Reproductive Life History Decisions in a Long-Distance Migrating Iteroparous Fish Model

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by
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AUTHORIZATION TO SUBMIT DISSERTATION

This dissertation of Laura E. Jenkins, submitted for the degree of Doctorate of Philosophy, with a Major in Biological Sciences and titled "Reproductive Life History Decisions in a Long-Distance Migrating Iteroparous Fish Model," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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ABSTRACT

Steelhead trout (*Oncorhynchus mykiss*, hereafter referred to as steelhead) display diverse life histories after initial or maiden spawning, with some individuals spawning again after 1 year (consecutive spawning) and others after two or more years (skip spawning). This study seeks to understand how reproductive performance compares between maiden and repeat spawners, how physiological condition is involved in the development of consecutive and skip spawning life histories, and how and when resources are allocated within and between reproductive events. An experimental model employing female, hatchery-origin steelhead was used to test for: (1) physiological trade-offs between maiden reproductive effort and future reproduction, (2) a conditional vs. fixed maturation strategy, (3) a critical period after maiden spawning for rematuration initiation, and (4) the sensitivity of ovarian development in relation to physiological condition. Female steelhead were captured following their upstream spawning migration, manually spawned, fed, sampled, and repeat spawned in captivity. This experimental model represents one of extreme energy depletion associated with an approximately 6 month-long, 800 km freshwater fasting spawning migration. Repeat spawners were larger and had greater absolute fecundity than maiden spawners. Skip spawners had larger eggs and greater total reproductive effort than maiden and consecutive spawners. When standardized for mass, consecutive spawners had more but smaller eggs than maiden spawners, indicating that egg size can vary and is determined early in oogenesis based on energy reserves. These observations imply that excess energy gained during the vitellogenic period is allocated to somatic growth. Mass growth and plasma triglyceride (TG) levels were greater in consecutive than skip spawners 10 weeks after maiden spawning, and muscle lipids and Fulton's condition factor were greater in consecutive spawners 20 weeks after maiden spawning. These findings suggest that consecutive spawners consumed more food than skip spawners during the period after spawning. Mass growth and TG were positively related to reproductive performance at repeat spawning in consecutive spawners, with the maximal correlation with egg size at 10 weeks, and the maximal correlation with total reproductive investment at 20 weeks. Consecutive maturation was not significantly associated with any measure of physiological condition at maiden spawning. However, post-spawning survival was positively associated with maiden spawning TG, representing the ability to access energy, and plasma osmolality, indicating the ability to maintain homeostasis. Surprisingly, consecutive maturation was positively related to maiden reproductive effort, which is not consistent with a theorized tradeoff between current and future reproduction in animals. Instead, this result suggests that both maiden reproductive effort and consecutive maturation may be condition dependent at time points prior to maiden spawning. This dissertation provides insight into how and when consecutive and skip spawning life history tactics develop physiologically in steelhead. Additionally, this dissertation provides evidence that female steelhead can modulate reproductive investment based on physiological condition early during oogenesis.

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INTRODUCTION

Life history theory is the study of patterns of survival, growth, and reproductive effort (Stearns 1992); patterns which interact with size, age, and developmental stage. Studies in life history theory can focus on traits at either the phenotypic or the genotypic level (Roff 1992). Herein, the focus is on phenotypic-level traits involved in reproductive decisions: physiological factors related to the maturation decision and energy allocation to and within reproductive events. Reproduction-related decisions are considered adaptations (Cole 1954) and are implemented by the physiological systems of individual animals. The adaptive nature of these traits (*i.e.*, ultimate causes) is discussed but is not the focus of the analysis. The major concepts driving this inquiry were: physiological trade-offs (Stearns 1992), condition-dependent strategies (Gross 1996), the critical period hypothesis of maturation (Thorpe 1994; Thorpe et al. 1998), and energy allocation decisions (Tyler et al. 1990; Stearns 1992; Tyler et al. 1994; Yoneda and Wright 2005). These concepts were explored in the context of spawning interval in a long-distance migrating, iteroparous, salmonid, the anadromous rainbow trout (*Oncorhynchus mykiss*, hereafter steelhead). The population studied exhibits extreme energy depletion because individuals fast for more than 6 months before spawning.

Physiological trade-offs

Physiological trade-offs result from "…limited resources in the environment prevent(ing) individuals from simultaneously maximizing all life-history traits…" (Brosset et al. 2016). Energy stores must be allocated between somatic processes (e.g., survival, maintenance, growth, locomotion, and retained somatic energy) and reproduction-related processes (e.g., breeding migration, gonadal development, and reproductive behavior). Physiological trade-offs are based on the "Principles of Allocation" (Stearns 1992). If energy or materials are limited in an individual, direct competition will result in splitting of the resources, whereby more resources allotted for one process leaves fewer resources available for others. In the absence of developmental constraints, selection should favor reproductive investment at individual breeding episodes that maximizes lifetime reproductive success in individuals. Trade-offs between current and future reproduction are predicted when energy is limited. Additionally, within gonadal development in females, resources must be partitioned between individual offspring, resulting in a trade-off between offspring number and offspring size (Smith and Fretwell 1974; Stearns 1992; Quinn et al. 2011). How these trade-offs are implemented and constrained by the physiological processes of gonadal development is not well understood. Reproduction and associated activities are highly energetically costly (Stearns 1992; Bronson 1995) and severe depletion of energy reserves has been observed across taxa (Wade and Schneider 1992). The impact of current reproduction on future reproduction is thought to be mediated by reductions in physiological condition. For example, reproductive effort has been shown to correlate negatively with parasite resistance and fat stores (Stearns 1992), indicating a tradeoff. However, how physiological condition interacts with reproductive life history decisions requires further definition.

Condition-dependent strategies

Condition has been defined as "…the relative capacity to maintain optimal functionality of all vital systems within the body…" (Hill 2011), which encompasses both the individual's energy reserves and its ability to maintain homeostasis and function or perform optimally. The influence of condition on life histories has been most studied in the context of condition-dependent life history strategies. Condition-dependent strategies have many names: alternative phenotypes, polyphenisms, or tactics within a flexible or conditional strategy that arise from a single genotype. This phenomenon (alternate phenotypes arising from single genotype) is also known as phenotypic plasticity (West-Eberhard 1989). Condition-dependent strategies involve a decision based on the individual's status, where fitness is generally unequal between tactics, equal at the switch point, and greater for an individual at a given status for the chosen tactic (Gross 1996). Physiologically driven condition-dependent strategies can be conceptualized using a reaction norm, or the relationship between physiological condition and the dependent variable: the expressed tactic or phenotype (Hutchings 2011). When the dependent variable is binominal, such as the decision to mature, an individual's physiological condition interacts with its genetically determined threshold to generate a decision. The threshold is subject to selection, providing a mechanism that explains the plasticity in size and age at maturity found in poikilotherms as adaptive (Bull and Shine 1979; Thorpe 2007; Taranger et al. 2010; Hutchings 2011). For sexual maturation, a threshold level of physiological condition is required for the completion of gonadal development, breeding, and activities associated with reproduction such as migration. Below the threshold level, an alternative phenotype will be adopted: deferred reproduction and a focus on feeding to increase energy stores in order to improve physiological condition for future reproduction. Though not truly a conditional strategy, survival is also condition-dependent. An individual in positive energy balance and health is likely to survive and an individual in negative energy balance and failing health is not. Physiological condition can be measured using both indices of energy reserves such as body lipid levels and or performance-based indices of functional ability such as maintenance of osmotic homeostasis.

Critical period hypothesis of salmonid maturation

The critical period hypothesis of maturation was developed to explain how and when the decision to mature sexually occurs in Atlantic salmon (*Salmo salar*) (Thorpe 1994), and has been extended for use in other salmonids. This hypothesis proposes that the initial maturation decision takes place during a seasonally defined critical period approximately one year before spawning and is permissively gated by threshold levels of metabolic fuels (Thorpe et al. 1998; Thorpe 2007). Maturation initiation in female salmonids is associated with transitions in early secondary oocyte growth (Campbell et al. 2006b; Lubzens et al. 2010; Taranger et al. 2010; Grieshaber et al. 2016). A second critical period approximately six months later is also postulated in which gonadal development can be arrested if energy reserves become insufficient (Thorpe et al. 1998). The aspect of metabolic fuel storage that gates entry into and or allows for continuation of a reproductive cycle is not known but is functionally defined as either the absolute level or the rate of change of body size (*i.e.*, growth) and/or lipid reserves (Thorpe 2007; Taranger et al. 2010). Although salmonid maturation decisions have been most studied in the context of puberty (*i.e.*, entry into the first reproductive event), it is reasonable to assume that similar processes operate during rematuration in iteroparous species.

Energy allocation decisions

The allocation of significant energy for ovarian development in salmonids takes place over the year before spawning (Campbell et al. 2006b). The relationship between the timing of the energy acquisition and the allocation of energy reserves to somatic processes (*i.e.*, growth) and ovarian development is a key question in salmonid biology (Bromage et al. 1992; Campbell et al. 2006a). Approximately 1 year prior to spawning a cohort of primary ovarian follicles transitions into a secondary growth phase, which may be stimulated by systemic factors associated with body growth (Campbell et al. 2006b; Lubzens et al. 2010). This cohort of oocytes then grows further during vitellogenesis, which is characterized by the bulk transfer of energy from soma to gonad (Lubzens et al. 2010). During vitellogenesis in salmonids, oocyte number is not expected to change significantly, whereas oocyte volume increases up to 50-fold and the gonadosomatic index increases to approximately 20% (Tyler et al. 1990). How and when the investment into ovarian development, and the partitioning of energy reserves between offspring, are sensitive to physiological condition requires further definition in salmonids.

Consecutive and skip spawning

In annually spawning iteroparous fishes, individuals within a population may spawn at intervals of one year (consecutive spawners) or two or more years (skip spawners). Skipped spawning is common in seasonally breeding fishes (Rideout and Tomkiewicz 2011) that are capital breeders (McBride et al. 2015), meaning that they “fund” reproduction with energy reserves rather than continuously gained energy. Skipped spawning is important because it impacts recruitment estimates used in fisheries management due to over-estimates of spawning adults and fecundity at the population scale (Rideout et al. 2005). Reproduction and associated activities severely deplete energy reserves in iteroparous species (Stearns 1992; Wade and Schneider 1992; Bronson 1995), and thus skipped reproduction is generally thought to result from the energetic cost of the previous breeding event. In iteroparous salmonids, based on the critical period hypothesis of salmonid maturation, the rematuration decision would be expected to occur near the time of maiden spawning. Although it is reasonable to assume that similar mechanisms operate during rematuration as in puberty, a major difference in rematuration is the level of energy depletion due to the previous reproductive event occurring during early oogenesis for consecutive spawners. Both life history pathways (consecutive, skip) are observed in repeat spawning steelhead in the wild (Keefer et al. 2008; Nielsen et al. 2011; Moore et al. 2014).

Kelt reconditioning

Reconditioning programs for post-spawn female steelhead (kelts) in the Columbia River Basin aim to maintain and enhance summer-run steelhead populations listed as threatened under the U.S. Endangered Species Act (ESA). Female steelhead are captured after spawning, fed, maintained in captivity, and then released back into the river to migrate upstream and spawn in the wild after approximately 6 months in captivity for consecutive spawners or 18 months for skip spawners. Columbia River Basin kelt reconditioning programs are currently being operated for several inland populations of summer-run steelhead (Abrahamse and Murdoch 2014; Hatch et al. 2016), and were adapted from programs developed for Atlantic salmon recovery (Crim et al. 1992; Moffett et al. 1996). Consecutive and skip spawning life histories are found in reconditioning programs for both steelhead (Pierce et al. 2017; Jenkins et al. 2018) and Atlantic salmon (Crim et al. 1992; Moffett et al. 1996). In order to quantify the benefits that can be expected from Columbia River Basin kelt reconditioning programs, it is necessary to (1) demonstrate that reconditioned kelts can generate viable gametes and (2) estimate the potential productivity of released consecutive and skip spawners compared with that of maiden spawners. Identifying how physiological condition relates to post-spawning survival, rematuration decisions, and energy allocation to reproduction at repeat spawning will provide a scientific basis for maximizing the success of kelt reconditioning programs.

