula:

$$Moisture\ (\%) = \frac{\text{Wet Weight (g)} - \text{Dry Weight (g)}}{\text{Wet Weight (g)}} \text{ x } 100$$

The coefficient of variation for triplicate percent moisture measurements for each individual fish ranged between 0.20 and 3.35. Mean percent moisture was calculated based on triplicate percent moisture measurements for each fish and related to the Fatmeter scores with linear regression using the lm () function in RStudio (Figure C.1).

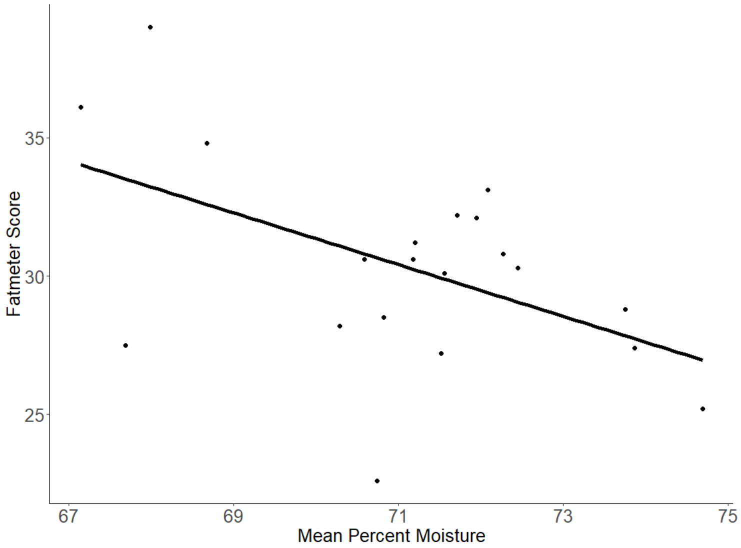


Figure C.1. The relationship between mean percent moisture and Fatmeter measurement for 25 westslope cutthroat trout from Washoe Park Hatchery ($R^2 = 0.25$, p-value = 0.02).

Fatmeter measurements weakly yet significantly related to mean percent moisture ($R^2 = 0.25$, p-value= 0.02). Based on the well-established inverse relationship between body moisture and lipid density in fishes, the significant relationship between Fatmeter measurements and mean percent moisture reported here suggests a significant relationship between Fatmeter measurements and whole-body energetic reserves. These findings are consistent with previous studies on juvenile steelhead (anadromous *O. mykiss*) (Hansen *et al.*, 2012) and juvenile pallid sturgeon (*Scaphirhynchus albus*) (Djokic 2022), which reported significant relationships between Fatmeter measurements and whole-body moisture or energy, but with low R^2. Due to the significant, but weak relationship between Fatmeter measurements and carcass percent moisture, I conclude that uncalibrated Fatmeter measurements can be cautiously applied as a proxy for juvenile WCT whole-body energetic reserves.

APPENDIX D

DERIVATION OF WEIGHT AT LENGTH EQUATION FOR A POPULATION OF WESTSLOPE CUTTHROAT TROUT IN SEKOKINI SPRINGS HATCHERY

The equation used to determine the ratio of predicted weight at length for calculating relative condition (K_n) was derived using a subset of experimental fish, with one observation per fish. This was done to avoid violating the assumptions of linear regression, as it requires independence of data. I randomly selected one of the replicate weight and length measurements for each individual fish to parameterize the length to weight relationship of the experimental hatchery population. I randomly assigned each fish to one of fourteen groups and based on the group the fish was assigned to, I used only that datapoint for later analysis.

With the parametrized dataset, I created a length vs. weight linear regression and derived the following weight-length equation from the relationship within this population.

$$\mathrm{Log}_{10}\,(\text{weight}) = -5.65 + 3.27 \text{ x } \mathrm{Log}_{10}\,(\text{total length})$$

is calculated with $K_n = W/ W_0 * 100$ where W_0 is the predicted length-specific mean weight for the population under study and W is the actual weight. The coefficients of the regression between $\log_{10}$-transformed weight and $\log_{10}$-transformed length were determined using the lm () function in Base R (n=64, SLR, $R^2 = 0.99$, p-value = <0.01), I calculated a predicted weight for each individual fish at each of the 13 Standard Inventories and singular Hatchery Intake Inventory using the regression equation and measured length.

APPENDIX E

GEOMETRIC MORPHOMETRIC METHODS USED TO ASSESS THE BODY SHAPE OF WESTSLOPE CUTTHROAT TROUT

Landmarks are anatomical locations that are recognizable and present on all specimens in the study regardless of unknown qualities such as age or sex, and that are identical among and within populations (Bookstein, 1991; Dryden and Mardia, 1998). Landmarks used in this study were chosen based on prior research on cutthroat trout specifically (Seiler and Keeley, 2009) and salmonids in general (Chavarie *et al.*, 2012) (Figure 1.5). At each Experimental Inventory, digital images of experimental fish were captured under consistent conditions to later identify landmark positions on each image. Photos were taken after each fish was anaesthetized and length, weight, energetic reserves, and visual health assessment measurements were completed. Fish were placed singly in a mesh cradle and 96 dpi images were taken with a Canon SLR camera and 60mm lens with an 18-megapixel sensor (Canon U.S.A. Inc., Huntington, NY, USA) positioned one meter above the approximate centroid of the fish and manually set to a 1/125 sec. exposure and f/5.6 aperture. A ruler, to provide scale, and a card with fish PIT number and date of image capture, were placed in the image frame of view. Overhead lighting in the hatchery provided consistent exposure for photographs.

For each image, TPSDig2 software (Rohlf, 2017) was used to manually record X and Y coordinates of each of the 28 landmarks. The 18 landmarks used for linear measurements were selected based on demonstrated direct and indirect relationships to foraging and swimming (Nakano *et al.*, 2020; Zimmerman *et al.* 2006, 2007, 2009; Chavarie *et al.*, 2013), and sensitivity to selection pressures in fishes (Webb, 1984; Kristjánsson *et al.*, 2002; Kahilainen *et al.*, 2005). The linear measurements were conducted manually in TPSDig2 by measuring the distance between predetermined pairs of the landmarks. Each measurement was scaled using the ruler in the image to ensure standardization of all linear measurements across all images.

Fish body shape was quantified using geometric morphometrics analyses on 21 of the landmarks recorded on images in TPSDig2. A matrix of X and Y coordinates of each landmark within an image was adjusted for scale using the ruler within the image and then imported into R. To generate standardized superimposition data with effects of size, position, and orientation on variation in body shape removed for subsequent analysis, a Generalized Procrustes Analysis (GPA) was conducted using the gpagen () function (Mitteroecker, 2009; Adams and Otárola-Castillo, 2013). This resulted in a set of Procrustes standardized coordinates (hereby "Procrustes coordinates") for each landmark and fish observation formed a multivariate coordinate matrix with three dimensions (p x k x n) where p is the number of landmarks, k is the number of dimensions, and n is the number of specimens. For all subsequent analysis, p remained constant at 21 landmarks, k remained constant at 2 dimensions (x and y), and n had a maximum number of specimens of 111 after photos were removed from the dataset due to poor photo quality (e.g., blurred from movement, fish lateral line not straight, fish mouth open, poor lighting).

APPENDIX F

CHEMICAL COMPOSITION OF SOLUTIONS USED IN FERTILIZATION OF WESTSLOPE CUTTHROAT TROUT AT SEKOKINI SPRINGS HATCHERY

The activating solution used during the fertilization of westslope cutthroat trout embryos (Table F.1). Components were mixed thoroughly with 1 L of hatchery water from a flow-through system (Cloud and Patton, 2008).

Table F.1. The composition of the physiological saline solution used to activate westslope cutthroat trout sperm during fertilization.

Compound	Mg/Liter
Sodium Chloride (NaCl)	8.00
Tris Base	1.20
Glycine	1.50

The extender solution used during the fertilization of westslope cutthroat trout embryos (Table F.2). Mix Part A and B separately thoroughly with 1 L each of hatchery water from a flow-through system. Mix A and B in ratio of 4(A):1(B) (Moore, 1987)

Table F.2. The composition of the physiological saline solution used as an extender of westslope cutthroat trout sperm during fertilization.

PART A	
Compound	Mg
Potassium Chloride	9.00
Sodium Chloride	2.50
Magnesium Sulfate	0.30
Sodium Phosphate	0.60

PART B	
Compound	Mg/Liter
Sodium Bicarbonate	5.00
Dextrose	5.00

APPENDIX G

CORRELATION MATRICES AMONG TRAITS AND REPRODUCTIVE ASSESSMENTS

Table G.1. Correlation coefficients between traits (WVHI: Weighted Health Index, VHI: Health Index; K_n: Relative Condition; PC: Principal Component [1,2,3,4,5,6,7,8]).at each date and the fertilization success, median percent eyed embryos, median percent hatch embryos, egg diameter, and egg weight of female WCT that successfully ovulated eggs and spawned. Relationships with a significance of below the cutoff of 0.05 are denoted with asterisks.

Length

	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
7/1/2019	-0.03	-0.09	-0.07	-0.19	-0.47*
8/26/2019	-0.18	0.04	0.07	0.01	-0.27
10/12/2019	-0.25	0.12	0.13	0.10	-0.15
12/2/2019	-0.31	0.14	0.13	0.10	-0.03
1/13/2020	-0.32	0.17	0.15	0.08	0.03
3/16/2020	-0.32	0.24	0.23	0.06	0.04
5/8/2020	-0.30	0.26	0.24	0.04	0.04
7/10/2020	-0.28	0.27	0.25	0.05	0.13
9/15/2020	-0.19	0.33	0.32	0.09	0.14
10/27/2020	-0.16	0.31	0.31	0.04	0.15
12/7/2020	-0.16	0.30	0.30	0.03	0.14
1/19/2021	-0.14	0.29	0.29	0.02	0.14
3/4/2021	-0.17	0.29	0.28	0.03	0.14
5/4/2021	-0.17	0.30	0.30	0.04	0.13

Weight

	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
7/1/2019	0.00	-0.04	0.01	-0.11	-0.40
8/26/2019	-0.22	0.12	0.17	0.13	-0.31
10/12/2019	-0.25	0.20	0.23	0.14	-0.13
12/2/2019	-0.34	0.17	0.18	0.16	-0.03
1/13/2020	-0.37	0.19	0.20	0.13	0.01
3/16/2020	-0.33	0.28	0.29	0.15	0.05
5/8/2020	-0.34	0.28	0.29	0.14	0.06

Table G.1. Continued

7/10/2020	-0.27	0.34	0.34	0.15	0.15
9/15/2020	-0.17	0.33	0.34	0.13	0.03
10/27/2020	-0.18	0.32	0.35	0.14	0.14
12/7/2020	-0.20	0.31	0.34	0.12	0.17
1/19/2021	-0.16	0.31	0.35	0.15	0.20
3/4/2021	-0.18	0.33	0.36	0.14	0.20
5/4/2021	-0.17	0.36	0.40	0.12	0.19

	Relative Condition				
	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
7/1/2019	0.13	0.13	0.15	0.21	0.49*
8/26/2019	-0.18	0.20	0.27	0.50*	-0.32
10/12/2019	0.01	0.28	0.32	0.19	0.41
12/2/2019	-0.05	0.07	0.12	0.28	0.36
1/13/2020	-0.05	-0.13	-0.01	0.11	0.07
3/16/2020	-0.05	0.02	0.16	0.61*	0.35
5/8/2020	-0.10	-0.07	0.04	0.43	0.20
7/10/2020	0.14	0.35	0.46	0.46	0.24
9/15/2020	0.14	-0.15	-0.05	0.33	-0.44
10/27/2020	0.03	-0.12	0.02	0.44	0.09
12/7/2020	-0.04	-0.19	-0.05	0.32	0.19
1/19/2021	-0.09	-0.02	0.13	0.16	0.08
3/4/2021	0.18	0.03	0.15	0.39	0.28
5/4/2021	0.02	0.30	0.39	-0.02	0.09

	Residual Daily Growth in Length				
	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
8/26/2019	-0.31	0.29	0.32	0.26	0.21
10/12/2019	-0.28	0.30	0.23	0.26	0.25