Snake River steelhead

The Snake River distinct population segment (DPS) of steelhead is listed as threatened under the ESA. Kelt reconditioning is currently underway to address recovery of the Snake River DPS (Hatch et al. 2017). Anadromous migration requires passage through 8 major hydroelectric dams. Lower Granite Dam (LGR) is the uppermost dam on the Snake River that must be navigated by Snake River steelhead. Wild-origin kelts migrating downstream after spawning were estimated to represent 17% of the pre-spawn population that migrated above LGR the previous fall (Evans et al. 2004). However, only 0.5-1.2% of the kelts collected at LGR were observed to return on repeat spawning migrations (Keefer et al. 2008), which included both consecutive and skip spawners. Although it is unknown how many steelhead historically returned to LGR as repeat spawners, the proportion may never have been high on the Clearwater River, a tributary to the Snake River. A study in the 1950s reported the proportion of repeat spawners returning to the Lewiston Dam was 1.63% prior to Snake River dam construction (Whitt 1954). However, this was more than 20 years after the initiation of Bonneville Dam construction in 1934, the first (and lowermost) dam constructed on the Columbia River. These observations suggest that dam construction may have already begun affecting the passage of pre- and post-spawn steelhead. Although the proportion of repeat spawning steelhead is low, physiological senescence was not observed in Snake River steelhead kelts (Penney and Moffitt 2014a).

Dworshak National Fish Hatchery-origin steelhead – An experimental model

An experimental system at Dworshak National Fish Hatchery (DNFH) on the Clearwater River at Ahsahka, Idaho, was developed for studying intersections between reproductive decisions, physiological condition, and reproductive development in repeat spawning female steelhead to test life history theory models and to evaluate kelt reconditioning as a recovery method. DNFH produces summer-run steelhead that are raised to age 1 in raceways before being released as smolts. Maturing adults return from the ocean, enter the Columbia River in late summer, and migrate more than 800 km upriver to DNFH where they are captured. Females are manually spawned in the spring at age 4 and placed in tanks. These are then fed, sampled when necessary, and repeat spawned in captivity. This experimental model offers access to fish that otherwise spend the majority of their lifespan in the wild, as well as uniformity in genetic background and age. Use of hatchery-origin (non-ESA listed) fish allows for quantification of reproductive measures, which is not an option for the population of wild-origin fish in this region due to their ESA listing as part of the Snake River DPS.

Objectives

The objectives of this study were: 1) to quantify reproductive performance in maiden, consecutive, and skip spawning female steelhead to better understand the timing of energy acquisition and its allocation to reproductive processes, and to quantify the benefits that can be expected from kelt reconditioning programs (Chapter 1), 2) to determine when the rematuration decision occurs, to determine how growth rates and measures of lipid reserves relate to the rematuration decision, and to assess the impact of recovery from maiden spawning on rematuration in female steelhead (Chapter 2), and 3) to determine whether and how post-spawning survival or rematuration were related to maiden reproductive investment or condition and whether reproductive investment at repeat spawning was condition-dependent during reconditioning, based on physiological condition measured early following maiden spawning (Chapter 3).

CHAPTER 1

Reproductive performance and energy balance in consecutive and skip repeat spawning female steelhead reconditioned in captivity[1]

Introduction

In iteroparous fishes, life history strategies should be selected to adjust reproductive investment at individual breeding episodes so as to maximize lifetime reproductive success in individuals. Individual developmental pathways are made up of sequential reproductive life history tactics influenced by rates of energy acquisition, conversion, and expenditure; physiological condition; and a genetic threshold for the energy stores needed to mature (Bull and Shine 1979; Thorpe et al. 1998; Thorpe 2007; McBride et al. 2015). Energy stores must be allocated between somatic processes (survival, maintenance, growth, locomotion, and retained somatic energy [condition]) and reproduction-related processes (migration, reproductive behavior, and gonadal development). Within gonadal development in females, stored energy must be further partitioned between fecundity and egg size (Smith and Fretwell 1974; Stearns 1992; Quinn et al. 2011). Allocation of limited energy reserves results in trade-offs between these processes (Stearns 1992). How these trade-offs are implemented in and constrained by the physiological and developmental processes of gonadal development is not well understood.

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[1] Jenkins, L.E., A.L. Pierce, N.D. Graham, R. Branstetter, D.R. Hatch, J.J. Nagler. 2018. Reproductive performance and energy balance in consecutive and skip repeat spawning female steelhead reconditioned in captivity. Transactions of the American Fisheries Society, 147(5): 959-971. See Appendix 1 for copyright information.

In salmonids, the production of eggs begins with the generation of primary ovarian follicles, each of which contains a developing oocyte (Lubzens et al. 2010). However, the commitment to spawning and allocation of significant energy to ovarian development takes place over the year before spawning (Campbell et al. 2006b). The initial stage occurs approximately 1 year prior to spawning and is characterized by the transition of a cohort of primary ovarian follicles into a secondary growth phase, which may be stimulated by systemic factors associated with body growth (Campbell et al. 2006b; Lubzens et al. 2010). This cohort of oocytes then grows further during vitellogenesis, which is characterized by the bulk transfer of energy from soma to gonad (Lubzens et al. 2010). During vitellogenesis, oocyte number is not expected to change significantly, whereas oocyte volume increases up to 50-fold and the gonadosomatic index increases to approximately 20% (Tyler et al. 1990). However, the relationship between the timing of the acquisition of energy reserves and the allocation of those reserves to reproductive processes is not fully understood and is a key question in salmonid biology (Bromage et al. 1992; Campbell et al. 2006a).

Steelhead *Oncorhynchus mykiss* (anadromous Rainbow Trout) show broad diversity and plasticity of life history patterns (Moore et al. 2014). Steelhead are capital breeders, funding their reproduction with energy reserves rather than continuously gained energy (McBride et al. 2015). A fasting, freshwater spawning migration follows one or multiple winters at sea (Burgner et al. 1992). Additional years at sea prior to the maiden (first) spawning result in increased energy acquisition, growth, body size, and reproductive performance, as fecundity and egg size are positively correlated with body length (Crespi and Teo 2002; Quinn et al. 2011). In addition, summer-run steelhead return to freshwater in the late summer before spawning the following spring, much earlier than winter-run steelhead, which return to freshwater immediately before spawning (Quinn et al. 2016). Repeat spawning also occurs over different intervals: 1 year (consecutive spawners) or 2 years (skip spawners) (Rideout et al. 2005; Keefer et al. 2008; Nielsen et al. 2011; Rideout and Tomkiewicz 2011; Moore et al. 2014). Repeat spawning requires recovering from previous spawning, accumulating energy stores, and initiating rematuration of the gonad. Maturation over different intervals in repeat spawners likely has implications for somatic growth and reproductive performance, as it does in maiden spawners. Somatic reserves spent on migration and maiden reproduction will need to be replenished for fish to spawn a second time. Consecutive spawners are maximally energy depleted near the time of maturation commitment and the initiation of ovarian development for the next spawning, and they spend only a few months recovering somatic reserves while continuing ovarian development and gathering sufficient energy to complete upstream migration and maturation. Skip spawners recondition for an entire year, recovering from maiden spawning and accumulating energy reserves before initiating gonadal development and resuming the allocation of energy to reproductive development and migration.

Reconditioning programs for steelhead kelts (spawned adults returning to the ocean) in the Columbia River basin aim to maintain and enhance populations of steelhead listed as threatened under the U.S. Endangered Species Act. Summer-run females are captured after spawning, fed, maintained in captivity, and then released back into the river in the fall to migrate upstream and spawn in the wild (approximately 6 months later for consecutive spawners and 18 months later for skip spawners). Columbia River basin reconditioning programs are currently being operated for several inland populations of summer-run steelhead (Abrahamse and Murdoch 2014; Hatch et al. 2016). Consecutive and skip spawning life histories are found in reconditioning programs for both steelhead and Atlantic Salmon *Salmo salar* (Crim et al. 1992; Moffett et al. 1996; Hatch et al. 2013; Pierce et al. 2017). It is necessary to quantify the benefits that can be expected from Columbia River basin kelt reconditioning programs (Alldredge et al. 2011). Among other things, this requires (1) estimation of the potential productivity of released consecutive and skip spawners compared with that of maiden spawners, and (2) demonstration that reconditioned females can generate viable gametes.

A tractable experimental system for studying steelhead kelt reconditioning at Dworshak National Fish Hatchery (DNFH) on the Clearwater River at Ahsahka, Idaho, was used to compare reproductive performance among maiden, consecutive, and skip spawning females. This hatchery produces summer-run steelhead that spend multiple winters at sea and that must migrate over 800 km upriver to the hatchery, near the maximum range for this species. Both the early return timing and the migration distance have been shown to result in severe energy depletion in postspawning fish (Penney and Moffitt 2014b). The purpose of this study was to quantify reproductive performance (*i.e.*, egg size, egg number, total egg mass) in maiden, consecutive, and skip spawning female steelhead. As these three groups differed in their schedules of energy availability during ovarian development, our first objective was to better understand the timing of energy acquisition and its allocation to reproductive processes. Our second objective was to quantify the benefits that can be expected from kelt reconditioning programs.

Methods

In this study, steelhead were collected and artificially spawned at DNFH between February and April 2013–2016, reconditioned to maturity (1 year for consecutive spawners, 2 years for skip spawners), and then spawned a second time. Reproductive performance measurements were collected at each spawning event.

Fish.—Returning maiden spawning female steelhead of hatchery origin were captured after ascending the adult fish ladder at DNFH beginning in February each year in 2013–2016. Fish were maintained for up to several weeks in holding ponds supplied with water from the North Fork Clearwater River before being spawned. During February–April 2013–2016, fully mature (ripe) maiden spawning females (*n* = 163, 148, 150, and 164 in 2013, 2014, 2015, and 2016, respectively) were selected for this study. Fish >70 cm were selected by DNFH staff for use as broodstock. Among these, fish with no visual wounds and that were in good or fair condition were selected for reconditioning, as previously described (Hatch et al. 2013).

Spawning and sampling.—Fish were anesthetized using AQUI-S 20E (AquaTactics, Kirkland, Washington; 75 mL/1,000 L water). Eggs were removed from the fish via "air spawning" (Leitritz and Lewis 1976): a 16-gauge needle was inserted through the midbody-cavity wall, just posterior to the pelvic fin, then oxygen at 17.2–20.7 kPa pressure was gently blown into the body cavity and ovulated eggs were collected from the urogenital opening. After spawning, fish were prophylactically treated for bacterial infection with oxytetracycline (Durvet, Blue Springs, Missouri; 20 mg/kg body weight) and for parasitic gill copepods *Salmincola californiensis* with emamectin (Sigma-Aldrich, St. Louis, Missouri; 200 µg/kg body weight), both via intraperitoneal injection. Fish were individually tagged with PIT tags inserted into the pelvic girdle and sampled for FL (cm) and wet body mass (kg) following air spawning. Wet mass was later adjusted by exclusion of the mass of any eggs remaining in the body cavity, as described below. Oxytetracycline injections continued at 10-week intervals. Emamectin injections were given at these intervals only when copepods were visible on the gills. Repeat spawners reconditioned in tanks (detailed below) were evaluated for ripeness weekly during the spawning season (January 12–April 1) and air spawned when ripe, as described above. For repeat spawners, references to year refer to the year of repeat spawning rather than the year of maiden spawning.

*Reconditioning husbandry.—*Fish were held in 4.6-m-diameter outdoor tanks, with a water height of 1.5 m, located at DNFH. Tanks were supplied with a total flow of approximately 200 L/min drawn from the North Fork Clearwater River that had a seasonally varying temperature profile (4.9–11.0°C). Fish were fed ad libitum a mixture of boiled krill *Euphausia superba* (Atlantic Pacific Products, Kingston, Rhode Island) and pellets (Biobrood, 6-mm pellet size; BioOregon, Longview, Washington) top-coated with menhaden oil (Argent Aquaculture, Redmond, Washington), along with freeze-dried decapsulated brine shrimp eggs (*Artemia* spp. cysts; American Brine Shrimp, Ogden, Utah) for increased palatability. Tanks were treated with formalin (flow-through treatment, 1:6,000 for 1 h daily; Syndel USA, Portland, Oregon) to control *Saprolegnia*.

*Survival.—*Survival to repeat spawning for female steelhead reconditioned at DNFH varied by maiden spawn year, averaging 10% (19% in 2013, 3% in 2014, and 8% in 2015). On average, 49% of the mortality occurred within 10 weeks of maiden spawning (39, 47, and 62% in 2014, 2015, and 2016, respectively). Because reproductive performance increases with fish size, we tested the effect of somatic mass at maiden spawning on survival. Survivors were smaller than mortalities in 2013, but no differences were detected in 2014 or 2015 (two-sample *t*-test; 2013: $t = 2.433$, df = 158, $P = 0.0161$; 2014: $t = 0.6694$, df = 144, $P = 0.5043$; 2015: $t = 0.2343$, df = 148, $P = 0.8151$). Because the smaller size of the 2013 survivors at maiden spawning would underestimate the reproductive performance of repeat spawners from that year, our approach in comparing spawning categories is conservative. Due to an equipment malfunction, all 2015 (skip spawning) and 2016 (consecutive and skip spawning) fish died in November 2016 and thus were not able to be repeat-spawned.

Reproductive performance measures.—Reproductive performance was quantified at maiden spawning for 2014–2016 females by measuring total, individual, and dry egg mass, fecundity, and fertilization success. These data were not collected for the 2013 maiden fish. Reproductive performance was quantified in females surviving to their second spawning (*i.e.*, 1 year later for consecutive spawners and 2 years later for skip spawners) during the 2014–2016 spawning seasons.

Individual egg mass, total egg mass, and fecundity were calculated gravimetrically (Fleming and Ng 1987). Dry egg mass was calculated by weighing 25 unfertilized eggs after desiccation to a constant mass at 110°C in an oven (24 h; (Brosset et al. 2016).

A small portion of eggs is occasionally retained in the body cavity following nonlethal spawning methods (Bromage et al. 1992), including air spawning. At 10 weeks after maiden spawning, fish were anesthetized, held vertically with the head up, and gently massaged on the ventral surface from anterior to posterior. Eggs were collected and enumerated. At the time of death, any additional retained eggs were enumerated during necropsy. Repeat spawners were air spawned, then necropsied to obtain and quantify any retained eggs. Total egg mass, fecundity, and somatic mass (*i.e.*, the mass excluding the mass of the eggs) were corrected for eggs retained in the body cavity following air spawning.

Fertilization success was determined using an in vitro fertilization assay developed for rainbow trout (Stoddard et al. 2005). After visual confirmation of motility, sperm was added to a subsample of 25–50 unfertilized eggs from each female, and sperm activation solution was added. The fertilized eggs were incubated for 12–14 h at 9–14.5°C in vertical stack incubators and then fixed in Stockard's solution. The fixed eggs were observed under a stereomicroscope, and fertilization was scored as the percentage of eggs with embryo cleavage.

Statistical analysis.—ANCOVA was used to compare the slopes of the linear regressions between (1) somatic mass and FL, (2) somatic mass or FL and reproductive performance measures, and (3) fecundity and individual egg mass for maiden and repeat spawners. Comparison of these slopes provided (1) an alternative analytical metric to fish shape (because with somatic mass, traditional measures like Fulton's condition factor are misleading) and (2) a method for determining how to standardize reproductive performance data for size, including whether to standardize by somatic mass or FL, along with enabling us to test hypotheses about reproductive performance trade-offs. The following ANCOVA models were used:

$$\gamma_{ij} = \alpha + \beta_1 D_j + \beta_2 X_{ij} + \beta_3 D_j X_{ij} + \varepsilon_{ij},$$

where γ_{ij} is the response variable, α is the intercept, β_1 is the group-specific effect of the spawning category, D_j is a dummy variable indicating the spawning category, β_2 is the slope given that $D_j = 0$, X_{ij} is the length, mass, or fecundity covariate, $\beta_3 D_j X_{ij}$ is the interaction term, and ε_{ij} is the random error.

The data conformed to the assumptions of ANCOVA (normality, homoscedasticity, and independence). In an analysis of FL versus somatic mass, the slopes did not differ significantly between maiden spawners of different years (ANCOVA: $F_{2,\,453} = 2.34799$, $P = 0.0967$) or between consecutive and skip repeat spawners ($F_{1,\,43} = 0.637243$, $P = 0.4291$), and thus the data were pooled as "maiden" and "repeat" spawners A significant interaction between the categorical (maiden, repeat) and continuous (somatic mass, total egg mass, individual egg mass, dry egg mass, fecundity) independent variables indicated a difference in slope between categories. Two 2014 maiden spawners (lengths, 65 and 90.5 cm) and one

2016 maiden spawner (mass, 5.88 kg) were excluded based on the ROUT outlier test (*Q* = 1.0%) from this and all further analyses, resulting in *n* = 459 for maiden, *n* = 25 for consecutive, and *n* = 22 for skip spawners. In the analyses of reproductive performance measures, 3 consecutive and 6 skip spawners were discovered to have an active bacterial infection in the body cavity at necropsy and were excluded from further analyses, resulting in *n* = 38 for repeat spawners. For the dry egg mass analysis, one 2014 consecutive spawner was excluded as an outlier, resulting in *n* = 37 for repeat spawners. Dry egg mass data were not available for one 2015 and two 2016 maiden spawners, resulting in *n* = 456 for maiden spawners.

Our initial intent was to compare reproductive performance between maiden and repeat spawning events in individual fish. However, we were unable to complete this analysis due to the mortality of many of our experimental fish. Therefore, the effects of pooled spawning category (maiden, consecutive, and skip) on somatic mass, FL, reproductive performance measures, and fertilization success were examined using univariate generalized linear models (GLMs) followed by Bonferroni-adjusted contrasts (JMP 13; SAS Institute, Cary, North Carolina). Because there were three contrasts between spawning categories, this resulted in a Bonferroni-adjusted alpha of 0.05 / 3 = 0.017. The following GLM models were used:

$$\gamma = \alpha + \beta_1 D_1 + \beta_2 D_2 + \varepsilon,$$

where γ is the response variable, D_1 and D_2 are binary dummy variables that encode the three levels of the categorical variable (maiden, consecutive, and skip spawners), α is the mean at the third level of the categorical variable, $\alpha + \beta_1$ is the mean at the first level of the categorical variable, $\alpha + \beta_2$ is the mean at the second level of the response variable, and ε is the random error. Repeat spawners experienced constant conditions in the reconditioning tanks, whereas the performance of maiden spawners within a given year may have been influenced by common river and ocean conditions experienced before spawning. We did not attempt to account for variation due to spawn year but instead assumed that the pooled maiden spawn years were representative of those for females typically spawned at DNFH. Reproductive performance data were analyzed both in absolute form and standardized to a standard body size. Standardization was performed to account for the significant positive relationships found between all reproductive performance measures and body size. Somatic mass was selected for standardization because the relationships (slopes) between somatic mass and reproductive performance measures differed to a lesser degree between maiden and repeat spawners than the relationships between FL and reproductive performance measures. To make the standardized data representative of the typical DNFH maiden spawner, standardization employed the slopes of the pooled regression relationships between somatic mass and the various reproductive performance measures. Thus, the standardized value = the original value for the individual − [(somatic mass of individual − 3.847 kg) × slope], where 3.847 kg is the pooled average somatic mass of a maiden spawner (Kinnison et al. 1998; Shimizu et al. 2009). Fertilization success data were arcsine-square-root transformed prior to analysis. Three consecutive spawner egg lots from 2014 froze and could not be fertilized. One maiden spawner egg lot was not collected in 2016. Fertilization success data were not size-standardized.

Spawn date fidelity was evaluated by subtracting the maiden spawning week from the repeat spawning week for individual fish, where week was enumerated beginning January 1 of each year. The difference between repeat and maiden spawning week was compared with zero using a one-sample *t*-test (https://www.graphpad.com/quickcalcs/OneSampleT1.cfm; GraphPad, La Jolla, California). Consecutive and skip repeat spawners were compared by means of a two-sample *t*-test. Unless otherwise indicated, all statistical analysis was conducted with PRISM 7.0 (GraphPad). Results are reported as significant when $P < 0.05$ or the Bonferroni threshold (0.017) as appropriate.

Ethics.—Fish care and sampling were conducted in accordance with a protocol reviewed and approved by the University of Idaho Animal Care and Use Committee.

Results

Body Size and Shape

Repeat spawners were significantly larger than maiden spawners in both somatic mass and FL (Table 1.1). Skip spawners had significantly greater mass than consecutive spawners. Linear regressions of somatic mass on FL showed significantly steeper slopes in repeat spawners than in maiden spawners (ANCOVA: $F_{1,\,502} = 55.7493$, $P < 0.0001$; Figure 1.1).

Gonadosomatic Relationships

Linear regressions of total egg mass, individual egg mass, and dry egg mass on somatic mass showed significantly steeper slopes in repeat spawners than in maiden spawners (ANCOVA: $F_{1,\,493} = 16.8596$, $P < 0.0001$; $F_{1,\,493} = 5.99037$, $P = 0.0147$; and $F_{1,\,487} = 6.96361$, $P = 0.0086$, respectively; Figure 1.2A–C). A nonsignificant tendency toward a steeper slope in repeat spawners was present when fecundity was regressed on somatic mass ($F_{1,\,493} = 2.32377$, $P = 0.1281$). Linear regressions of total egg mass, individual egg mass, dry egg mass, and fecundity on FL showed significantly steeper slopes in repeat than in maiden spawners ($F_{1,\,493} = 19.7526$, $P < 0.0001$; $F_{1,\,493} = 5.4403$, $P = 0.0201$; $F_{1,\,487} = 6.42565$, $P = 0.0116$; and $F_{1,\,493} =$

4.52329, $P = 0.0339$, respectively; Figure 1.3). The ratios between the repeat spawner and maiden spawner slopes were consistently smaller in the mass–reproductive performance relationships than in the length–reproductive performance relationships (total egg mass: 1.98 versus 2.66; individual egg mass: 1.93 versus 2.86; dry egg mass: 1.94 versus 3.33; and fecundity: 1.53 versus 2.02).

Reproductive Performance

Total egg mass was significantly greater in repeat spawners than in maiden spawners and was greater in skip spawners than in consecutive spawners (Table 1.2). Size-standardized total egg mass was greater in skip spawners than in maiden and consecutive spawners (Table 1.3).