Table G.1. Continued

12/2/2019	-0.24	0.08	-0.02	0.05	0.43
1/13/2020	-0.11	0.18	0.10	-0.09	0.38
3/16/2020	0.13	0.35	0.39	-0.02	0.11
5/8/2020	0.16	-0.05	-0.06	0.06	-0.03
7/10/2020	0.28	-0.19	-0.20	0.10	0.46
9/15/2020	0.21	0.37	0.43	0.22	0.21
10/27/2020	0.11	-0.06	0.00	-0.29	0.10
12/7/2020	0.09	-0.14	-0.16	-0.10	-0.17
1/19/2021	-0.13	-0.01	0.00	-0.11	-0.30
3/4/2021	-0.40	-0.05	-0.03	-0.08	0.16
5/4/2021	-0.08	0.16	0.26	0.25	-0.05

Residual Daily Growth in Weight

	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
8/26/2019	-0.30	0.26	0.31	0.21	-0.22
10/12/2019	-0.19	0.31	0.29	0.09	0.12
12/2/2019	-0.29	0.02	0.00	0.09	0.22
1/13/2020	-0.23	0.18	0.11	-0.26	0.38
3/16/2020	0.36	0.51	0.55	0.22	0.24
5/8/2020	0.00	-0.22	-0.21	0.02	0.06
7/10/2020	0.47	0.14	0.09	0.11	0.51*
9/15/2020	0.27	0.13	0.17	0.00	-0.34
10/27/2020	-0.04	-0.11	-0.02	0.05	0.60*
12/7/2020	-0.10	-0.24	-0.23	-0.1	0.10
1/19/2021	0.31	-0.06	-0.09	0.14	0.10
3/4/2021	-0.32	0.25	0.26	-0.04	0.08
5/4/2021	-0.01	0.42	0.46	-0.07	0.07

Latency

	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight

Table G.1. Continued

8/26/2019	-0.44	-0.54*	-0.50	0.18	-0.05
1/13/2020	0.18	-0.23	-0.26	-0.75	-0.21
9/15/2020	-0.09	0.23*	0.15	0.12	-0.30
1/19/2021	-0.67	-0.31	-0.37	-0.14	-0.30

Energetic Reserves

	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
7/1/2019	-0.03	0.34	0.40	0.64*	0.21
8/26/2019	0.08	0.13	0.08	0.27	0.07
1/13/2020	0.08	0.04	0.11	0.08	-0.03
9/15/2020	0.24	0.21	0.10	-0.35	0.09
1/19/2021	0.11	-0.22	-0.19	-0.16	-0.21

Linear Measurements

	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
Upper Jaw Residual Length 8/26/2019	-0.07	0.19	0.25	0.10	0.41
Upper Jaw Residual Length 1/13/2020	-0.13	0.08	0.17	-0.02	0.29
Upper Jaw Residual Length 9/15/2020	0.27	-0.25	-0.17	0.07	-0.03
Upper Jaw Residual Length 1/19/2021	0.21	0.21	0.26	-0.13	0.05
Lower Jaw Residual Length 8/26/2019	-0.28	0.43	0.51	0.28	0.63
Lower Jaw Residual Length 1/13/2020	0.10	0.16	0.22	0.21	0.27
Lower Jaw Residual Length 9/15/2020	0.15	0.18	0.24	0.28	-0.17

Table G.1. Continued

Lower Jaw Residual Length 1/19/2021	0.21	-0.03	0.02	-0.22	-0.07
Anal Fin Residual Length 8/26/2019	-0.14	-0.08	-0.02	-0.16	-0.12
Anal Fin Residual Length 1/13/2020	0.03	-0.21	-0.19	-0.34	-0.11
Anal Fin Residual Length 9/15/2020	0.38	0.22	0.35	0.48	-0.16
Anal Fin Residual Length 1/19/2021	0.09	0.00	0.20	0.65	0.04

	Body Shape				
	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
PC 1 8/26/2019	-0.22	-0.02	-0.03	-0.43	-0.12
PC 1 1/13/2020	-0.19	0.38	0.34	0.08	0.71*
PC 2 8/26/2019	0.04	-0.17	-0.24	0.00	-0.22
PC 2 1/13/2020	-0.35	-0.37	-0.36	-0.14	-0.41
PC 3 8/26/2019	-0.04	0.45	0.51	0.30	-0.01
PC 3 1/13/2020	-0.46	0.17	0.17	-0.13	-0.29
PC 4 8/26/2019	-0.17	-0.44	-0.35	-0.26	0.11
PC 4 1/13/2020	0.38	-0.37	-0.36	-0.31	-0.38
PC 5 8/26/2019	0.32	0.32	0.27	0.07	0.54
PC 5 1/13/2020	-0.15	-0.24	-0.31	-0.16	0.01
PC 6 8/26/2019	-0.38	-0.01	0.00	-0.09	-0.13
PC 6 1/13/2020	-0.38	-0.25	-0.27	-0.11	0.47
PC 7 8/26/2019	0.50	0.27	0.22	-0.02	0.22
PC 7 1/13/2020	0.36	-0.22	-0.18	-0.01	0.36
PC 8 8/26/2019	-0.24	-0.11	-0.07	0.21	0.18
PC 8 1/13/2020	-0.25	-0.28	-0.17	-0.58	-0.12

Table G.1. Continued

	Visual Health Assessment				
	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
	-0.17	0.09	0.19	0.07	-0.10
VHI 7/1/2019	0.02	-0.32	-0.31	0.34	0.29
VHI 8/26/2019	0.25	-0.14	-0.15	0.1	-0.23
VHI 1/13/2020	-0.21	-0.24	-0.18	0.02	0.00
VHI 9/15/2020	0.09	0.01	-0.04	0.00	-0.11
VHI 1/19/2021	0.05	0.38	0.53	0.11	-0.03
WVHI 7/1/2019	-0.04	-0.15	-0.17	-0.03	0.11
WVHI 8/26/2019	0.04	0.19	0.13	-0.03	-0.18
WVHI 1/13/2020	0.00	0.09	0.19	0.16	-0.09
WVHI 9/15/2020	0.16	0.18	0.20	0.11	-0.22
WVHI 1/19/2021	0.02	0.06	0.02	-0.30	0.17

Table G.2. Correlation coefficients between traits (WVHI: Weighted Health Index, VHI: Health Index; K_n: Relative Condition; PC: Principal Component [1,2,3,4,5,6,7,8]) at each date and the total ovulated eggs and total hatch embryos of all females, including those that survived until the Reproductive Inventory, but did not successfully ovulate eggs. The four females that did not ovulate were included in analyses as having a total ovulate eggs and total hatch embryos of "0". Relationships with a significance of below the cutoff of 0.05 are denoted with asterisks.

	Length	
	Total Ovulated Eggs	Total Hatch Embryos
7/1/2019	0.21	0.16
8/26/2019	0.36	0.37
10/12/2019	0.50*	0.52*
12/2/2019	0.60*	0.61*
1/13/2020	0.63*	0.64*
3/16/2020	0.63*	0.65*
5/8/2020	0.64*	0.65*
7/10/2020	0.62*	0.63*

Table G.2. Continued

9/15/2020	0.63*	0.66*
10/27/2020	0.65*	0.67*
12/7/2020	0.63*	0.65*
1/19/2021	0.64*	0.65*
3/4/2021	0.63*	0.65*
5/4/2021	0.64*	0.67*

	Weight	
	Total Ovulated Eggs	Total Hatch Embryos
7/1/2019	0.09	0.08
8/26/2019	0.28	0.33
10/12/2019	0.50*	0.56*
12/2/2019	0.56*	0.59*
1/13/2020	0.58*	0.61*
3/16/2020	0.6*	0.65*
5/8/2020	0.62*	0.66*
7/10/2020	0.66*	0.70*
9/15/2020	0.61*	0.66*
10/27/2020	0.62*	0.68*
12/7/2020	0.65*	0.70*
1/19/2021	0.65*	0.70*
3/4/2021	0.66*	0.71*
5/4/2021	0.66*	0.72*

	Residual Daily Growth in Length	
	Total Ovulated Eggs	Total Hatch Embryos
8/26/2019	0.44*	0.55*
10/12/2019	0.56*	0.61*
12/2/2019	0.48*	0.43*
1/13/2020	0.14	0.15
3/16/2020	0.08	0.16

Table G.2. Continued

5/8/2020	0.07	0.05
7/10/2020	-0.16	-0.21
9/15/2020	0.17	0.25
10/27/2020	0.35	0.31
12/7/2020	-0.16	-0.13
1/19/2021	-0.27	-0.22
3/4/2021	-0.16	-0.16
5/4/2021	0.00	0.06

	Residual Daily Growth in Weight	
	Total Ovulated Eggs	Total Hatch Embryos
8/26/2019	0.37	0.47*
10/12/2019	0.69*	0.73*
12/2/2019	0.29	0.25
1/13/2020	0.08	0.12
3/16/2020	0.09	0.20
5/8/2020	0.08	-0.01
7/10/2020	0.08	0.10
9/15/2020	-0.06	-0.02
10/27/2020	-0.05	-0.03
12/7/2020	0.07	0.01
1/19/2021	-0.09	-0.12
3/4/2021	0.05	0.11
5/4/2021	0.21	0.30

	Relative Condition	
	Total Ovulated Eggs	Total Hatch Embryos
7/1/2019	-0.32	-0.24
8/26/2019	-0.26	-0.13
10/12/2019	-0.07	0.02
12/2/2019	-0.33	-0.29

Table G.2. Continued

1/13/2020	-0.42*	-0.37
3/16/2020	-0.31	-0.27
5/8/2020	-0.31	-0.31
7/10/2020	0.12	0.20
9/15/2020	-0.47*	-0.49*
10/27/2020	-0.46*	-0.41*
12/7/2020	-0.15	-0.17
1/19/2021	0.08	0.08
3/4/2021	-0.07	-0.05
5/4/2021	-0.09	-0.05
	Visual Health Assessment	
	Total Ovulated Eggs	Total Hatch Embryos
VHI 7/1/2019	0.28	0.28
VHI 8/26/2019	-0.10	-0.25
VHI 1/13/2020	-0.34	-0.41
VHI 9/15/2020	0.14	0.04
VHI 1/19/2021	-0.13	-0.09
WVHI 7/1/2019	0.20	0.29
WVHI 8/26/2019	0.16	0.02
WVHI 1/13/2020	-0.09	-0.04
WVHI 9/15/2020	0.11	0.07
WVHI 1/19/2021	0.08	0.17
	Energetic Reserves	
	Total Ovulated Eggs	Total Hatch Embryos
7/1/2019	-0.33	-0.18
8/26/2019	-0.48*	-0.37
1/13/2020	-0.23	-0.17
9/15/2020	0.49*	0.47*
1/19/2021	-0.11	-0.24

Table G.2. Continued

	Latency	
	Total Ovulated Eggs	Total Hatch Embryos
8/26/2019	0.40	0.19
1/13/2020	0.27	0.18
9/15/2020	0.11	0.29
1/19/2021	0.49	0.40
	Body Shape	
	Total Ovulated Eggs	Total Hatch Embryos
PC 1 8/26/2019	0.13	0.19
PC 1 1/13/2020	0.17	0.35
PC 2 8/26/2019	-0.32	-0.37
PC 2 1/13/2020	-0.17	-0.26
PC 3 8/26/2019	0.32	0.44
PC 3 1/13/2020	-0.18	-0.08
PC 4 8/26/2019	0.18	0.06
PC 4 1/13/2020	0.23	0.09
PC 5 8/26/2019	-0.04	0.12
PC 5 1/13/2020	-0.30	-0.37
PC 6 8/26/2019	0.05	0.09
PC 6 1/13/2020	0.26	0.16
PC 7 8/26/2019	-0.30	-0.14
PC 7 1/13/2020	0.35	0.22
PC 8 8/26/2019	0.14	0.14
PC 8 1/13/2020	0.26	0.11
	Linear Measurements	
	Total Ovulated Eggs	Total Hatch Embryos
Upper Jaw Residual Length 8/26/2019	0.19	0.21

Table G.2. Continued

Upper Jaw Residual Length 1/13/2020	-0.47*	-0.45*
Upper Jaw Residual Length 9/15/2020	-0.16	-0.19
Upper Jaw Residual Length 1/19/2021	0.31	0.38
Lower Jaw Residual Length 8/26/2019	0.22	0.34
Lower Jaw Residual Length 1/13/2020	-0.48*	-0.47*
Lower Jaw Residual Length 9/15/2020	-0.13	-0.13
Lower Jaw Residual Length 1/19/2021	0.17	0.17
Anal Fin Residual Length 8/26/2019	0.03	0.00
Anal Fin Residual Length 1/13/2020	0.06	-0.08
Anal Fin Residual Length 9/15/2020	0.15	0.16
Anal Fin Residual Length 1/19/2021	0.30	0.27

Table G. 3. Correlation coefficients between traits (WVHI: Weighted Health Index, VHI: Health Index; K_n: Relative Condition; PC: Principal Component [1,2,3,4,5,6,7,8]) at each date and the reproductive performance metrics of male WCT. Relationships with a significance of below the cutoff of 0.05 are denoted with asterisks.