Individual egg mass was significantly greater in skip spawners than in consecutive and maiden spawners (Table 1.2). Size-standardized individual egg mass was significantly greater in skip spawners than in maiden and consecutive spawners; it was also lower in consecutive than maiden spawners, though this difference was marginally nonsignificant ($P = 0.026$, compared with the Bonferroni-adjusted significance threshold of 0.017; Table 1.3).

Dry egg mass was significantly greater in repeat spawners than in maiden spawners, and greater in skip spawners than in consecutive spawners (Table 1.2). Skip spawners had significantly greater size-standardized dry egg mass than consecutive and maiden spawners (Table 1.3).

Fecundity was significantly greater in repeat spawners than in maiden spawners (Table 1.2). Fecundity was similar between groups when standardized for size; it was greater in consecutive than in maiden spawners, though the difference was marginally nonsignificant ($P = 0.029$, compared with the Bonferroni-adjusted significance threshold of 0.017; Table 1.3).

Linear regressions of size-standardized individual egg mass with size-standardized fecundity showed similar negative slopes in maiden and consecutive spawners (ANCOVA: $F_{1, 477} = 2.224$, $P = 0.1365$; Figure 1.6). The relationship had a similar trend, but there was a nonsignificant slope in skip spawners ($P = 0.0701$).

Fertilization success was significantly greater, on average, in maiden and consecutive spawners than in skip spawners (Figure 1.4).

Spawn Date Fidelity

Consecutive spawners, on average, spawned in the same week in which they spawned as maiden spawners (one-sample *t*-test: $t = 0.1970$, df = 24, $P = 0.8455$). Skip spawners spawned significantly earlier (by an average of 3 weeks) than they did as maiden spawners ($t = 5.7681$, df = 21, $P < 0.0001$). Spawn date fidelity differed significantly between consecutive and skip spawners (two-sample *t*-test: $t = 3.943$, df = 45, $P = 0.0003$; Figure 1.5).

Discussion

Body Size and Shape

Repeat spawners were longer, heavier overall, and heavier at a given length than maiden spawners. These differences are attributable to differing schedules of acquisition and allocation of energy stores. In this population of summer-run steelhead, maiden spawners fast during the 6- to 9-month freshwater period prior to spawning (Penney and Moffitt 2014a) and allocate somatic energy to maintenance, survival, migration, and ovarian growth. In contrast, repeat spawners operate off a steady energy income (satiation feeding) during the entire year prior to spawning and are not required to invest stored energy in migration. Following maiden spawning, both the consecutive and skip spawners in our study would have been severely energy depleted. The substantially (1.35-fold) greater somatic mass of consecutive versus maiden spawners shows that they were able to overcome this deficit and invest additional energy into somatic growth while allocating considerable energy to ovarian growth. Repeat spawners had 46% greater somatic mass than maiden spawners on average, although they were only 5% longer; they were, however, thicker in shape (*i.e.*, had greater mass per length), similar to captive Atlantic Salmon kelts after reconditioning, where repeat spawners were 53% heavier but only 4.2% longer (Moffett et al. 1996). In contrast, wild, repeat spawning Miramichi River (New Brunswick) Atlantic Salmon increased more in length, with skip spawners (21%) and consecutive spawners (8%) being greater in length than two-sea-winter maiden spawners (Chaput and Benoit 2012), and ocean-reconditioned consecutive spawning Sacramento River steelhead increased 13.4% in length (Null et al. 2012), suggesting that captive and ocean reconditioning produce different growth patterns.

Somatic Size and Reproductive Performance Relationships

The relationships between somatic mass and the reproductive performance metrics were more similar than the relationships between FL and the reproductive performance metrics when compared between maiden and repeat spawners. This is likely due to the difference in body shape that emerged between maiden and repeat spawners. Although reproductive performance measures (*i.e.*, fecundity, egg size) are often correlated with length in salmonids, including *O. mykiss* (Crespi and Teo 2002; Quinn et al. 2011), this is likely due to the greater availability of length data in fisheries data sets. Our results, however, indicate that somatic mass is more closely related to reproductive performance measures and is likely a better indicator of energy stores than is length; this is consistent with previous analyses investigating relationships between fecundity and body size in fish (Koops et al. 2004).

Reproductive Performance Standardized for Size

Differences between spawning categories in size-standardized reproductive performance measures suggest that reproductive investment, egg size, and fecundity were modulated by energy reserves over the year before spawning. Size-standardized total egg mass, individual egg mass, and dry individual egg mass were greater in skip spawners than in maiden and consecutive spawners. Individual egg mass was 5% lower in consecutive spawners than in maiden spawners, with the marginal non-significance likely being due to low sample size. Dry egg mass followed a similar pattern (1% lower on average). Similarly, consecutive spawning wild Atlantic Salmon in the Miramichi River had smaller-diameter eggs than did maiden and skip spawners when standardized by length, and skip spawners had larger-diameter eggs than maiden or consecutive spawners (Reid and Chaput 2012). The beginning of the fasting spawning migration of summer-run steelhead coincides with the onset of vitellogenesis (Pierce et al. 2017), and thus the vast majority of energy transfer from the soma to the ovary takes place after migration begins (Lubzens et al. 2010). There was a strong trend toward reduction in size-standardized egg size in consecutive spawners compared with maiden spawners, even though consecutive spawners were fed to satiation during vitellogenesis while maiden spawners fasted. In addition, although both consecutive and skip spawners were fed to satiation during vitellogenesis, resulting in increased somatic size in both groups, size-standardized egg size differed. These results suggest that egg size is not sensitive to energy intake during vitellogenesis. Skip spawners would have had substantially greater energy reserves than consecutive spawners, and likely maiden spawners as well, during the period around the initiation of rematuration approximately 1 year before spawning. This is because skip spawners would have completely recovered from their maiden migration and spawning, whereas consecutive spawners would be maximally energy depleted, and the growth of maiden spawners would be reduced during the winter (Burgner et al. 1992). This resulted in a substantial increase in reproductive investment, measured as total egg mass, in skip spawners: 20.0% versus maiden spawners and 14.3% versus consecutive spawners, compared with only a 5% increase in consecutive versus maiden spawners. Thus, we hypothesize that reproductive investment and egg size are set by energy reserves early in the secondary oocyte growth period, soon after the proposed timing of the initial maturation commitment (Bromage et al. 1992; Campbell et al. 2006a). Similarly, energy investment into egg production appears to be determined early in Atlantic Cod *Gadus morhua*, as surplus energy during vitellogenesis has been shown to be invested in somatic growth rather than egg production (Yoneda and Wright 2005). In Rainbow Trout, removal of one ovary before the beginning of vitellogenesis resulted in complete recovery of the gonadosomatic index by hypertrophy of the remaining ovary (Tyler et al. 1994). The physiological mechanisms underlying this “set point” for reproductive investment are not yet clear.

Size-standardized fecundity was 8.7% higher in consecutive spawners and 8.0% in skip spawners than in maiden spawners. This indicates that changes in reproductive effort were due to modulation of both offspring number and the energy invested into individual offspring through the processes of vitellogenesis. The increase in fecundity in consecutive spawners was offset by a decrease in egg size, whereas skip spawners exhibited increases in both fecundity and egg size. This suggests that fecundity is set first in the allocation of reproductive effort and egg size is adjusted to fecundity. This is consistent with laboratory studies suggesting that fecundity is set early in ovarian development in Rainbow Trout (Tyler et al. 1990; Bromage et al. 1992). Similarly, a field study on hatchery-origin, winter-run steelhead found that size-standardized egg size—but not fecundity—differed between life history types (*i.e.*, two-sea-winter and three-sea-winter maiden spawners; (Quinn et al. 2011), as did the previously mentioned study on Atlantic Salmon (Reid and Chaput 2012) with respect to the size-standardized egg size and fecundity of maiden, consecutive, and skip spawners. However, these results are not consistent with theoretical predictions that fecundity will be adjusted to produce optimum-sized eggs (Sargent et al. 1987). In contrast to these results, reconditioned Atlantic Salmon kelts showed lower mean fecundity in repeat spawners but had similar egg diameter (Moffett et al. 1996). Juvenile growth in freshwater has been proposed to shift the egg size–fecundity trade-off in salmonids (Thorpe et al. 1984; Morita et al. 1999). All DNFH steelhead are raised under constant conditions and grow rapidly before being released as 1-year-old smolts. Thus, the variation in egg size found in the present study suggests that there is considerable plasticity in egg size independent of juvenile life history.

Absolute Reproductive Performance

Fecundity was greater in repeat spawners (24% in consecutive spawners and 30% in skip spawners) than in maiden spawners, suggesting that reconditioned kelts have the potential to produce more offspring than maiden spawners. Individual egg mass was significantly (15%) greater in skip spawners than in maiden and consecutive spawners. Despite size-standardized egg size being lower in consecutive spawners than in maiden spawners, absolute egg size was similar. In salmonids, larger egg size is expected to lead to larger juveniles that survive at higher rates (Crespi and Teo 2002). Larger eggs are considered “well-provisioned,” with greater energy reserves that result in a survival advantage and larger size and gape at first feeding that result in a competitive advantage. The benefit of larger egg size increases in environments of decreasing quality (Rollinson and Hutchings 2013). The benefit of egg size is difficult to quantify without a thorough understanding of the contextual ecology and habitat (substrate size, conspecifics, nutrient availability for juveniles, etc.). Total egg mass increased with spawning iteration and interval when compared among groups in the absolute form. This measure of reproductive performance integrates egg size and fecundity and provides a reasonable means of quantifying the productivity of consecutive and skip spawning reconditioned kelts; consecutive and skip spawners had 1.28 and 1.52 times the total egg mass of maiden spawners, respectively.

Fertilization Success

Maiden and consecutive spawners had significantly greater fertilization success, exceeding 80% on average, than skip spawners, which is consistent with the mean survival to the eyed stage in *O. mykiss* reported as early as 1953 (Bromage et al. 1992). When fertilization success is below 80%, *O. mykiss* spawners are referred to as "subfertile" (Stoddard et al. 2005). Subfertility was found in 50% of skip spawners, which significantly lowered the average for that group despite its having wide variation. Subfertility was also found in maiden spawners (20% in 2014, 36% in 2015, and 4% in 2016) and in consecutive spawners (10% for all years combined). Skip spawning is a normal life history pattern for *O. mykiss*, and there is no reason to associate subfertility with skip spawning as a life history pattern. Rather, it is likely that reduced fertilization success resulted from the misidentification of ripeness and/or undetected infection. Manual stripping of ovulated eggs can cause modest to significant reduction in fertilization success in the days following ovulation, particularly as the number of days postovulation and water temperature increase (Bromage et al. 1992). Stripping a fish too early was a concern as well, due to the inability to quantify unovulated eggs. Atlantic Salmon reconditioning efforts in Canada have reported high fertilization success (93.7–95.6%; (Crim et al. 1992). Survival of fry from repeat spawners (64.5%) to 780 d after fertilization was similar to that of fry of maiden spawners (72.3%) (Moffett et al. 1996). More recently, wild

Atlantic Salmon offspring survival to "just before hatching" was slightly reduced (88.3%) in consecutive spawners compared with maiden and skip spawners (94%), which were similar to each other (Reid and Chaput 2012).

Spawn Date Fidelity

Skip spawners spawned earlier, on average, than their initial spawning dates and earlier than consecutive spawners. The earlier spawning, however, was not outside the average range of spawning for maiden spawners. Skip spawners migrated earlier than maiden spawners in wild Atlantic Salmon, consistent with potentially earlier spawning (Niemelä et al. 2006). In naturally repeat spawning Columbia River summer-run steelhead, skip spawners were detected earlier and consecutive spawners later than peak maiden spawner detection timing at Bonneville Dam, although this was long before spawning (Keefer and Caudill 2014).

Conclusions

In a controlled laboratory setting, it was previously established that fecundity is set prior to the onset of vitellogenesis in rainbow trout (Tyler et al. 1990). The size-standardized results from this study suggest that egg size is also set early in oogenesis, as consecutive spawners, forced to allocate greater quantities of energy to somatic recovery following spawning, showed smaller egg size despite having access to energy throughout the entire oogenic period. Fish in all groups maintained or increased fecundity while adjusting egg size. This suggests that fecundity is decoupled from physiologically perceived energy stores during early oogenesis and that spawners in this steelhead population modulate reproductive performance by adjusting investment into individual offspring rather than adjusting the number of offspring they develop, at least within the range of egg sizes found in the current study.

Management Implications

Repeat spawners are important to steelhead populations (Moore et al. 2014), increasing their stability during bottleneck years because having multiple cohorts available to interbreed spreads the risk of poor ocean or river effects. Releasing captive-reconditioned wild steelhead, particularly ones with larger eggs, more eggs, and greater total egg mass per fish, will increase the productivity of steelhead populations. Larger eggs are increasingly beneficial in variable and poor-quality environments, and more eggs would be expected to increase the numbers of adult offspring. Assuming reproductive effort is allocated appropriately between fecundity and egg size, the increase in total egg mass provides a means of quantifying the productivity increase of consecutive and skip spawners (1.28- and 1.52-fold over that of maiden spawners, respectively). Spawn timing was not substantially changed by reconditioning, suggesting that after release reconditioned steelhead will be able to find mates and spawn at a time that is appropriate for the population. Optimal feeding conditions during the period after capture would be expected to result in the maximal reproductive performance of kelts released to spawn. Conversely, continued feeding of the fish into the fall does not appear to translate into increased reproductive performance, but it could be used to increase the somatic energy stores required for migration.

Future Work

In general, more data are needed on skip spawners for this steelhead population. This is particularly true for fertilization success, but for all factors our understanding would be enriched by more detailed studies on how reproductive performance is regulated. This is challenging data to collect, however, and requires maintaining large adult fish in captivity for 1 to 2 years.

Tracking physiological factors such as growth and condition over the year after maiden spawning and the year before repeat spawning in both consecutive and skip spawners will allow a deeper look at the trends we observed in this study. Analysis of physiological factors indicating energy stores, condition, and growth up to and after spawning would provide insight into when reproductive decisions are made. Additionally, intentionally restricting feed in hatchery-origin kelts at different time periods and assessing its effect on life history, reproductive performance, and body size would deepen our understanding of when and how energy reserves influence reproductive development.

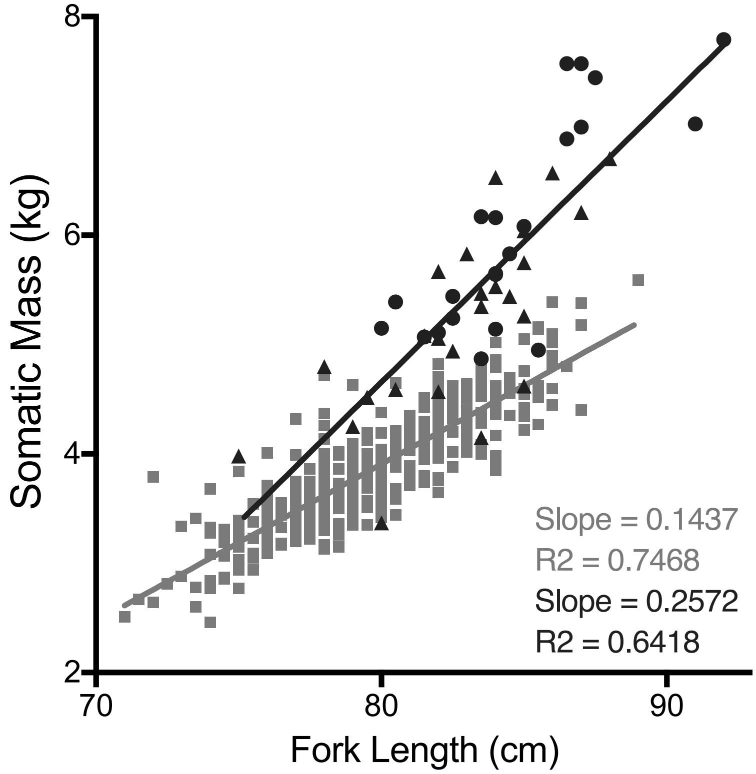

FIGURE 1.1. Relationships between fork length and somatic mass in female steelhead from the Clearwater River, Idaho, spawned in 2014–2016. Fish were classified as maiden (gray squares, *n* = 459), consecutive (black triangles, *n* = 25), or skip spawners (black circles, *n* = 22). The combined regression result for repeat (consecutive and skip) spawners is indicated by the black line, that for maiden spawners by the gray line. The slopes for maiden and repeat spawners differed significantly (ANCOVA: $F_{1,\,502} = 55.7493$, $P < 0.0001$).

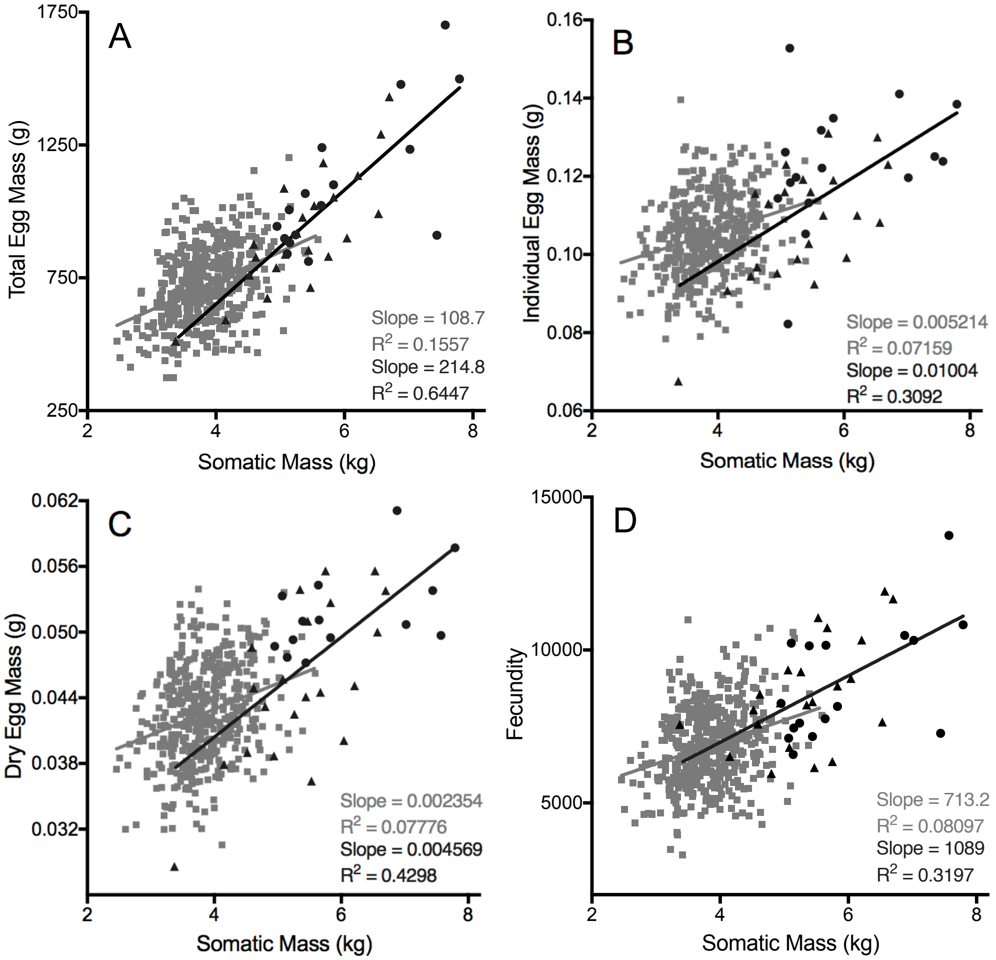

FIGURE 1.2. Relationships between somatic mass and **(A)** total egg mass, **(B)** individual egg mass, **(C)** dry egg mass, and **(D)** fecundity in spawning female steelhead. Fish were classified as maiden (gray squares, *n* = 459 in panels [A], [B], and [D], 456 in panel [C]), consecutive (black triangles, *n* = 22 [21]), or skip spawners (black circles, *n* = 16 [14]). The slopes for maiden and repeat spawners differed significantly in panels (A), (B), and (C) (ANCOVA: $F_{1, 493} = 16.8596$, $P < 0.0001$; $F_{1, 493} = 5.99037$, $P = 0.0147$; $F_{1, 487} = 6.96361$, $P = 0.0086$, respectively), but not in panel (D) ($F_{1, 493} = 2.32377$, $P = 0.1281$).

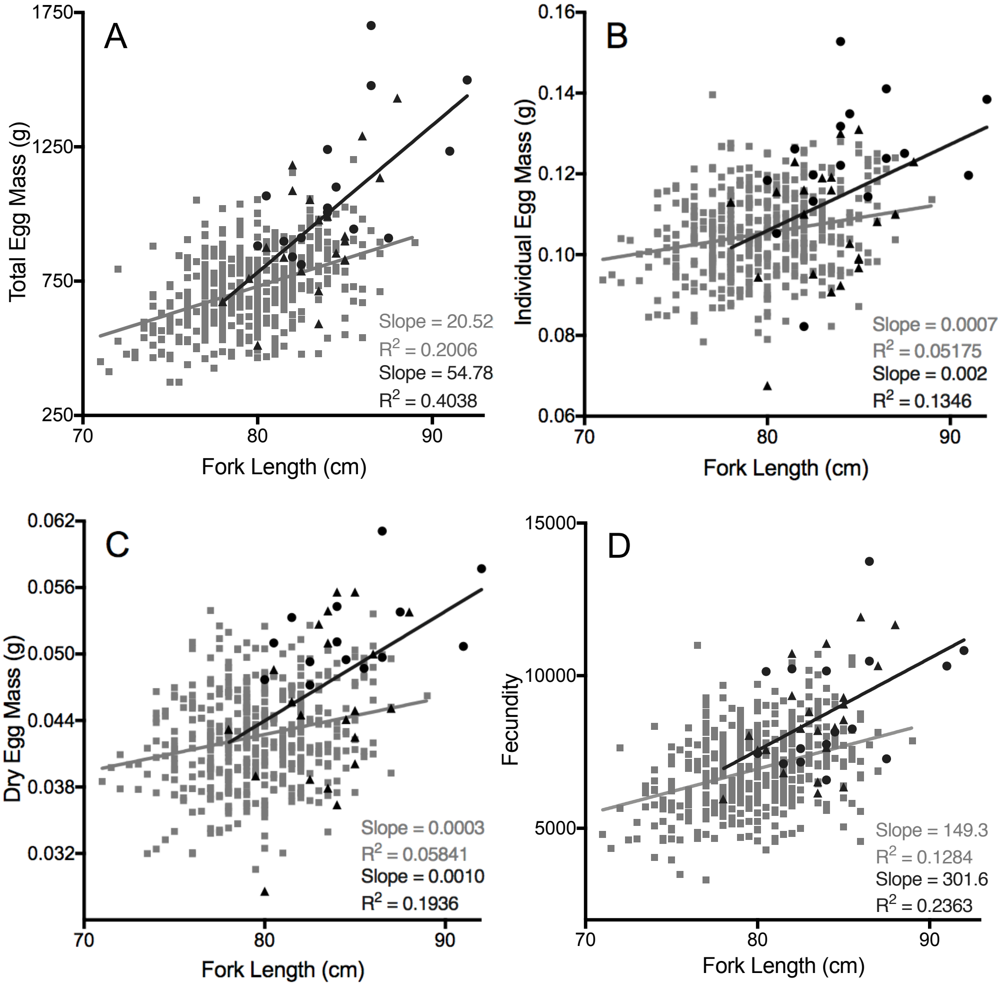

FIGURE 1.3. Relationships between fork length and **(A)** total egg mass, **(B)** individual egg mass, **(C)** dry egg mass, and **(D)** fecundity in maiden and repeat spawning female steelhead. Fish were classified as in Figure 2. The slopes for maiden and repeat spawners differed significantly for all relationships (ANCOVA: $F_{1,\,493} = 19.7526$, $P < 0.0001$; $F_{1,\,493} = 5.4403$, $P = 0.0201$; $F_{1,\,487} = 6.42565$, $P = 0.0116$; $F_{1,\,493} = 4.52329$, $P = 0.0339$ in panels [A]–[D], respectively).