	Length				
	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
7/1/2019	0.01	0.14	-0.04	-0.06	0.32

Table G.3. Continued

8/26/2019	0.04	0.15	0.07	0.11	0.19
10/12/2019	0.01	0.17	0.01	0.07	0.26
12/2/2019	-0.02	0.11	0.06	0.09	0.39
1/13/2020	-0.03	0.10	0.11	0.11	0.46*
3/16/2020	-0.05	0.07	0.08	0.07	0.53*
5/8/2020	-0.11	0.02	0.07	0.05	0.58*
7/10/2020	-0.21	-0.01	0.03	0.02	0.56*
9/15/2020	-0.26	-0.07	0.05	0.04	0.53*
10/27/2020	-0.26	-0.1	0.04	0.03	0.53*
12/7/2020	-0.28	-0.14	0.02	0.00	0.51*
1/19/2021	-0.29	-0.13	-0.02	-0.04	0..52*
3/4/2021	-0.29	-0.13	-0.02	-0.04	0.49*
5/4/2021	-0.27	-0.13	-0.03	-0.05	0.50*

	Weight				
	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
7/1/2019	-0.05	0.13	-0.08	-0.12	0.18
8/26/2019	0.10	0.13	0.11	0.13	0.50
10/12/2019	0.01	0.18	-0.02	0.02	0.21
12/2/2019	0.00	0.11	0.05	0.05	0.11
1/13/2020	-0.02	0.07	0.07	0.05	0.07
3/16/2020	-0.10	0.01	0.05	0.00	0.10
5/8/2020	-0.16	-0.05	0.09	0.00	0.22
7/10/2020	-0.19	-0.05	0.09	0.01	0.29
9/15/2020	-0.23	-0.08	0.12	0.04	0.15
10/27/2020	-0.23	-0.1	0.09	0.01	0.16
12/7/2020	-0.28	-0.16	0.03	-0.05	0.16
1/19/2021	-0.28	-0.17	0.01	-0.07	0.15
3/4/2021	-0.27	-0.17	0.00	-0.08	0.18

Table G.3. Continued

5/4/2021	-0.26	-0.17	-0.01	-0.09	0.24

	Relative Condition				
	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
7/1/2019	-0.24	-0.22	-0.23	-0.07	0.38
8/26/2019	-0.26	0.07	0.03	-0.03	0.24
10/12/2019	-0.12	-0.10	-0.14	0.09	0.28
12/2/2019	-0.09	0.07	-0.06	0.14	0.42*
1/13/2020	-0.18	-0.03	-0.08	0.09	0.50*
3/16/2020	-0.37	-0.23	-0.25	-0.11	0.62*
5/8/2020	-0.37	-0.14	-0.18	-0.15	0.68*
7/10/2020	-0.14	-0.01	-0.05	-0.43*	0.62*
9/15/2020	-0.15	0.16	0.04	0.06	0.60*
10/27/2020	-0.02	-0.15	-0.25	0.06	0.60*
12/7/2020	-0.02	-0.12	-0.12	0.14	0.57*
1/19/2021	-0.30	-0.06	-0.05	0.15	0.58*
3/4/2021	-0.27	-0.09	-0.12	-0.14	0.58*
5/4/2021	-0.21	0.03	0.01	0.00	0.59*

	Residual Daily Growth in Length				
	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
8/26/2019	0.03	0.10	0.22	0.38	0.19
10/12/2019	-0.25	0.17	-0.13	-0.08	-0.16
12/2/2019	-0.09	-0.18	0.18	0.10	0.37*
1/13/2020	-0.06	-0.02	0.28	0.16	0.50*
3/16/2020	-0.12	-0.16	-0.05	-0.10	0.48*
5/8/2020	-0.32	-0.48*	-0.09	-0.15	0.54

Table G.3. Continued

7/10/2020	-0.42*	-0.33	-0.14	-0.09	0.28
9/15/2020	-0.21	-0.05	0.25	0.27	-0.10
10/27/2020	0.07	-0.41*	0.17	0.11	0.24
12/7/2020	-0.20	-0.47*	-0.2	-0.26	0.28
1/19/2021	-0.07	-0.22	-0.11	-0.20	-0.19
3/4/2021	-0.07	-0.07	-0.01	0.03	-0.07*
5/4/2021	0.29	0.23	-0.14	-0.16	-0.59

Residual Daily Growth in Weight

	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
8/26/2019	0.24	0.14	0.26	0.35	0.37
10/12/2019	-0.25	0.24	-0.13	-0.06	0.24
12/2/2019	-0.01	-0.14	0.16	0.03	0.42*
1/13/2020	-0.06	-0.18	0.16	0.01	0.51*
3/16/2020	-0.33	-0.43*	-0.06	-0.2	0.62*
5/8/2020	-0.19	-0.41*	0.08	-0.02	0.68*
7/10/2020	-0.23	-0.19	0.10	0.09	0.62*
9/15/2020	-0.24	-0.07	0.29	0.25	0.59*
10/27/2020	0.16	-0.18	-0.33	-0.27	0.59*
12/7/2020	-0.25	-0.25	-0.31	-0.33	0.57*
1/19/2021	0.01	-0.30	-0.24	-0.29	0.58*
3/4/2021	0.07	-0.17	-0.06	-0.09	0.58*
5/4/2021	0.10	0.13	-0.01	-0.03	0.59*

Latency

	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
8/26/2019	-0.26	0.04	-0.64	-0.65	0.46
1/13/2020	-0.06	0.16	-0.03	0.01	-0.05
9/15/2020	-0.13	0.13	-0.55	-0.34	-0.36

Table G.3. Continued

1/19/2021	-0.11	0.22	-0.44	-0.36	-0.25

Energetic Reserves

	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
7/1/2019	-0.33	0.13	-0.14	-0.01	-0.17
8/26/2019	-0.12	-0.18	0.01	0.10	-0.20
1/13/2020	-0.12	-0.10	-0.27	-0.32	0.26
9/15/2020	0.05	-0.26	0.07	-0.04	0.02
1/19/2021	0.18	0.01	-0.08	-0.15	-0.27

Linear Measurements

	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
Upper Jaw Residual Length 8/26/2019	0.04	-0.01	0.01	0.00	-0.13
Upper Jaw Residual Length 1/13/2020	0.18	0.30	0.11	0.22	-0.24
Upper Jaw Residual Length 9/15/2020	-0.22	0.06	0.05	0.12	0.12
Upper Jaw Residual Length 1/19/2021	-0.04	0.00	-0.4*	-0.29	0.14
Lower Jaw Residual Length 8/26/2019	0.06	-0.04	-0.10	-0.07	-0.06
Lower Jaw Residual Length 1/13/2020	0.07	0.06	0.14	0.18	-0.13
Lower Jaw Residual Length 9/15/2020	-0.28	0.02	0.04	0.09	0.17
Lower Jaw Residual Length 1/19/2021	0.04	0.04	-0.19	-0.07	0.10

Table G.3. Continued

Anal Fin Residual Length 8/26/2019	0.03	0.15	-0.37*	-0.45*	-0.13
Anal Fin Residual Length 1/13/2020	0.28	-0.03	0.35	0.32	0.16
Anal Fin Residual Length 9/15/2020	-0.23	-0.10	0.38	0.34	0.04
Anal Fin Residual Length 1/19/2021	-0.33	-0.37	0.12	0.09	0.26

Body Shape

	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
PC 1 8/26/2019	0.24	-0.17	0.30	0.32	-0.09
PC 1 1/13/2020	0.29	-0.03	0.23	0.18	-0.14
PC 2 8/26/2019	-0.10	-0.51	-0.07	-0.10	-0.05
PC 2 1/13/2020	0.13*	-0.24	-0.06	-0.14	-0.01
PC 3 8/26/2019	0.08	-0.24	0.12	0.09	0.02
PC 3 1/13/2020	-0.28	-0.30	-0.15	-0.18	0.23
PC 4 8/26/2019	-0.07	-0.24	-0.10	0.02	0.16
PC 4 1/13/2020	-0.25	-0.28	0.31	0.30	-0.02
PC 5 8/26/2019	-0.04	-0.23	0.01	0.15	-0.06
PC 5 1/13/2020	-0.09	-0.45	0.43	0.49	-0.09
PC 6 8/26/2019	-0.32	0.05	-0.17	-0.15	-0.15
PC 6 1/13/2020	-0.45	-0.33	-0.13	-0.06	-0.16
PC 7 8/26/2019	0.27	0.15	0.18	0.18	-0.02
PC 7 1/13/2020	-0.15	-0.32	-0.10	-0.11	-0.35
PC 8 8/26/2019	-0.18	-0.28	0.25	0.25	0.11
PC 8 1/13/2020	0.04	0.13	0.22	0.15	0.06

Table G.3. Continued

	Visual Health Indices				
	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Median Spermatocrit
VHI 7/1/2019	-0.04	-0.05	-0.09	-0.13	-0.20
VHI 8/26/2019	0.00	0.10	0.07	0.01	0.14
VHI 1/13/2020	-0.05	0.17	-0.25	-0.27	-0.20
VHI 9/15/2020	0.11	0.11	-0.05	-0.11	-0.02
VHI 1/19/2021	-0.12	0.05	0.00	-0.04	-0.28
WVHI 7/1/2019	-0.01	-0.01	-0.26	-0.30	0.01
WVHI 8/26/2019	0.09	0.08	0.10	0.02	0.27
WVHI 1/13/2020	-0.20	-0.05	-0.28	-0.31	0.06
WVHI 9/15/2020	0.18	0.28	-0.21	-0.27	0.05
WVHI 1/19/2021	-0.03	0.25	0.05	0.03	-0.33

APPENDIX H

CORRELATION MATRICES AMONG ALL REPRODUCTIVE ASSESSMENTS BY SEX

Table H.1. Correlation coefficients between all reproductive traits of all female WCT that successfully ovulated. In this table, females that did not successfully ovulate, and thus had a total ovulated eggs and total hatch embryo value of 0, were not included. This table sought to examine relationships between the quality of eggs produced, with the quantity of eggs produced, and ultimate reproductive performance, which could not be done for females that did not successfully ovulate. Relationships with a significance of below the cutoff of 0.05 are denoted with asterisks.

	Total Ovulated Eggs	Total Hatch Embryos	Fertilization Success	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Egg Diameter	Egg Weight
Total Ovulated Eggs	1	0.96*	-0.18*	0.21*	0.18*	-0.23*	0.21*
Total Hatch Embryos	0.96*	1	-0.21*	0.53*	0.51*	-0.07*	0.27*
Fertilization Success	-0.18	-0.21	1	-0.05*	-0.05*	-0.01	-0.16*
Median Percent Eyed Embryos	0.21*	0.53*	-0.05	1	0.97*	0.23*	0.08
Median Percent Hatch Embryos	0.18*	0.51*	-0.04	0.97*	1	0.33*	0.10
Egg Diameter	-0.23*	-0.07	-0.01	0.23*	0.33*	1	0.38*
Egg Weight	0.21*	0.27	-0.16*	0.08	0.10*	0.38*	1

Table H.2. Correlation coefficients between all reproductive traits of all male WCT. Relationships with a significance of below the cutoff of 0.05 are denoted with asterisks.