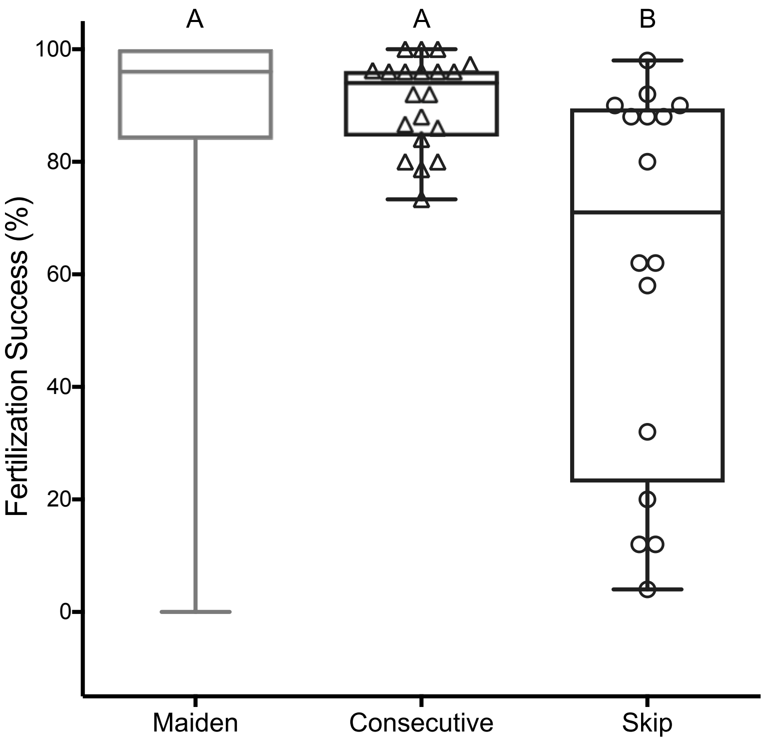

FIGURE 1.4. Fertilization success in female steelhead from the Clearwater River, Idaho, spawned in 2014–2016. Fish were classified as maiden (*n* = 458), consecutive (*n* = 20), or skip spawners (*n* = 16). The box heights indicate the interquartile ranges, the horizontal lines within them indicate the medians, and the whiskers show the data ranges. All points are shown for repeat spawners. Groups with different lowercase letters are significantly different (GLM followed by contrasts).

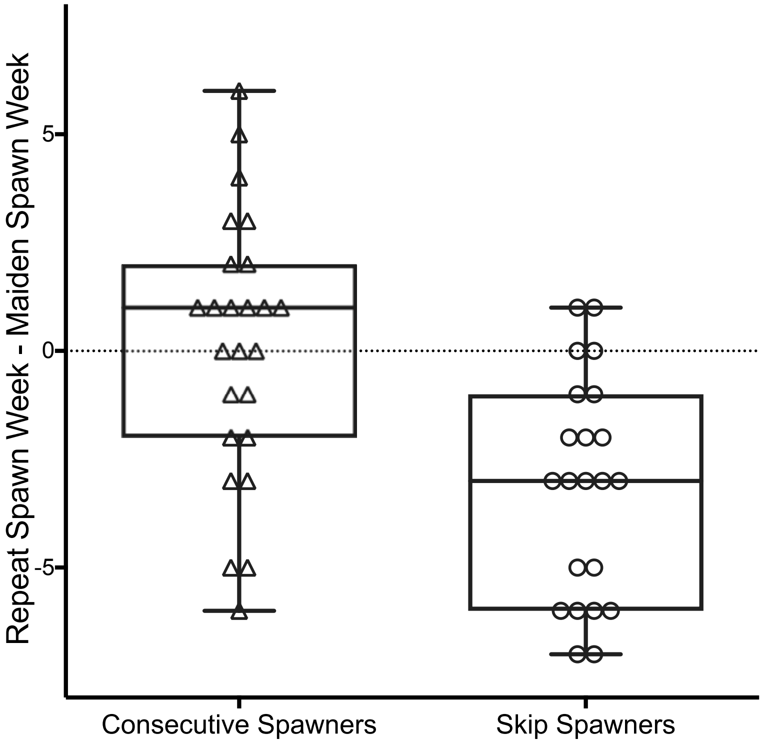

FIGURE 1.5. Spawn week fidelity in repeat spawning female steelhead from the Clearwater River, Idaho, spawned in 2013–2016. Fish were grouped as consecutive ($n = 25$) or skip spawners ($n = 22$). The box heights indicate the interquartile ranges, the horizontal lines within them indicate the medians, and the whiskers show the data ranges; all points are shown. The repeat spawn week did not differ significantly from the maiden spawn week in consecutive spawners (one-sample *t*-test: $t = 0.1970$, df = 24, $P = 0.8455$) but was significantly earlier in skip spawners ($t = 5.7681$, df = 21, $P < 0.0001$). The repeat spawn week was significantly earlier in skip spawners than in consecutive spawners (two-sample *t*-test: $t = 3.943$, df = 45, $P = 0.0003$).

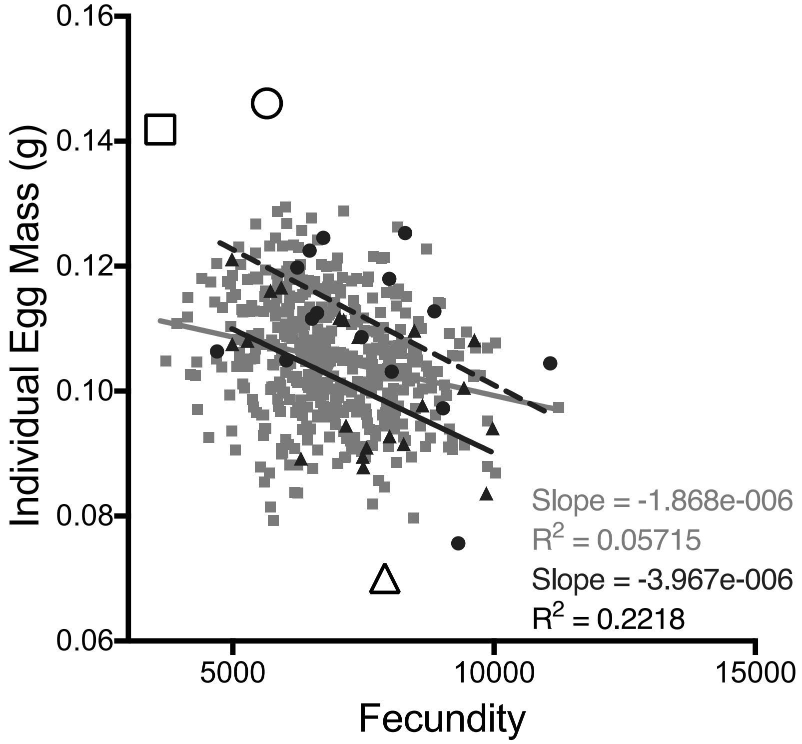

FIGURE 1.6. Relationship between fecundity and individual egg mass in female steelhead from the Clearwater River, Idaho, spawned in 2014–2016. Females were grouped as maiden (gray squares, *n* = 459), consecutive (black triangles, *n* = 22), or skip spawners (black circles, *n* = 16). The negative relationship between fecundity and egg mass was significant for maiden (gray line, $P < 0.0001$) and consecutive spawners (black line, $P = 0.0168$) but not for skip spawners (line not shown, $P = 0.0701$). The slopes for maiden and consecutive spawners did not differ significantly (ANCOVA: $F_{1, 477} = 2.224$, $P = 0.1365$). The exclusion of three outliers (enlarged open shapes) did not change the significance of the relationships.

TABLE 1.1. Somatic mass and fork length (FL) of female steelhead from the Clearwater River, Idaho. Mass and length were examined using univariate GLM, followed by contrasts of spawning categories (maiden, consecutive, and skip). Groups sharing a letter do not differ significantly. For repeat spawners, references to year refer to the year of repeat spawning rather than the year of maiden spawning. Combined data for all repeat spawners are listed for reference but were not included in the statistical analysis.

		Somatic mass (kg)		FL (cm)	
Spawning category	*n*	Mean	SD	Mean	SD
Maiden (all)	459	3.847 x	0.512	79.60 y	3.089
2014	146	3.721	0.512	79.07	3.105
2015	150	3.985	0.555	80.50	3.178
2016	163	3.835	0.436	79.22	2.777
Consecutive (all)	25	5.211 y	0.853	82.76 z	2.941
2014	11	5.395	0.922	82.27	3.849
2015	2	5.280	1.075	82.25	3.889
2016	12	5.031	0.800	83.29	1.852
Skip (all)	22	6.052 z	0.977	84.75 z	3.011
2015	20	6.104	1.012	84.90	3.114
2016	2	5.540	0.141	83.25	1.061
Repeat (all)	47	5.605	0.998	83.69	3.108

TABLE 1.2. Reproductive performance across life history groups in female steelhead from the Clearwater River, Idaho. Total egg mass, individual egg mass, dry egg mass, and fecundity were examined using univariate GLM, followed by independent contrasts of spawning categories (maiden, consecutive, skip). Indications of significance, references to year, and combined repeat spawner data are as in Table 1.1.

Spawning category	*n*	Total egg mass (g)		Individual egg mass (g)		Dry egg mass [a] (g)		Fecundity (number of eggs)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Maiden (all)	459	722.2 x	141.0	0.105 y	0.010	0.0426 x	0.0043	6,887 y	1,283
2014	146	665.3	128.8	0.101	0.001	0.0401	0.0041	6,612	1,149
2015	150	702.7	123.4	0.107	0.009	0.0433	0.0039	6,620	1,218
2016	163	791.3	138.9	0.108	0.001	0.0442	0.0039	7,378	1,315
Consecutive (all)	22	921.0 y	221.4	0.108 y	0.0151	0.0453 y	0.0071	8,557 z	1,753
2014	8	1,049.0	228.3	0.119	0.0084	0.0501	0.0056	8,858	2,069
2015	2	830.0	99.0	0.097	0.0034	0.0400	0.0008	8,559	726
2016	12	851.1	202.1	0.102	0.0155	0.0435	0.0072	8,355	1,727
Skip (all)	16	1,097.0 z	264.9	0.123 z	0.0161	0.0515 z	0.0067	8,953 z	1,944
2015	14	1,122.0	271.3	0.123	0.0168	0.0520	0.0039	9,166	1,989
2016	2	917.0	148.9	0.123	0.0132	0.0508	0.0050	7,463	414
Repeat (all)	38	994.9	253.0	0.114	0.0171	0.0480	0.0068	8,724	1,821

[a] For this variable, *n* = 149 for maiden spawners in 2015 and 161 for maiden spawners in 2016; therefore, for all maiden spawners, *n* = 456. For consecutive spawners in 2014, *n* = 7, such that for all repeat spawners *n* = 37.