	Median Spermatocrit	Median Percent Eyed Embryos	Median Percent Hatch Embryos	Fertilization Success
Median Spermatocrit	1	-0.05	-0.22	0.03
Median Percent Median Eyed	-0.05	1	0.94*	-0.1
Median Percent Hatch Embryos	-0.22	0.94	1	-0.07
Fertilization Success	0.03	-0.10	-0.07	1

APPENDIX I

CALCULATION OF GROWTH RATE OF WESTSLOPE CUTTHROAT TROUT FOR EACH TIME INTERVAL DURING REARING

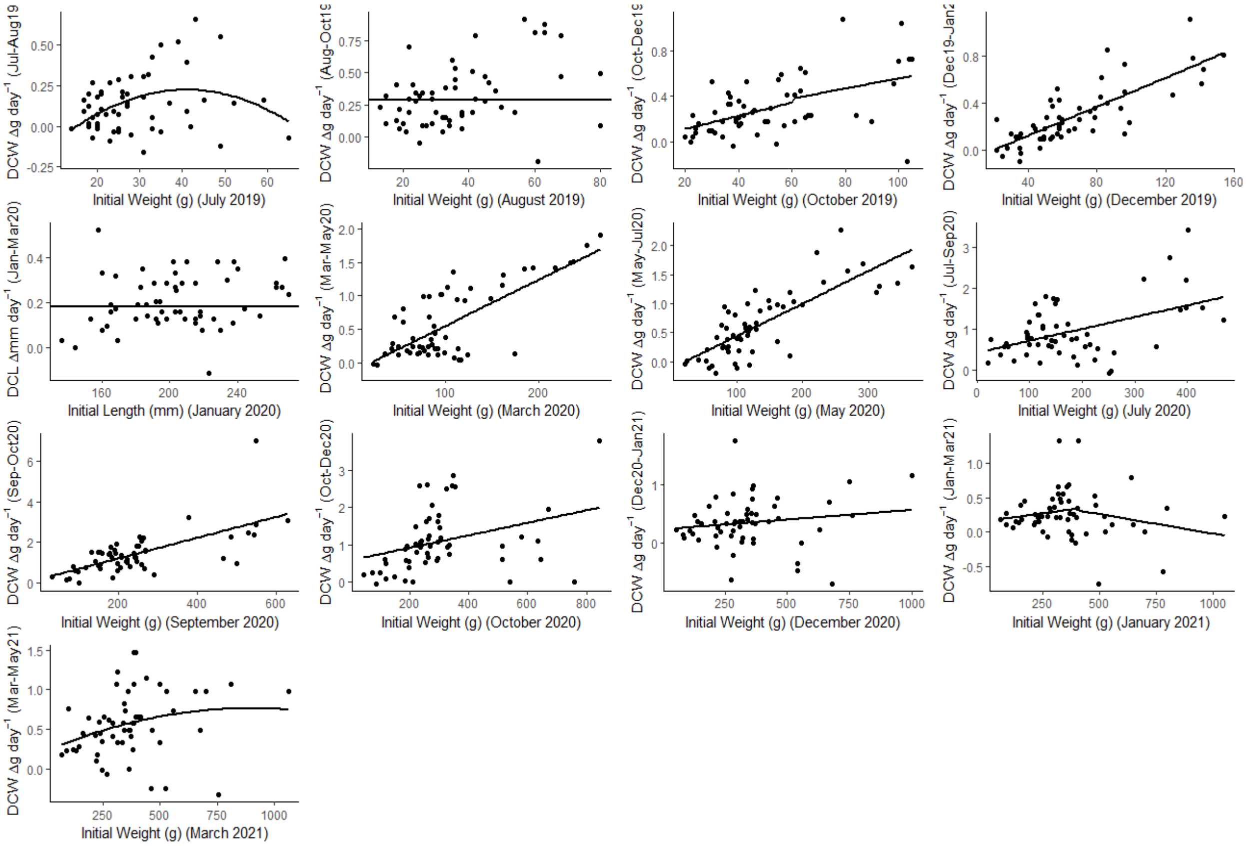


Figure I.1. Plots denoting the relationships between the initial weight (g) at the beginning of each time period and the daily change in weight (Δg d^{-1}; DCW) between the initial time point and the next subsequent inventory. The relationships were: quadratic (Weight in July 2019 vs. DCW July-August 2019; Weight in March 2021 vs. DCW March-May 2021), piecewise linear (Weight in October 2019 vs. DCW October-December 2019; Weight in January 2021 vs. DCW January-March 2021), or linear (all other relationships).

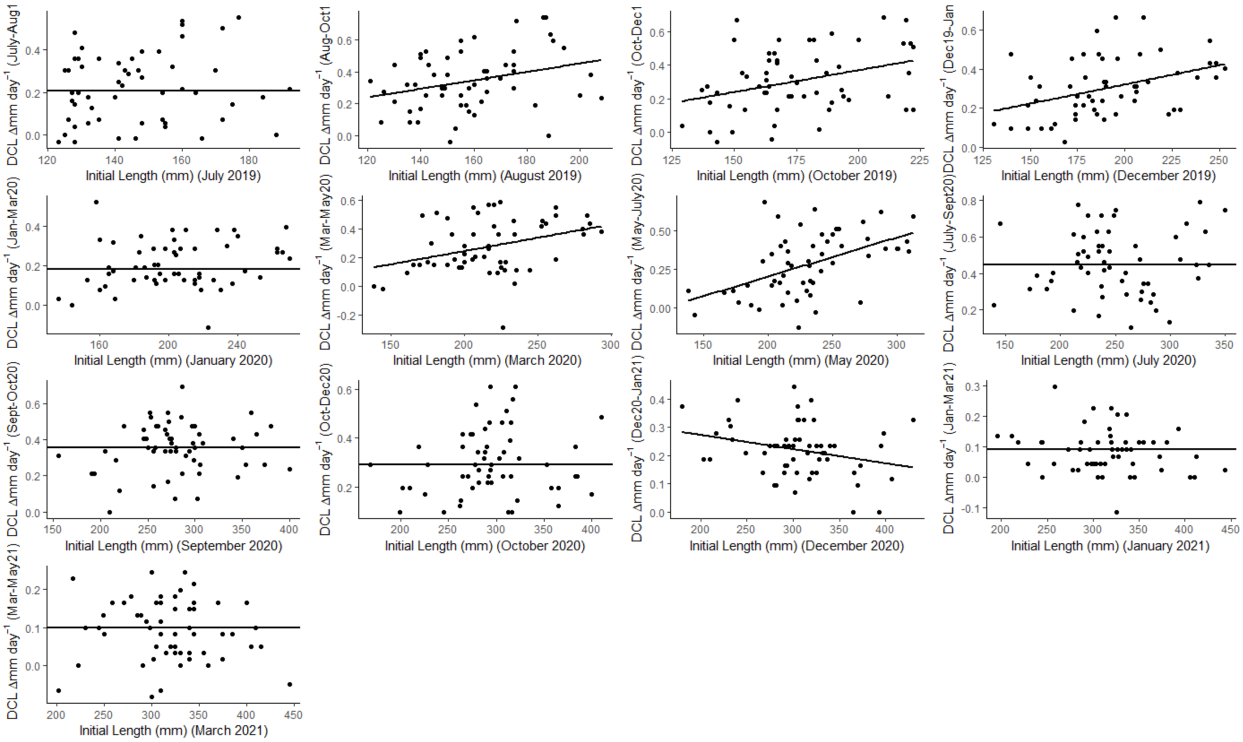


Figure I.2. Plots denoting the relationships between the initial length (mm) at the beginning of each time period and the daily change in length (Δmm d^{-1}; DCL) between the initial time point and the next subsequent inventory. The relationships were: linear (Length in August 2019 vs. DCL August-October 2019; Length in October 2019 vs. DCL October-December 2019; Length in March 2020 vs. DCL March -May 2020; Length in May 2020 vs. DCL May -July 2020; Length in December 2020 vs. DCL December 2020 -January 2021), or there was no best fit line, so the median was used instead (all other relationships).

REFERENCES CITED

Adams, D. C., Collyer, M. L., Kaliontzopoulou, A., and Sherratt, E. 2019. Geomorph: Software for geometric morphometric analyses. R package version 3.0. 5. In *R Package*.

Adams, D. C., and Otárola-Castillo, E. 2013. geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution*, *4*(4): 393-399.

Adriaenssens, B., and Johnsson, J., I. 2009. Personality and life-history productivity: consistent or variable association? *Trends in Ecological Evolution,* 24:179–180.

Al-Chokhachy, R., Heki, L., Loux, T. and Peka, R., 2020. Return of a giant: coordinated conservation leads to the first wild reproduction of Lahontan Cutthroat Trout in the Truckee River in nearly a century. *Fisheries*, *45*(2), pp.63-73.

Al-Chokhachy, R., and Sepulveda, A. J. 2019. Impacts of Nonnative Brown Trout on Yellowstone Cutthroat Trout in a Tributary Stream. *North American Journal of Fisheries Management*, 39 (1), 17–28.

Al-Chokhachy, R., Kovach, R. P., Sepulveda, A., Strait, J., Shepard, B. B., and Muhlfeld, C. C. 2019. Compensatory growth offsets poor condition in native trout populations. *Freshwater Biology*, *64* (12): 2120-2130.

Ali, O.A., O'Rourke, S.M., Amish, S.J., Meek, M.H., Luikart, G., Jeffres, C., Miller, M.R. 2016. RAD Capture (Rapture): Flexible and Efficient Sequence-Based Genotyping. *Genetics*, 202(2):389-400.

Allendorf, F.W. and Leary, R.F., 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation biology*, *2*(2), pp.170-184.

Allendorf, F.W., Leary, R.F., Hitt, N.P., Knudsen, K.L., Boyer, M.C., and Spruell, P. 2005. Cutthroat trout hybridization and the U.S. Endangered Species Act: one species, two policies. *Conservation Biology*, 19:1326-1328

Anderson, L.E. and Lee, S.T., 2013. Untangling the recreational value of wild and hatchery salmon. *Marine Resource Economics*, *28*(2), pp.175-197.

Andrews, T.C., Shepard, B.B., Litt, A.R., Kruse, C.G., Nelson, M.L., Clancey, P., Zale, A.V., Taper, M.L. and Kalinowski, S.T., 2016. Performance of juvenile cutthroat trout translocated as embryos from five populations into a common habitat. *North American Journal of Fisheries Management*, *36*(4), pp.926-941.

Araki, H., Ardren, W.R., Olsen, E., Cooper, B., and Blouin, M.S., 2007. Reproductive success of captive-bred steelhead trout in the wild: evaluation of three hatchery programs in the Hood River. *Conservation Biology*, 21: 181–190.

Araki, H., Cooper, B., Blouin, M.S., 2007b. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. *Science*, 318: 100–103.

Araki, H., Berejikian, B. A., Ford, M. J., and Blouin, M. S. 2008. Fitness of hatchery-reared salmonids in the wild. *Evolutionary Applications*, 1: 342–55.

Araki, H. and Schmid, C., 2010. Is hatchery stocking a help or harm? Evidence, limitations, and future directions in ecological and genetic surveys. *Aquaculture*, *308*, pp. S2-S11.

Araújo, M. S., Bolnick, D. I., and Layman, C. A. 2011. The ecological causes of individual specialization. *Ecology Letters*, *14*(9): 948-958.

Arostegui, M. C., Quinn, T., P. 2019. Reliance on lakes by salmon, trout and charr (*Oncorhynchus*, *Salmo* and *Salvelinus*): an evaluation of spawning habitats, rearing strategies, and trophic polymorphisms. *Fisheries*, 20: 775–784

Averett, R. C., and C. MacPhee. 1971. Distribution and growth of indigenous fluvial and adfluvial Cutthroat Trout (*Salmo clarki*) in the St. Joe River. *Idaho*. *Northwest Science* 45: 38– 47.