TABLE 1.3. Size-standardized reproductive performance across life history groups in female steelhead from the Clearwater River, Idaho. Data were standardized to the average somatic mass of a maiden spawner (3.847kg) using the linear regression of each metric from combined maiden spawner data. Reproductive performance measures were analyzed using univariate GLM, as in Table 1.2. Indications of significance, references to year, and combined repeat spawner data are as described in Table 1.1.

	N	Total egg mass (g)		Individual egg mass (g)		Dry egg mass [a] (g)		Fecundity (number of eggs)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Maidens (all)	459	722.1 y	129.6	0.1051 y	0.0096	0.04260 y	0.00414	6,886	1,230
Maiden 2014	146	679.1	122.7	0.1014	0.0094	0.04044	0.00398	6,703	1,119
Maiden 2015	150	687.6	105.6	0.1059	0.0089	0.04301	0.00371	6,521	1,128
Maiden 2016	163	792.5	126.5	0.1078	0.0094	0.04419	0.00384	7,386	1,256
Consecutives (all)	22	758.1 y	157.9	0.1001[b], y	0.0128	0.04179 y	0.00600	7,485[c]	1,515
Consecutive 2014	8	833.4	190.3	0.1088	0.0075	0.04519	0.00480	7,444	1,884
Consecutive 2015	2	673.7	18.3	0.0894	0.0022	0.03619	0.00175	7,530	45.57
Consecutive 2016	12	721.9	132.8	0.0961	0.0135	0.04075	0.00615	7,505	1,456
Skips (all)	16	866.5 z	196.5	0.1121 z	0.0152	0.04655 z	0.00636	7,440	1,629
Skip 2015	14	885.6	200.0	0.1119	0.0160	0.04652	0.00671	7,610	1,675
Skip 2016	2	732.3	133.5	0.1137	0.0124	0.04678	0.00469	6,249	312.8
Repeats (all)	38	803.7	180.9	0.1051	0.0149	0.04385	0.00651	7,466	1,542

[a] See note a to Table 1.2.

[b] Although individual egg mass was lower for consecutive spawners than for maiden spawners, this difference was marginally nonsignificant ($P = 0.026$, versus the Bonferroni-adjusted threshold of 0.017).

[c] Although fecundity was greater for consecutive spawners than for maiden spawners, this difference was marginally nonsignificant ($P = 0.029$, versus the Bonferroni-adjusted threshold of 0.017)

CHAPTER 2

Elevated plasma triglycerides and mass growth are early signals of rematuration in iteroparous female steelhead

Introduction

Skipped reproduction occurs when an iteroparous animal remains reproductively inactive through a potential breeding cycle. Skipped reproduction is best characterized in poikilothermic vertebrates, where it is often associated with energetically demanding accessory activities that accompany reproduction, such as migration, brooding, or live bearing (Bull and Shine 1979). Skipped reproduction is also observed in birds (Goutte et al. 2010; Crossin et al. 2012; Reed et al. 2015), and analogous phenomena such as delayed and skipped estrous are found in mammals (Frisch and Revelle 1970; Early et al. 1999; Jones and Lubbers 2001), which are primarily thought to be caused by insufficient metabolic fuels (Wade and Schneider 1992; Bronson 1995; Erikstad et al. 1998). Reproduction and associated activities are highly energetically costly (Stearns 1992; Bronson 1995) and may result in severe depletion of energy reserves (Wade and Schneider 1992). Thus, skipped reproduction could result from the energetic cost of the previous breeding event.

Skipped reproduction is common in seasonally breeding, iteroparous, egg laying fishes, where it is referred to as skipped spawning (Rideout and Tomkiewicz 2011). Individuals within a population may spawn at one-year intervals (consecutive spawners) or skip one or more years (skip spawners). Many of the fish species that exhibit skip spawning are so-called “capital breeders” that fund reproduction from energy stores acquired prior to the majority of reproductive investment (McBride et al. 2015). This has resulted in the idea of a threshold level of energy reserves required to successfully complete gonadal development, spawning, and associated activities such as migration. In this “reaction norm” framework, an individual’s “condition,” or level of energy reserves, interacts with a genetically determined threshold to generate a decision to either initiate maturation (or rematuration) or to remain reproductively inactive for the given reproductive cycle (Hutchings 2011). The threshold is subject to selection, providing a life history theory mechanism that explains the plasticity in size and age at maturity found in many fish species as adaptive (Bull and Shine 1979; Thorpe 2007; Taranger et al. 2010; Hutchings 2011). However, the physiological mechanisms through which energetic status influences reproductive decisions in fishes are not fully understood.

The critical period hypothesis of maturation was developed for Atlantic salmon, and proposes that the initial maturation decision takes place during a seasonally defined critical period approximately one year before spawning, and is permissively gated by stores of metabolic fuels (Thorpe et al. 1998; Thorpe 2007). The window for initiation of maturation is thought to be associated with transitions in early secondary oocyte growth in females (Campbell et al. 2006b; Lubzens et al. 2010; Taranger et al. 2010; Grieshaber et al. 2016). The aspect of metabolic fuel storage that gates entry into a reproductive cycle is not known but is functionally defined as either the absolute level or the rate of change of body size (growth) and/or lipid reserves (Thorpe 2007; Taranger et al. 2010). Supporting the potential role of growth in the maturation decision, studies in salmonids suggest that the growth regulatory hormone insulin-like growth factor-1 (IGF-1) may stimulate the initiation of maturation (Baker et al. 2000; Taylor et al. 2008; Taranger et al. 2010; Wilkinson et al. 2010). Lipid reserves are also thought to be of prime importance, although the signaling networks involved are not yet clear in fishes (Taranger et al. 2010). Triglycerides are the primary form in which lipids are stored for energy (Sheridan 1994). Of the circulating lipids: triglycerides, lipoproteins (*i.e.*, vitellogenin for yolk formation in the oocyte), non-esterified fatty acids, and cholesterol, triglycerides are a reasonable representation of lipids available for energy use in fish. Nevertheless, to our knowledge, the timing of the maturation decision window and the relative importance of lipid reserves and growth in the initiation of maturation have not been precisely delineated in any species. In addition, maturation decisions in fishes have been most studied in the context of puberty. Although it is reasonable to assume that similar mechanisms operate during rematuration as in puberty, the impact of energy depletion from the previous spawning event on rematuration decisions and reproductive development could be unique and have not been examined.

Steelhead provide a model species for studying the reproductive decisions underlying consecutive and skip spawning. Steelhead spawn in the spring in cold freshwater streams, the resulting juveniles migrate to the ocean to feed and grow, and maturing adults then return to their natal stream to spawn (Burgner et al. 1992; Quinn 2005). Unlike the closely related semelparous Pacific salmon species, steelhead are iteroparous (Burgner et al. 1992; Quinn 2005). Individuals of steelhead populations can display a diverse suite of life histories, including both freshwater resident and anadromous forms, variation in size and age at seaward migration, variation in size and age at initial maturation, and consecutive and skip repeat spawning (Nielsen et al. 2011; Courter et al. 2013; Moore et al. 2014). In coastal “winter run” steelhead populations, adults return to freshwater with fully developed gonads in the winter or early spring shortly before spawning, whereas in “summer run” populations such as those in the interior Columbia River Basin, adults enter freshwater with immature gonads in late summer and complete gonadal development while fasting and migrating to spawning areas to spawn the following spring (Quinn et al. 2016). Summer run steelhead are thus capital breeders, with the energetic demands of migration, gonadal development, and spawning resulting in an extreme energy deficit and high post-spawning mortality (Penney and Moffitt 2014b). Reconditioning of post-spawning female steelhead (kelts) has been developed as a restoration tool for populations of interior summer run Columbia River Basin steelhead listed as threatened under the U.S. Endangered Species Act (Hatch et al. 2013; Trammell et al. 2016). Kelts are captured after spawning, held and fed in freshwater, and then released to spawn again. The consecutive and skip spawning life histories are observed in reconditioned kelts, varying in proportion by location and year (Hatch et al. 2016; Pierce et al. 2017).

The objectives of this chapter were to determine when the rematuration decision occurs, to determine how growth rates and measures of lipid reserves relate to the rematuration decision, and to assess the impact of recovery from maiden spawning on rematuration in female steelhead. To facilitate this study an experimental system using non-ESA-listed, hatchery-origin summer run steelhead returning to DNFH was used as described in Chapter 1.

Materials and Methods

*Fish.—*Female steelhead were captured after returning on their maiden spawning migration at DNFH as described in Chapter 1. In February-April 2015 and 2016, N=150 and 164 females, respectively, were spawned, selected for this study, and individually tagged as described in Chapter 1. Fish husbandry was as described in Chapter 1.

*Sampling.—*At the time of maiden spawning and at 10-week intervals thereafter, fish were sampled for FL and wet mass, as described in Chapter 1, muscle lipid level (ML, %), and blood. Mass at maiden spawning was taken after eggs were removed, was corrected for any residual eggs remaining in the body cavity and is referred to as somatic mass as described in Chapter 1. Subsequent measurements of body mass included any new ovarian growth. ML was measured by microwave energy meter (Fish Fatmeter model 692, Distell Inc., West Lothian, UK) using the Trout-1 setting, as previously validated for rainbow trout (Caldwell et al. 2013). Blood (3mL) was taken from the caudal vein using a heparinized (ammonium heparin, 10 mg/mL, Sigma-Aldrich, St. Louis, MO) 20 gauge, 3.8cm needle and syringe. Blood was centrifuged at 8300G for 5 min. The plasma was removed, frozen on dry ice in the field, and then later stored at -80ºC. Sampling continued at 10-week intervals until spawning occurred again approximately 1 year (50 weeks) later for consecutive spawning 2015 fish, or until approximately 1 year plus 30 weeks after spawning for 2015 fish that skipped spawning, or until 30 weeks after spawning for 2016 spawn year fish.

Survival.—Mortality occurred during reconditioning as expected for steelhead kelts (Hatch et al. 2013). On average, 55% of mortality occurred within 10 weeks of spawning (47% and 62% for 2015 and 2016, respectively). Survival to 30 weeks after spawning was 29% in 2015 (43/150 fish), 18% in 2016 (30/163), 70% for 2015 skip spawners from 30 weeks after maiden spawning to 1 year plus 30 weeks (21/30), and 84% for 2015 skip spawners from 1 year after spawning to 1 year plus 30 weeks (21/25). A necropsy was performed on each mortality. In November 2016, approximately 35 weeks after maiden spawning of the 2016 spawn fish and 15 weeks prior to repeat spawning of the 2015 skip spawners and 2016 consecutive spawners, all fish died due to an equipment malfunction.

Assays.—Plasma estradiol-17*β* (E2) levels were measured by ELISA (Biosense, Cayman Chemical, Ann Arbor, MI). Steroids were extracted from plasma using ether extraction, re-suspended in assay buffer, and assayed in triplicate. Intra- and inter- assay %CV were 8.0% was 7.1%, respectively. Plasma triglyceride (TG) levels were measured using a

VetTest (Idexx, Westport, ME), according to validation for use in *Oncorhynchus* spp. (Meador et al. 2006).