Azuma, T., Ohta, H., Oda, S., Muto, K., Yada, T. and Unuma, T., 2003. Changes in fertility of rainbow trout eggs retained in coelom. *Fisheries science*, *69*(1), pp.131-136.

Bardon-Albaret, A. and Saillant, E., 2017. Egg quality traits and predictors of embryo and fry viability in red snapper *Lutjanus campechanus*. *Aquaculture Reports*, *7*, pp.48-56.

Baschant and Tuckermann. 2010. The role of the glucocorticoid receptor in inflammation and immunity. *Journal of Steroid Biochemistry and Molecular Biology*,*120* (2-3), 69-75.

Bascinar, N. and Okumuş, İ., 2004. The early development of brook trout *Salvelinus fontinalis* (Mitchill): Survival and growth rates of alevins. *Turkish Journal of Veterinary and Animal Sciences*, *28*(2), pp.297-301.

Baskett, M. L., and Waples, R. 2013. Evaluating alternative strategies for minimizing unintended fitness consequences of cultured individuals on wild populations. *Conservation Biology*, 27:83–94.

Bayley, P. B. 1995. Understanding large river-floodplain ecosystems. *BioScience* 45: 153–158.

Bayse, S. M., Regish, A. M., and McCormick, S. D. 2018. Proximate composition, lipid utilization and validation of a non-lethal method to determine lipid content in migrating American shad *Alosa sapidissima*. *Journal of Fish Biology, 92*(6), 1832-1848.

Behnke, R.J. 2002. Trout and salmon of North America. The Free Press, New York.

Bennett, S., Al-Chokhachy, R., Roper, B. B., and Budy, P. 2014. Annual variation of spawning Cutthroat Trout in a small western USA stream: a case study with implications for the conservation of potamodromous trout life history diversity. *North American Journal of Fisheries Management*, 34: 1033–1046.

Berejikian, B. A., Johnson, T., Endicott, R. S., Lee-Waltermire, J. 2008. Increases in steelhead (*Oncorhynchus mykiss*) redd abundance resulting from two conservation hatchery strategies in the Hamma River, Washington. *Canadian Journal of Fisheries of Aquatic Sciences,* 65(4): 754–64.

Berejikian, B.A., Larsen, D.A., and Swanson, P. 2012. Development of natural growth regimes for hatchery-reared steelhead to reduce residualism, fitness loss, and negative ecological interactions. *Environmental Biology of Fish*, 94: 29–44.

Biro, P. A, Morton, A. E., Post, J. R., and Parkinson, E. A. 2004. Over-winter lipid depletion and mortality of age-0 rainbow trout (*Oncorhynchus mykiss*). *Canadian Journal of Fisheries and Aquatic Sciences*, 61(8): 1513-1519.

Biro, P. A., Post, J. R., and Beckmann, C. 2021. Autumn lipid reserves, overwinter lipid depletion, and high winter mortality of rainbow trout in experimental lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 78(6): 738-743.

Blackwell, B. G., Brown, M.L., Willis, D. W. 2000. Relative weight (Wr) status and current use. *Fisheries Assessment and Management Reviews in Fisheries Science,* 8: 1–44.

Blazer, V. S., H. L. Walsh, R. P. Braham, and Smith, C. 2018. Necropsy-based wild fish health assessment. *Journal of Visualized Experiments,* 139: 57946.

Bobe, J. and Labbé, C., 2010. Egg and sperm quality in fish. *General and comparative endocrinology*, *165*(3), pp.535-548.

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. A., Svanbäck, R. 2002. Measuring individual-level resource specialization. *Ecology*, 83: 2936–2941.

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., and Forister, M. L. 2003. The ecology of individuals: incidence and implications of individual specialization. *The American Naturalist, 161*(1), 1-28.

Bookstein, F. L., 1991. Morphometric Tools for Landmark Data: Geometry and Biology, Cambridge University Press, Cambridge.

Brandl, S.J., Robbins, W.D., and Bellwood, D. R. 2015. Exploring the nature of ecological specialization in a coral reef fish community: morphology, diet, and foraging microhabitat use. *Proceedings of the Royal British Society*, 282: 20151147.

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (Oncorhynchus mykiss). *Aquaculture*, *100*(1-3), pp.141-166.

Burrows, R.E., 1951. An evaluation of methods of egg enumeration. *The Progressive Fish-Culturist*, *13*(2), pp.79-85.

Burton T., Killen S. S., Armstrong J. D., and Metcalfe, N. B. 2011. What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? *Proceedings of the Royal British Society*, 278: 3465–3473.

Campbell, M.R., Dillon, J. and Powell, M.S., 2002. Hybridization and introgression in a managed, native population of Yellowstone cutthroat trout: genetic detection and management implications. *Transactions of the American Fisheries Society*, *131*(3), pp.364-375.

Campbell, P.M., Pottinger, T.G. and Sumpter, J.P., 1992. Stress reduces the quality of gametes produced by rainbow trout. *Biology of reproduction*, *47*(6), pp.1140-1150.

Campbell, T., Simmons, J., Sáenz, J., Jerde, C.L., Cowan, W., Chandra, S. and Hogan, Z., 2019. Population connectivity of adfluvial and stream-resident Lahontan cutthroat trout: Implications for resilience, management, and restoration. *Canadian Journal of Fisheries and Aquatic Sciences*, *76*(3), pp.426-437.

Carim, K.J., Eby, L.A., Barfoot, C.A. and Boyer, M.C., 2016. Consistent loss of genetic diversity in isolated cutthroat trout populations independent of habitat size and quality. *Conservation genetics*, *17*(6), pp.1363-1376.

Carim, K.J., Vindenes, Y., Eby, L.A., Barfoot, C. and Vøllestad, L.A., 2017. Life history, population viability, and the potential for local adaptation in isolated trout populations. *Global Ecology and Conservation*, *10*, pp.93-102.

Carim, K.J., Relyea, S., Barfoot, C., Eby, L.A., Kronenberger, J.A., Whiteley, A.R. and Larkin, B., 2021. Ultrasound imaging identifies life history variation in resident Cutthroat Trout. *PloS one*, *16*(2), p.e0246365.

Carl, L.M., and Stelfox, J.D. 1989. A meristic, morphometric, and electrophoretic analysis of cutthroat trout (*Salmo clarki*), from two mountain lakes in Alberta. *Canadian Field-Naturalist*. 103:80-84.

Chavarie, L., Howland, K. L., and Tonn, W. M. 2013. Sympatric polymorphism in lake trout: The coexistence of multiple shallow water morphotypes in Great Bear Lake. *Transactions of the American Fisheries Society*, 142(3): 814–823.

Chavarie, L., Howland, K., Gallagher, C., and Tonn, W. 2016a. Fatty acid signatures and stomach contents of four sympatric Lake Trout: Assessment of trophic patterns among morphotypes in Great Bear Lake. *Ecology of Freshwater Fish*, 25: 109–124.

Chavarie, L., Howland, K., Venturelli, P., Kissinger, B. C., Tallman, R., and Tonn, W. 2016b. Life-history variation among four shallow-water morphotypes of lake trout from Great Bear Lake, Canada. *Journal of Great Lakes Research*, 10.1016/j.jglr.2015.07.006.

Chavarie, L., Hoffmann, J., Muir, A. M., Krueger, C. C., Bronte, C. R., Howland, K. L., Gallagher, C. P., Sitar, S. P., Hansen, M. J., Vinson, M. R., Baker, L. F., Loseto, L. L., Tonn, W. M., and Swanson, H. K. 2020. Dietary versus nondietary fatty acid profiles of lake trout ecotypes from Lake Superior and Great Bear Lake: Are fish really what they eat? *Canadian Journal of Fisheries and Aquatic Sciences*, 77(7): 1209– 1220.

Chavarie, L. Howland, K, L., Harris, L. N., Gallagher, C. P., Hansen, M. J., Tonn, W. M., Muir, A. M., Krueger, C.C. 2021. Among-individual diet variation within a lake trout ecotype: Lack of stability of niche use. *Ecology and Evolution*, 11: 1457-1475.

Christie, M. R., Marine, M., French, R., Waples, R. S., and Blouin, M. 2012. Effective size of a wild salmonid population is greatly reduced by hatchery supplementation. Heredity, 109, 254.

Christie, M. R., Ford, M. J., Blouin, M. S. 2014. On the reproductive success of early-generation hatchery fish in the wild. *Evolutionary Applications*, 7: 883–896.

Christie, M. R., Marine, M. L., Fox, S. E., French, R. A., and Blouin, M. S. 2016. A single generation of domestication heritably alters the expression of hundreds of genes. *Natural Communications*, 7: 6.

Ciereszko, A. and Dabrowski, K., 1993. Estimation of sperm concentration of rainbow trout, whitefish and yellow perch using a spectrophotometric technique. *Aquaculture*, *109*(3-4), pp.367-373.

Collyer, M.L., Adams, D.C. 2018. RRPP: an r package for fitting linear models to high-dimensional data using residual randomization. *Methods Ecological Evolution*, 9 (7): 1772–1779.

Colt, J., and Shearer, K. D. 2001. Evaluation of the use of the Torry Fish Fatmeter to nonlethally estimate lipid in adult salmon. *Report of Research to the US Army Corps of Engineers, Portland District, Contract Report W66QKZ00805700, Seattle*.

Cook, K. V., Hinch, S. G., Watson, M. S., Patterson, D. A., Reid, A. J., and Cooke, S. J. 2018. Experimental capture and handling of chum salmon reveal thresholds in injury, impairment, and physiology: best practices to improve bycatch survival in a purse seine fishery. *Fisheries Research*, 206: 96-108.

Cooper, E. L. 1961. Growth of wild and hatchery strains of brook trout. *Transactions of the American Fisheries Society*, *90*(4): 424-438.

Conrad, J. L., Weinersmith, K. L., Brodin, T., Saltz, J. B., and Sih, A. 2011. Behavioral syndromes in fishes: a review with implications for ecology and fisheries management. *Journal Fisheries Biology,* 78: 395–435.

Contreras-Sanchez, W. M., Schreck, C.B., Fitzpatrick, M.S., and Pereira, C.B., 1998. Effects of stress on the reproductive performance of rainbow trout (Oncorhynchus mykiss). *Biology of Reproduction*, *58*(2), pp.439-447.

Crossin, G. T., Hinch, S. G., Cooke, S. J., Welch, D. W., Patterson, D. A., Jones, S. R. M., and Farrell, A. P. 2008. Exposure to high temperature influences the behavior, physiology, and survival of sockeye salmon during spawning migration. *Canadian Journal of Zoology*, *86*(2): 127-140.

Damsgard, B., Evensen, T. H., Overli, O., Gorissen, M., Ebbesson, L. O. E., Rey, S., and Hoglund, E. 2019. Proactive avoidance behavior and pace-of-life syndrome in Atlantic salmon. *Royal Society of Open Science*, 6 (3):181859.

Danner, G.R., 2008. Salmonid embryo development and pathology. In *American Fisheries Society Symposium* (Vol. 65, pp. 000-000).

Dingemanse, N. J., and Wolf, M. 2013. Between-individual differences in behavioral plasticity within populations: causes and consequences. *Animal Behavior,* 85: 1031–1039.

Distell. 2003. Distell Fish Fatmeter, user and technical manual model 692, version 2.4. Distell West Lothian, Scotland.

Djokic, M. A., Heishman, J., Kappenman, K. M., Gaylord, T. G., and Verhille, C. E. 2022. A microwave energy meter to estimate energetic reserves in juvenile sturgeon. *Journal of Applied Ichthyology*.

Dorner, B., Catalano, M. J., and Peterman, R. M. 2017. Spatial and temporal patterns of covariation in productivity of Chinook salmon populations of the Northeastern Pacific. *Canadian Journal of Fisheries and Aquatic Sciences*, 75(7): 1082-1095.

Draghi, J. A., and Whitlock, M. C. 2012. Phenotypic plasticity facilitates mutational variance, genetic variance, and evolvability along the major axis of environmental variation. *Evolution*, 66: 2891–2902.

Drinan, D. P., Kalinowski, S. T., Vu, N. V., Shepard, B. B., Muhlfeld, C. C., and Campbell. M. R. 2011. Genetic variation in Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi:* implications for conservation. *Conservation Genetics,* 12:1513–1523.

Drinan, D.P., Webb, M.A., Naish, K.A., Kalinowski, S.T., Boyer, M.C., Steed, A.C., Shepard, B.B. and Muhlfeld, C.C., 2015. Effects of hybridization between nonnative rainbow trout and native westslope cutthroat trout on fitness-related traits. *Transactions of the American Fisheries Society*, *144*(6), pp.1275-1291.

Dryden, I. L., and K. V. Mardia. 1998. *Statistical shape analysis*. Wiley, Chichester

Ertel, B.D., Heim, K.C., Arnold, J.L., Detjens, C.R. and Koel, T.M., 2017. Preservation of native cutthroat trout in northern Yellowstone. *Yellowstone Sci*, *25*(1), pp.35-41.

Fausch, K. D., Rieman, B. E., Young, M. K., Dunham, J. B. 2006. Strategies for conserving native salmonid populations at risk from nonnative fish invasions: tradeoffs in using barriers to upstream movement. Gen. Tech. Rep. RMRS-GTR-174. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 44 p.

Flagg, T.A., 2015. Balancing conservation and harvest objectives: a review of considerations for the management of salmon hatcheries in the US Pacific Northwest. *North American Journal of Aquaculture*, *77*(3), pp.367-376.

Flagg, T.A., Nash, C.E., Beckman, B.R., Berejikian, B.A., Colt, J., Dickhoff, W.W., Fairgrieve, W.T., Iwamoto, R.N., Larsen, D.A., Mahnken, C.V.W. and Maynard, D.J., 1999. A conceptual framework for conservation hatchery strategies for Pacific salmonids.

Fleming, I. A. and Einum, S. 1997. Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. *Journal of Marine Science,* 54, 1051–1063.

Fraser, D. J., and L. Bernatchez. 2005. Adaptive migratory divergence among sympatric brook charr populations. *Evolution,* 59: 611– 624.

Frost, A. J., Winrow-Giffen, A., Ashley, P. J., and Sneddon L. U. 2007. Plasticity in animal personality traits: does prior experience alter the degree of boldness? *Proceedings of the Royal British Society,* 274: 333–339.

FWP. 2007. Memorandum of Understanding and Conservation Agreement for Westslope Cutthroat Trout in Montana. Montana Fish, Wildlife and Parks, Helena, Montana.

Garland, T., 2014. Trade-offs. *Current Biology, 24*(2), pp. R60-R61.

Ghaedi, G., Falahatkar, B., Yavari, V., Sheibani, M.T. and Broujeni, G.N., 2015. The onset of stress response in rainbow trout Oncorhynchus mykiss embryos subjected to density and handling. *Fish Physiology and Biochemistry, 41*(2), pp.485-493.

Gillespie, G. J., and M. G. Fox. 2003. Morphological and life history differentiation in populations of pumpkinseed (*Lepomis gibbosus*). *Journal of Fish Biology*, 62: 1099– 1115.

Goetz, F.A., Baker, B., Buehrens, T. and Quinn, T.P., 2013. Diversity of movements by individual anadromous coastal cutthroat trout *Oncorhynchus clarkii clarkii*. *Journal of Fish Biology, 83*(5), pp.1161-1182.

Granath, K.L., Smoker, W.W., Gharrett, A.J., and Hard, J.J. 2004. Effects on embryo development time and survival of intercrossing three geographically separate populations of Southeast Alaska Coho salmon, *Oncorhynchus kisutch*. Environ. Biol. Fishes, **69**(1-4): 299–306.

Green, B.S., 2008. Maternal effects in fish populations. *Advances in marine biology*, *54*, pp.1-105.

Greene, C.M., Hall, J.E., Guilbault, K.R. and Quinn, T.P., 2010. Improved viability of populations with diverse life-history portfolios. *Biology Letters, 6*(3), pp.382-386.

Grisak, G., and Marotz, B. 2002. South Fork Flathead Watershed Westslope Cutthroat Trout Conservation Program, Annual Report. 10.2172/963010.

Grisak, G. and Marotz, B., 2003. *South Fork Flathead Watershed Westslope Cutthroat Trout Conservation Program, Annual Report 2002* (No. DOE/BP-00005043-1). Bonneville Power Administration (BPA), Portland, OR (United States).

Halvorsen, M.B., Casper, B.M., Woodley, C.M., Carlson, T.J., and Popper, A.N. 2012. Threshold for onset of injury in Chinook salmon from exposure to impulsive pile driving sounds. *Plos One*, 7(6): e38968.

Hanson, K. C., Ostrand, K. G., Gannam, A. L., and Ostrand, S. L. 2010. Comparison and Validation of Nonlethal Techniques for Estimating Condition in Juvenile Salmonids. *Transactions of the American Fisheries Society*, 139.6: 1733-741.

Harbicht, A.B., Fraser, D.J. and Ardren, W.R., 2020. Minor shifts towards more natural conditions in captivity improve long-term survival among reintroduced Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *77*(5), pp.931-942.

Harig, A.L. and Fausch, K.D., 2002. Minimum habitat requirements for establishing translocated cutthroat trout populations. *Ecological Applications*, *12*(2), pp.535-551.

Harder, A.M., Willoughby, J.R., Ardren, W.R., Christie, M.R. 2020. Among family variation in survival and gene expression uncovers adaptive genetic variation in a threatened fish. *Molecular Ecology* 29: 1035-1049.

Harder, A.M., and Christie, M. R. 2022. Genomic signatures of adaptation to novel environments: hatchery and life history-associated loci in landlocked and anadromous Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences*. e-First https://doi.org/10.1139/cjfas-2021-0066

Hartman, K. J., and F. J. Margraf. 2006. Relationships among condition indices, feeding, and growth of Walleye in Lake Erie. *Fisheries Management and Ecology*, 13: 121–130.

Hartman, K. J., and F. J. Margraf. 2008. Common relationships among proximate composition components in fishes. *Journal of Fish Biology*, 73: 2352–2360.

Helfrich, L. A., Liston, C. R., Mefford, B., and Bark, R. 2001. Survival and injury of splittail and Chinook salmon passed through a large hidrostal pump. *North American Journal of Fisheries Management*, *21*(3): 616-623.

Hilborn, R., Quinn, T.P., Schindler, D.E. and Rogers, D.E., 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences*, *100*(11), pp.6564-6568.

Hilderbrand, R.H., 2002. Simulating supplementation strategies for restoring and maintaining stream resident cutthroat trout populations. *North American Journal of Fisheries Management*, *22*(3), pp.879-887.

Hitt, N. P., Snook, E. L., and Massie, D. L. 2017. Brook trout use of thermal refugia, and foraging habitat influenced by brown trout. *Canadian Journal of Fisheries and Aquatic Sciences*, 74(3), 406–418.

Homel, K. M., R. E. Gresswell, and J. L. Kershner. 2015. Life history diversity of Snake River finespotted Cutthroat Trout: managing for persistence in a rapidly changing environment. *North American Journal of Fisheries Management*, 35:789–801.

Horan, D.L., Kershner, J.L., Hawkins, C.P. and Crowl, T.A., 2000. Effects of habitat area and complexity on Colorado River cutthroat trout density in Uinta Mountain streams. *Transactions of the American Fisheries Society*, *129*(6), pp.1250-1263.

Houde, A.L.S., Fraser, D.J., O'Reilly, P. and Hutchings, J.A., 2011. Maternal and paternal effects on fitness correlates in outbred and inbred Atlantic salmon (Salmo salar). *Canadian Journal of Fisheries and Aquatic Sciences, 68*(3), pp.534-549.

Hoyle, I., Oidtmann, B., Ellis, T., Turnbull, J., North, B., Nikolaidis, J., T. G. Knowles. 2007. A validated macroscopic key to assess fin damage in farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 270, 1–4:142-148.

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

Jirka, K. J., and Kraft, C. E. 2017. Diet Niche Width and Individual Specialization of Brook Trout in Adirondack Lakes, *Transactions of the American Fisheries Society*, 146(4): 716-731.

Jonsson, B., and Jonsson, N. 2005. Lipid energy reserves influence life-history decision of Atlantic salmon (*Salmo salar*) and brown trout (*S. trutta*) in fresh water. *Ecology of Freshwater Fish,* 14, 296–301.

Jonsson, N. and Jonsson, B., 1999. Trade-off between egg mass and egg number in brown trout. *Journal of Fish Biology*, *55*(4), pp.767-783.

Jonsson, N., Jonsson, B., and Hansen, L.P. 1997. Changes in proximate composition and estimates of energetic costs during upstream migration and spawning in Atlantic salmon (*Salmo salar*). *Journal of Animal Ecology*, 66: 425–436.

Kaga, T., Sato, S., Nagasawa, T., Fukuwaka, M. A., Nomura, T., and Urawa, S. 2009. Rapid estimation of lipid content of immature chum and pink salmon in the ocean with a handheld microwave meter. *North Pacific Anadromous Fish Commission, Document, 1208.*

Kahilainen, K., Alajärvi, E., and Lehtonen, H. 2005. Planktivory and diet-overlap of densely rakered whitefish (*Coregonus lavaretus*) in a subarctic lake. *Ecology of Freshwater Fish*, *14*(1): 50-58.

Kaitala, A., Kaitala, V. and Lundberg, P., 1993. A theory of partial migration. *The American Naturalist*, *142*(1), pp.59-81.

Kaufman, S. D., Johnston, T. A., Leggett, W. C., Moles, M. D., Casselman, J. M., and Schulte-Hostedde, A. I. 2007. Relationships between body condition indices and proximate composition in adult walleyes. *Transactions of the American Fisheries Society*, *136*(6): 1566-157.

Keeley, E.R., Parkinson, E.A. and Taylor, E.B., 2005. Ecotypic differentiation of native rainbow trout (Oncorhynchus mykiss) populations from British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences, 62*(7), pp.1523-1539.

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

Killen, S. S., Adriaenssens, B., Marras, S., Claireaux, G., and Cooke, S. J. 2016. Context dependency of trait repeatability and its relevance for management and conservation of fish populations. *Conservation Physiology*, 4, 1-19.

Killen, S. S., Glazier, D. S., Rezende, E. L., Clark, T. D., Atkinson, D., Willener, A. S., and Halsey, L. G. 2016. Ecological influences and morphological correlates of resting and maximal metabolic rates across teleost fish species. *American Naturalist*, 187(5): 592–606.

Komrakova, M. and Holtz, W., 2009. Factors responsible for successful chilled storage of unfertilized rainbow trout (Oncorhynchus mykiss) eggs. *Aquaculture*, *286*(3-4), pp.156-163.

Knott, J., Mueller, M., Pander, J., and Geist, J. 2019. Fish passage and injury risk at a surface bypass of a small-scale hydropower plant. *Sustainability*, *11*(21): 6037.

Kristjánsson, B. K., Skúlason, S., and Noakes, D. L. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus*). *Evolutionary Ecology Research*, *4*(5): 659-672.

Lavergne, S., Mouquet, N., Thuiller, W., Ronce, O. 2010. Biodiversity and climate change: integrating evolutionary and ecological responses of species and communities. *Annual Review of Ecology, Evolution, and Systematics*, 41: 321-350.

Leary, R.F., Dotson, T., Genter, D., Hill, B., Holton, G., Huston, J., Knudson, K.L., Rumsey, S., and Sage, G.K., 1995. Westslope cutthroat trout restoration program: Past and present distribution, brood stock program, and conservation genetics committee report. *Accessed March 2*, p.2003.

Lubzens, E., Young, G., Bobe, J. and Cerdà, J., 2010. Oogenesis in teleosts: how fish eggs are formed. *General and comparative endocrinology*, *165*(3), pp.367-389.

Luis-Miguel, C., and Hoffmann A. A. 2017. Evolution of phenotypic plasticity in extreme environments. *Philosophical Transactions of the Royal Society*, 372.

Martin, L.B., Hasselquist, D., and Wikelski, M. 2006. Investment in immune defense is linked to pace of life in house sparrows. *Oecologia*, 147 (4): 565-575.

Martinelli, L., Harris, O., Collins, M., and Kebus, M. 2020. Efficacy of a Modified Health Assessment Utilized on Two Genetically Distinct Stocks of Rainbow Trout. *Journal of Aquatic Animal Health*, 32(2): 59-64.

Maynard, D.J., Flagg, T.A. and Mahnken, C.V., 1995. A review of seminatural culture strategies for enhancing the post release survival of anadromous salmonids. In *American Fisheries Society Symposium* (Vol. 15, pp. 307-314).

Maynard, D.J., McDowell, G.C., Tezak, E.P. and Flagg, T.A., 1996. Effect of diets supplemented with live food on the foraging behavior of cultured fall chinook salmon. *The Progressive Fish Culturist*, *58*(3), pp.187-191.

McClure, M.M., Utter, F.M., Baldwin, C., Carmichael, R.W., Hassemer, P.F., Howell, P.J., Spruell, P., Cooney, T.D., Schaller, H.A. and Petrosky, C.E., 2008. Evolutionary effects of alternative artificial propagation programs: implications for viability of endangered anadromous salmonids. *Evolutionary Applications*, *1*(2), pp.356-375.

McKinstry, C. A., Carlson, T. J., and Brown, R. S. 2007. *Derivation of Mortal Injury Metric for Studies of Rapid Decompression of Depth-Acclimated Physostomous Fish*. United States. USDOE.

Metcalfe, N. B., Van Leeuwen, T. E., and Killen, S. S. 2016. Does individual variation in metabolic phenotype predict fish behavior and performance? *Journal of Fish Biology*, 88: 298–321.

Mittelbach, G. G. 1984. Predation and resource partitioning in two sunfishes (*Centrarchidae*). *Ecology* 65: 499– 513.

Mittelbach, G.C., Osenberg, C.W., and Wainwright, P.C. 1999. Variation in feeding morphology between pumpkinseed populations: phenotypic plasticity or evolution? *Evolutionary Ecology Research* 1: 111– 128.

Mitteroecker, P., and Gunz, P. 2009. Advances in geometric morphometrics. *Evolutionary Biology*, *36*(2): 235-247.

Mittelbach, G.G., Ballew, N.G. and Kjelvik, M.K., 2014. Fish behavioral types and their ecological consequences. *Canadian Journal of Fisheries and Aquatic Sciences*, *71*(6), pp.927-944.

Mobrand, L. E., Barr, J., Blankenship, L., Campton, D. E., Evelyn, T. T. P., Flagg, T. A., Mahnken C. V. 2005. Hatchery reform in Washington State: principles and emerging issues. *Fisheries* 30:11–22.

Muhlfeld, C. C., McMahon, T. E., Belcer, D., and Kershner. J. J. 2009. Spatial and temporal spawning dynamics of native Westslope Cutthroat Trout, *Oncorhynchus clarkii lewisi*, introduced Rainbow Trout, *Oncorhynchus mykiss*, and their hybrids. *Canadian Journal of Fisheries and Aquatic Sciences*, 66:1153–1168.

Muhlfeld, C. C., Kovach, R. P., Jones, L. A., Al-Chokhachy, R., Boyer, M. C., Leary, R. F., and Allendorf, F. W. 2014. Invasive hybridization in a threatened species is accelerated by climate change. *Nature Climate Change*, 4(7), 620-624.

Musseau, C., Vincenzi, S., Santoul, F., Boulêtreau, S., Jesenšek, D. and Crivelli, A.J. 2020. Within-individual trophic variability drives short-term intraspecific trait variation in natural populations. *Journal of Animal Ecology*, 89: 921–932.

Nagler, J.J., Parsons, J.E. and Cloud, J.G., 2000. Single pair mating indicates maternal effects on embryo survival in rainbow trout, Oncorhynchus mykiss. *Aquaculture*, *184*(1-2), pp.177-183.

Nakano, S., Fausch, K. D., Koizumi, I., Kanno, Y., Taniguchi, Y., Kitano, S., and Miyake, Y. 2020. Evaluating a pattern of ecological character displacement: charr jaw morphology and diet diverge in sympatry versus allopatry across catchments in Hokkaido, Japan. *Biological Journal of the Linnean Society*, *129*(2): 356-378.

Näslund, J., 2015. The pace of life of juvenile brown trout-inter-and intra-individual variation in growth and behaviour. (PhD thesis). Gothenburg, Sweden: University of Gothenburg.

Naughton, G. P., Keefer, M. L., Clabough, T. S., Jepson, M. A., Lee, S. R., Peery, C. A., and Caudill, C. C. 2011. Influence of pinniped-caused injuries on the survival of adult Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Oncorhynchus mykiss*) in the Columbia River basin. *Canadian Journal of Fisheries and Aquatic Sciences*, *68* (9): 1615-1624.

Northcote, T.G., 1992. Migration and residency in stream salmonids- some ecological considerations and evolutionary consequences. *Nordic Journal of Freshwater Research. Drottningholm*, *67*, pp.5-17.

Novinger, D.C. and Rahel, F.J., 2003. Isolation management with artificial barriers as a conservation strategy for cutthroat trout in headwater streams. *Conservation Biology*, *17*(3), pp.772-781.

Novotny, J. F., and J. W. Beeman. 1990. Use of a fish health condition profile in assessing the health and condition of juvenile Chinook Salmon. *Progressive Fish Culturist*, 52:162–170.

Ohlberger, J., Scheuerell, M. D., and Schindler, D. E. 2016. Population coherence and environmental impacts across spatial scales: A case study of Chinook salmon. *Ecosphere*, 7(4), e01333

Ostberg, C. O., Duda, J. J., Graham, J. H., Zhang, S., Haywood, K. P., Miller, B., and Lerud, T. L. 2011. Growth, Morphology, and Developmental Instability of Rainbow Trout, Yellowstone Cutthroat Trout, and Four Hybrid Generations. *Transactions of the American Fisheries Society*, 140(2), 334–344.

Øverli, Ø., Korzan, W. J., Ho¨glund, E., Winberg, S., Bollig, H., Watt, M., Forster, G. L., Barton, B. A., Øverli, A., Renner, K. J., and Summers, C. H. 2004. Stress coping style predicts aggression and social dominance in rainbow trout. *Hormones and Behavior*, 45: 235–241.

Øverli, Ø., Pottinger, T. G., Carrick, T. R., Øverli, E., and Winberg, S. 2002. Differences in behavior between rainbow trout selected for high- and low-stress responsiveness. *Journal of Experimental Biology*, 205: 391– 395.

Parsons, K. J., and Robinson, B. W. 2006. Replicated evolution of integrated plastic responses during early adaptive divergence. *Evolution*, 60: 801– 813.

Parsons, K. J., and Robinson, B. W. 2007. Foraging performance of diet-induced morphotypes in pumpkinseed sunfish (*Lepomis gibbosus*) favors resource polymorphism. *Journal of Evolutionary Biology*, 20: 673– 684.

Pellegrini, A.F.A., Wisenden, B.D. and Sorensen, P.W. 2010. Bold minnows consistently approach danger in the field and lab in response to either chemical or visual indicators of predation risk. *Behavioral Ecology and Sociobiology*, 64, 381–387.

Penaluna, B.E., Abadía-Cardoso, A., Dunham, J.B., García-Dé León, F.J., Gresswell, R.E., Luna, A.R., Taylor, E.B., Shepard, B.B., Al-Chokhachy, R., Muhlfeld, C.C. and Bestgen, K.R., 2016. Conservation of native Pacific trout diversity in western North America. *Fisheries*, *41*(6), pp.286-300.

Peterson, B.G., Carl, P., Boudt, K., Bennett, R., Ulrich, J., Zivot, E., Lestel, M., Balkissoon, K. and Wuertz, D., 2014. PerformanceAnalytics: Econometric tools for performance and risk analysis. *R package version*, *1*(3).

Pickering, A. D.1993. Endocrine-induced pathology in stressed salmonid fish. *Fish. Res*, 1735-50.

Poff, N. L., Hart, D.D. 2002. How Dams Vary and Why It Matters for the Emerging Science of Dam Removal: An ecological classification of dams is needed to characterize how the tremendous variation in the size, operational mode, age, and number of dams in a river basin influences the potential for restoring regulated rivers via dam removal, *BioScience*, Volume 52, Issue 8, August 2002, Pages 659–668.

Polverino, G., Santostefano, F., Díaz-Gil, C., and Mehner, T. 2018. Ecological conditions drive pace-of-life syndromes by shaping relationships between life history, physiology, and behavior in two populations of eastern mosquitofish. *Scientific Reports- Nature*. 8: 14673.

Pope, K. L., and C. G. Kruse. 2007. Condition. Pages 423–471 in M. L. Brown and C. S. Guy, editors. Analysis and interpretation of freshwater fisheries data. *American Fisheries Society*, Bethesda, Maryland.

Pottinger, T. G., and Carrick, T. R. 2001. Stress responsiveness affects dominant-subordinate relationships in rainbow trout. *Hormones and Behavior*, 40(3): 419–427.

Puchany, A.R., 2021. *Success of westslope cutthroat trout and arctic grayling conservation translocations in Yellowstone National Park, Montana and Wyoming, USA* (Doctoral dissertation, Montana State University-Bozeman, College of Letters & Science).

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle, Washington, and American Fisheries Society, Bethesda, Maryland.

Quist, M.C., Hubert, W. A., and Isaak, D.J. 2004. Fish assemblage structure and relations with environmental conditions in a Rocky Mountain watershed. *Canadian Journal of Zoology*, 82:1554-1565.

Rahel, F.J., Keleher, C.J. and Anderson, J.L., 1996. Potential habitat loss and population fragmentation for cold water fish in the North Platte River drainage of the Rocky Mountains: response to climate warming. *Limnology and Oceanography*, *41*(5), pp.1116-1123.

R Core Team. 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/

R’eale, D., Garant, D., Humphries, M. M., Bergeron, P., Careau, V. and Montiglio, P.-O. 2010. Personality and the emergence of the pace-of-life syndrome concept at the population level. *Philosophical Transactions of the Royal British Society*, 365, 4051-4063.

Reist, J. D. 1985. An empirical evaluation of several univariate methods that adjust for size variation in morphometric data. *Canadian Journal of Zoology*, 63: 1429–1439.

Reist, J. D., Power, M., and Dempson, J. B. 2013. Arctic Charr (*Salvelinus alpinus*): a case study of the importance of understanding biodiversity and taxonomic issues in northern fishes. *Biodiversity*, 14: 45–56

Ricklefs, R. E., and Wikelski, M. 2002. The physiology/lifehistory nexus. *Trends in Ecological Evolution*, 17: 462–468.

Rieman, B. E., and Apperson, K. A. 1989. Status and analysis of salmonid fisheries: Westslope Cutthroat Trout synopsis and analysis of fishery information. Idaho Department of Fish and Game, Project F-73-R-11, Subproject No. II, Job No. I, Boise.

Rime, H., Guitton, N., Pineau, C., Bonnet, E., Bobe, J. and Jalabert, B., 2004. Post-ovulatory ageing and egg quality: a proteomic analysis of rainbow trout coelomic fluid. *Reproductive Biology and Endocrinology*, *2*(1), pp.1-10.

Riva-Rossi, C., Pascual, M. A., Babaluk, J. A., García-Asorey, M., and Halden, N. M. 2007. Intra-population variation in anadromy and reproductive life span in rainbow trout introduced in the Santa Cruz River, Argentina. *Journal of Fish Biology*, 70 (6): 1780-1797.

Roberts, J.J., Fausch, K.D., Hooten, M.B. and Peterson, D.P., 2017. Nonnative trout invasions combined with climate change threaten persistence of isolated cutthroat trout populations in the southern Rocky Mountains. *North American Journal of Fisheries Management*, *37*(2), pp.314-325.

Rohlf, F. J. 2017. Program TpsDig, version 2.31. *Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, NY.*

Rosengren, M., Kvingedal, E., Näslund, J., Johnsson, J.I. and Sundell, K., 2017. Born to be wild: effects of rearing density and environmental enrichment on stress, welfare, and smolt migration in hatchery-reared Atlantic salmon. *Canadian Journal of Fisheries and Aquatic Sciences*, *74*(3), pp.396-405.

Roughgarden, J. 1972. Evolution of Niche Width. *The American Naturalist*, Vol. 106, No. 952, pp. 683-718.
Roughgarden, J. 1974. Niche Width: Biogeographic Patterns among Anolis Lizard Populations. *The American Naturalist*, Vol. 108, No. 962, pp. 429-442.

Roughgarden, J. 1979. Theory of population genetics and evolutionary ecology: an introduction. Macmillan, New York, New York, USA.

RStudio Team. 2021. RStudio: Integrated Development Environment for R. Boston, MA. Retrieved from http://www.rstudio.com/

Ruiz-Gomez, M. de L., Huntingford, F.A., Øverli, Ø., Th ¨ornqvist, P.O., and H ¨oglund, E.2011. Response to environmental change in rainbow trout selected for divergent stress coping styles. *Physiological Behavior*. 102: 317–322.

Ryman, N., and Laikre, L. 1991. Effects of supportive breeding on the genetically effective population size. *Conservation Biology*, 5(3): 325– 329.

Sakai, K., Nomura, M., Takasima, F. and Oto, H., 1975. The over-ripening phenomenon of rainbow trout, 2: Changes in the percentage of eyed eggs, hatching rate and incidence of abnormal alevins during the process of over-ripening. *Bulletin of the Japanese Society of Scientific Fisheries*.

Schindler, D. E., Hilborn, R., Chasco, B., Boatright, C. P., Quinn, T. P., Rogers, L. A., and Webster, M. S. 2010. Population diversity and the portfolio effect in an exploited species. *Nature*, 465(7298): 609–612.

Schlager, S. 2017. Morpho and Rvcg–Shape Analysis in R: R-Packages for geometric morphometrics, shape analysis and surface manipulations. In *Statistical shape and deformation analysis* (217-256). Academic Press.

Schluter, D. 2000. The ecology of adaptive radiation. Oxford University Press, Oxford.

Schlichting, C.D. and Pigliucci, M. 1998. Phenotypic Evolution: A Reaction Norm Perspective. Sinauer Associates, Sunderland.

Schloesser, R. W., and Fabrizio, M. C. 2017. Condition indices as surrogates of energy density and lipid content in juveniles of three fish species. *Transactions of the American Fisheries Society*, *146*(5): 1058-1069.

Schreck, C. 2010. Stress and fish reproduction: The roles of allostasis and hormesis. *General and Comparative Endocrinology, 165*(3), 549-556.

Scott, A.P. and Baynes, S.M., 1982. Plasma levels of sex steroids in relation to ovulation and spermiation in rainbow trout (*Salmo gairdneri*). In *Proceedings of the International Symposium on Reproductive Physiology of Fish* (pp. 103-106).

Seebacher F. and C.E. Franklin. 2012. Determining environmental causes of biological effects: the need for a mechanistic physiological dimension in conservation biology. *Philosophical Transactions of the Royal Society*, 367:1607–1614.

Seebacher, F., White, C.R. and Franklin, C.E., 2015. Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, *5*(1), pp.61-66.

Seiler, M. B., and Keeley, E. R. 2009. Intraspecific taxonomy and ecology characterize morphological divergence among cutthroat trout (*Oncorhynchus clarkii ssp. Richardson*) populations. *Biological Journal of the Linnean Society*, *96*(2): 266-281.

Shepard, B.B., Clancey, P., Nelson, M.L., Kruse, C.G., Al-Chokhachy, R., Drinan, D., Taper, M.L. and Zale, A.V., 2021. Evaluation of Remote Site Incubators to Incubate Wild-and Hatchery-Origin Westslope Cutthroat Trout Embryos. *North American Journal of Fisheries Management*, *41*(3), pp.844-855.

Shepard, B. B., May, B. E., and Urie, W. 2005. Status and conservation of westslope cutthroat trout within the western United States. North American Journal of Fisheries Management 25:1426-1440.

Shepard, B.B., Sanborn, B., Ulmer, L. and Lee, D.C., 1997. Status and risk of extinction for westslope cutthroat trout in the upper Missouri River basin, Montana. *North American journal of fisheries management*, *17*(4), pp.1158-1172.

Shepard, B., Zale, A.V., Andrews, T., Taper, M.L. and Kalinowski, S.T., 2019. Performance of Westslope Cutthroat Trout Released into the Upper Cherry Creek Drainage Using Remote Stream Incubators. *Intermountain Journal of Sciences*, *25*(1-4).

Sidlauskas, B. L., Mol, J. H., and Vari, R. P. 2011. Dealing with allometry in linear and geometric morphometrics: a taxonomic case study in the *Leporinus cylindriformis* group (Characiformes: Anostomidae) with description of a new species from Suriname. *Zoological Journal of the Linnean Society*, *162*(1): 103-130.

Singh, A.S. and Masuku, M.B., 2014. Sampling techniques & determination of sample size in applied statistics research: An overview. *International Journal of economics, commerce, and management*, *2*(11), pp.1-22.

Skúlason, S., Parsons, K. J., Svanbäck, R., Räsänen, K., Ferguson, M. M., Adams, C. E., Amundsen, P.- A., Bartels, P., Bean, C. W., Boughman, J. W., Englund, G., Guðbrandsson, J., Hooker, O. E., Hudson, A. G., Kahilainen, K. K., Knudsen, R., Kristjánsson, B. K., Leblanc, C. L., Jónsson, Z., and Snorrason, S. S. 2019. A way forward with eco evo devo: An extended theory of resource polymorphism with post-glacial fishes as model systems. *Biological Reviews*, 94, 1786– 1808.

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

Smith, T. B., and Skúlason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *The Annual Review of Ecology, Evolution, and Systematics*, 27:111–133.

Springate, J.R.C., Bromage, N.R., Elliott, J.A.K. and Hudson, D.L., 1984. The timing of ovulation and stripping and their effects on the rates of fertilization and survival to eying, hatch and swim-up in the rainbow trout (Salmo gairdneri R.). *Aquaculture*, *43*(1-3), pp.313-322.

Stapp, P., and Hayward, G. D. 2002. Estimates of Predator Consumption of Yellowstone Cutthroat Trout (*Oncorhynchus clarki bouvieri*) in Yellowstone Lake. *Journal of Freshwater Ecology*, 17(2), 319–329

Strzyżewska-Worotyńska, E., Szarek, J., Babińska, I., and Gulda, D. 2017. Gills as morphological biomarkers in extensive and intensive rainbow trout (*Oncorhynchus mykiss*) production technologies. *Environmental Monitoring and Assessment,* 6;189(12): 611.

Su, G., Logez, M., Xu, J., Tao, S., Villéger, S. and Brosse, S., 2021. Human impacts on global freshwater fish biodiversity. *Science*, *371*(6531), pp.835-838.

Sutton, S. G., T. P. Bult, and R. L. Haedrich. 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic Salmon parr. *Transactions of the American Fisheries Society*, 129:527–538.

Tierney, K. B., and Farrell, A. P. 2004 The relationships between fish health, metabolic rate, swimming performance and recovery in return-run sockeye salmon, *Oncorhynchus nerka* (*Walbaum*). *Journal of Fish Diseases*, 27: 663–671.

Toscano, B.J., Gownaris, N.J., Heerhartz, S.M. and Monaco, C.J., 2016. Personality, foraging behavior and specialization: integrating behavioral and food web ecology at the individual level. *Oecologia*, *182*(1), pp.55-69.

Vander Zanden, J. M., Shuter, B. J., Lester, N. P., and Rasmussen, J. B. 2000. Within- and among-population variation in the trophic position of a pelagic predator, lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences*, 57(4): 725-731.

Via, S. 2002. The ecological genetics of speciation. *American Naturalist,* 159: S1-S7. 10.1086/338368.

Villéger, S., Miranda, J.R., Hernández, D.F. and Mouillot, D., 2010. Contrasting changes in taxonomic vs. functional diversity of tropical fish communities after habitat degradation. *Ecological applications*, *20*(6), pp.1512-1522.

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E. 2007. Let the concept of trait be functional! *Oikos*, 116, 882–892.

Vogt, A., Gormley, T. R., Downey, G., and Somers, J. 2002. A comparison of selected rapid methods for fat measurement in fresh herring (*Clupea harengus*). *Journal of Food Composition and Analysis*, *15*(2): 205-215.

Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback Gasterosteus aculeatus L. (*Gasterosteidae*) body shape, Biological Journal of the Linnean Society, 61(1) 1: 3–50.

Waples, R.S., 1991. Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(S1), pp.124-133.

Waters, C. D., Hard, J. J., Fast, D. E., Knudsen, C. M., Bosch, W. J., and Naish, K. A. 2020. Genomic and phenotypic effects of inbreeding across two different hatchery management regimes in Chinook salmon. *Molecular Ecology*, 29(4), 658–672.

Webb, P. W. 1984. Form and function in fish swimming. *Scientific American*, *251*(1), 72-83.

Westerberg, M., Staffan, F., Magnhagen, C., 2004. Influence of predation risk on individual competitive ability and growth in Eurasian perch *Perca fluviatilis*. *Animal Behavior,* 67: 273-279.

Westneat, D. F., Wright, J., and Dingemanse, N. J. 2015. The biology hidden inside residual within-individual phenotypic variation. *Biological Reviews*, *90*(3): 729-743.

White, J. R., Meekan, M. G., McCormick, M. I., and Ferrari, M. C. O. 2013. A comparison of measures of boldness and their relationships to survival in young fish. *PLoS One*, 8: e68900.

White, S.J., Kells, T.J. and Wilson, A.J., 2016. Metabolism, personality, and pace of life in the Trinidadian guppy, *Poecilia reticulata*. *Behaviour*, *153*(13-14), pp.1517-1543.

Williams, J.E., Haak, A.L., Neville, H.M. and Colyer, W.T., 2009. Potential consequences of climate change to persistence of cutthroat trout populations. *North American journal of fisheries management*, *29*(3), pp.533-548.

Willoughby, J. R., and Christie, M. R. 2019. Long-term demographic and genetic effects of releasing captive-born individuals into the wild. *Conservation Biology,* 33(2): 377-388.

Willoughby, J. R., Harder, A. M., Tennessen, J. A., Scribner, K. T., and Christie, M. R. 2018. Rapid genetic adaptation to a novel environment despite a genome-wide reduction in genetic diversity. *Molecular Ecology, 27*(20): 4041-4051.

Wohl, E. E. 2001. Virtual rivers. Yale University Press, New Haven, Connecticut.

Wolf, M., van Doorn, G., Leimar, O., Weissing, F. J. 2007. Life-history trade-offs favor the evolution of animal personalities. *Nature*, 447: 581–584.

Yeannes, M. I., Almandos, M. E., 2003. Estimation of fish proximate composition starting from water content. *Journal of Food Composition and Analysis*, 16 (1): 81-92

Young, M.K. and Harig, A.L., 2001. A critique of the recovery of greenback cutthroat trout. *Conservation Biology*, *15*(6), pp.1575-1584.

Young, J. L., Hinch, S. G., Cooke, S. J., Crossin, G. T., Patterson, D. A., Farrell, A. P., and Van Der Kraak, G. 2006. Physiological and energetic correlates of en route mortality for abnormally early migrating adult sockeye salmon (*Oncorhynchus nerka*) in the Thompson River, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences,* 63:1067–1077.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elsevier, New York.

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

Zimmerman, M. S., C. C. Krueger, and R. L. Eshenroder. 2007. Morphological and ecological differences between shallow- and deep-water Lake Trout in Lake Mistassini, Quebec. *Journal of Great Lakes Research,* 33:156– 169.

Zimmerman, M. S., S. N. Schmidt, C. C. Krueger, M. J. Vander Zanden, and R. L. Eshenroder. 2009. Ontogenetic niche shifts and resource partitioning of Lake Trout morphotypes. *Canadian Journal of Fisheries and Aquatic Sciences*, 66:1007–1018.

Zymonas, N.D. and McMahon, T.E., 2009. Comparison of pelvic fin rays, scales, and otoliths for estimating age and growth of bull trout, Salvelinus confluentus. *Fisheries Management and Ecology*, *16*(2), pp.155-164.