Morphometric Analysis.—Fulton's condition factor (K) was calculated as:

$$K = 100 * \text{body mass (g)} * (\text{fork length (cm)})^{-3}$$

Mass specific growth rate (MSGR) was calculated as:

$$\% \text{ body mass gain} * \text{day}^{-1} = 100 * (\ln(\text{body mass final}) - \ln(\text{body mass initial}))\text{days}^{-1}$$

Length specific growth rate (LSGR) was calculated in the same manner as that for mass.

Statistical Analysis.— Rematuration status was assigned in early autumn, 30 weeks after spawning, based on two E2 concentration groups (high levels = maturing, low levels = not maturing), and confirmed by spawning of survivors, or at necropsy for pre-spawn mortalities by examining developing ovaries for large oocytes as described in Chapter 1. Based on these results, fish were categorized as either rematuring and non-rematuring and were compared in a time series at 10-week intervals starting at spawning. Additionally, fish rematuring either one or two years after maiden spawning were categorized as either consecutive spawning or skip spawning fish, respectively, and compared at the same relative time points during the year prior to repeat spawning.

E2 levels were $\log_{10}$-transformed and ML levels were arcsine square root-transformed prior to analysis. Two-way ANOVA was employed to test for significant effects of maturation group, time, and group*time interaction on E2 levels, MSGR, LSGR, TG levels, and K. Where significant effects were found, one-way ANOVA was used to assess the effects of time and maturation group, followed by Tukey's HSD or a Two-sample T-test. A Kruskal-Wallis test was used to analyze ML data due to truncation of the data, followed by Dunn's multiple comparison test or the Mann-Whitney test. The Rout Outlier Test was used to detect and remove outliers. Unless otherwise indicated, all statistical analysis was conducted with

PRISM software version 7.0 (GraphPad Inc., La Jolla, CA). Results are reported as significant when $P < 0.05$.

*Ethics.—*Fish care and sampling were conducted in accordance with a protocol reviewed and approved by the University of Idaho Animal Care and Use Committee.

Results

Rematuration

The proportion of survivors to 30 weeks after maiden spawning that matured in consecutive years was 30% (13/43) in 2015 and 40% (12/30) in 2016. Of 2015 fish that survived 1 year plus 30 weeks after spawning, and did not spawn in consecutive years, 86% (18/21) were rematuring as skip spawners in 2016. No evidence of arrested maturation (*i.e.*, premature decreases in E2) was detected in any individual. However, one of the 30 non-rematuring 2015 fish showed a distinctly non-representative negative trajectory starting in early summer (20 weeks post-spawn), followed by death 1 year after spawning, and was excluded from the data.

Time Course of Recovery from Spawning

Two-Way ANOVA found significant effects of maturation group, time, and group*time interactions, with the exception of significant group*time interaction effects for LSGR (both years) and MSGR in 2016 (Table 2.1).

TG level was greater in rematuring than non-rematuring fish at 10 weeks in both years, remaining that way with the exception of week 20 in 2016 and week 50 in 2015 (Figure 2.1). TG increased in rematuring fish from 10 to 30 weeks in both years. In non-rematuring fish, TG decreased from 0 to 10 weeks (2015: 1.8-fold; 2016: 1.8-fold). After the 10-week time point, TG in non-rematuring fish returned to maiden levels at 20 (2016) or 30 (2015) weeks and for all following time points.

ML level was greater in rematuring than non-rematuring fish at week 20 and 30 (week 30, P=0.0585) in 2015 and at weeks 10-30 in 2016 (Figure 2.2). At week 50 in 2015, ML level was greater in non-rematuring than in rematuring fish. ML level increased from week 10 to week 20 in both groups and years.

K was greater in rematuring than non-rematuring fish at weeks 20-40 in 2015 and at weeks 10-30 in 2016 (Figure 2.3). K increased progressively from week 10 to week 30 in rematuring fish in both years. K increased progressively from 20 to 50 weeks (2015) and 10 to 30 weeks (2016) in non-rematuring fish.

Mass specific growth rate (MSGR) was greater in rematuring than non-maturing fish during weeks 0-10 after maiden spawning in both years (Figure 2.4), continuing for weeks 10-20 and weeks 20-30 in 2015. MSGR was positive for rematuring and negative for non-rematuring fish during weeks 0-10 in both years (Figure 2.4). MSGR increased strongly from weeks 0-10 to weeks 10-20 in both rematuring and non-rematuring fish in both years and remained high through weeks 20-30. In 2015, MSGR declined progressively from weeks 20-30 through weeks 40-50 in rematuring fish, and from weeks 30-40 to weeks 40-50 in non-rematuring fish, returning to levels similar to weeks 0-10. MSGR was greater in non-rematuring fish than rematuring fish over weeks 40-50 in 2015.

Length specific growth rate (LSGR) was negative during weeks 0-10 and increased during weeks 10-20 in both rematuring and non-rematuring fish in both years (Figure 2.5). LSGR subsequently decreased from weeks 30-40 to 40-50 in non-rematuring fish in 2015. LSGR was greater in rematuring than non-rematuring fish at weeks 10-20, 20-30, and 40-50 in 2015 and weeks 20-30 in 2016.

E2 levels were greater in rematuring than non-rematuring fish starting at 20 weeks after maiden spawning, remaining that way for the study duration in both years (Figure 2.6). E2 levels decreased (1.5-3-fold) from spawning to week 10 in both groups and years. In 2015, rematuring E2 levels increased progressively from week 10 to 40, and then decreased at week 50 to levels similar to week 0. The pattern was similar in 2016, except higher rematuring E2 levels were attained at week 20, and there was no subsequent increase at week 30. In non-rematuring fish, E2 levels remained below spawning levels for the recovery year (both years), decreasing from weeks 10-20, but increasing from weeks 20-40 in 2015.

Time Course of Reproductive Rematuration

Two-Way ANOVA found significant effects of maturation group, time, and group*time interactions (Table 2.2).

TG levels were greater in skip than consecutive spawners at weeks 0 and 10 (Figure 2.7). TG levels increased progressively from week 10 to 30 in consecutive spawners (2.0-fold), whereas levels were constant in skip spawners from weeks 0 to 20, followed by a small (1.3-fold) but significant increase at week 30.

ML levels were greater in skip than consecutive spawners at all time points (Figure 2.8). ML levels increased at week 20 in skip and week 30 in consecutive spawners versus week 0 in the year prior to repeat spawning.

K was greater in skip than consecutive spawners at all time points in the year prior to repeat spawning (Figure 2.9). K increased progressively in consecutive spawners from week 10 to 30 and in skip spawners from week 10 to 20.

MSGR was greater in skip spawners during weeks 0-10, but greater in consecutive spawners during weeks 20-30 (Figure 2.10). MSGR increased from weeks 0-10 to 10-20 in both consecutive and skip spawners. During weeks 20-30, MSGR in skip spawners decreased to levels similar to weeks 0-10.

LSGR was greater in skip than consecutive spawners (which had negative LSGR) during weeks 0-10 (Figure 2.11). LSGR increased from weeks 0-10 to 10-20 in both consecutive and skip spawners.

E2 levels were greater in consecutive than skip spawners at week 0, but greater in skip spawners from 10 to 30 weeks (Figure 2.12). From 0-10 weeks, E2 levels increased in skip and decreased in consecutive spawners. Thereafter, E2 levels increased for both groups.

Discussion

The sequence of events over the year after maiden spawning tracked in this study (Figs 2.1-2.6) illustrates the timing of reproductive decisions and differences in energy acquisition and allocation between consecutive and skip spawning female steelhead. TG levels and growth increased over the first 10 weeks after spawning in consecutive versus skip spawners, which suggests that there was greater feeding in consecutive spawners over this time period and that the reproductive decision occurred before 10 weeks post-spawning. Consecutive spawners accumulated greater energy reserves and grew faster than skip spawners over the summer growing season in the year following maiden spawning, suggesting that maturation stimulated feeding. The sequence of events over the year prior to repeat spawning (Figs 2.7-12) illustrates the effect of recovery from maiden spawning on rematuration, energy reserves, and growth. The increase in E2 occurred earlier during the maturation year in skip spawners, suggesting that in consecutive spawners reproductive development was delayed by the energetic or physiological demands of maiden spawning. Skip spawners had substantially greater energy reserves and E2 levels during oogenesis, which likely allowed for greater reproductive investment in skip versus consecutive spawners, as reported in Chapter 1.

Energy Reserves

Energy reserves were assessed using three metrics, focusing on lipids as these have been proposed as particularly important in salmonid maturation (Thorpe et al. 1998): TG levels, representing short-term lipid energy availability, ML levels, representing long term lipid energy stores, and K, a measure of body shape used as a proxy for whole body lipid and other energy stores in fishes (Sutton et al. 2000; Hanson et al. 2010). Energy reserves generally increased more rapidly during recovery from spawning in consecutive spawners but attained higher levels during the extended reconditioning period in skip spawners.

*Year after maiden spawning.—*After spawning, TG levels were greater in rematuring fish from 10-40 weeks, with minor variation in statistical significance between years, and then decreased immediately before spawning. The divergence in circulating TG levels at 10 weeks is most likely due to greater food intake, assimilation, and more rapid somatic recovery in consecutive spawners. However, we cannot exclude the possibility that lipid metabolism differed between maturing and non-maturing female steelhead during this time period. Our interpretation that the increase in TG in rematuring fish was due to greater food intake is supported by the mass SGR results discussed below. If this interpretation is correct, then physiological differences manifesting in the form of increased feeding motivation found between rematuring and non-rematuring fish by 10 weeks after spawning imply that the rematuration decision is linked to the difference in feeding motivation. This suggests two possible mechanisms: the decision either occurs late during the first ten weeks or afterward, with increased feeding early on after spawning causing improved energetic status and leading to initiation of rematuration, or the decision occurs prior to or early during the first ten weeks, with initiation of rematuration stimulating increased feeding and nutrient assimilation. The second possibility is supported by studies in Atlantic salmon and rainbow trout showing increased feed intake, growth, and plasma levels of IGF-1 in maturing fish during early stages of maturation approximately one year before spawning (Kadri et al. 1996; Stead et al. 1999; Wilkinson et al. 2010), and by the presence of developed follicles in rainbow trout immediately after ovulation and oocytes in early secondary growth in post-spawned steelhead (kelts) (De Mones et al. 1989; Penney and Moffitt 2014a), suggesting that secondary oocyte growth begins prior to ovulation of the previous batch.

In female brown trout (*Salmo trutta*), pre-spawning plasma TG levels (mean 189 mg/dL) were similar to spawning levels in the present study and decreased approximately 50% after spawning (Gauthey et al. 2015). TG elevation in consecutive spawners at subsequent time points in the present study, as well as the decrease immediately before spawning, are consistent with both greater feeding and mobilization of lipid reserves during vitellogenesis. The major fates of circulating TGs would be expected to be storage in muscle, mesenteric, and liver lipid depots (Sheridan 1994) and incorporation into the developing ovary during exogenous vitellogenesis (Norberg and Haux 1985). This is supported by studies that have observed increases in TG during exogenous vitellogenesis in reconditioned repeat spawning Atlantic salmon (Johnston et al. 1987), and prior to peak vitellogenesis in rainbow trout (Bon et al. 1997). In non-rematuring fish, TG levels increased to 200-300 mg/dL by week 30 and remained in this range through the following winter and summer, suggesting that TGs are maintained in this range by homeostatic processes in actively feeding steelhead kelts.

ML levels and K increased more rapidly in consecutive than skip trajectory fish, diverging at 20 weeks following the increase in TG levels. The greater ML levels and K in rematuring fish in the year following maiden spawning is consistent with reconditioned wild steelhead approximately 6 months after spawning (Pierce et al. 2017) and fully fed versus feed restricted rainbow trout over the first 20 weeks after spawning (Caldwell et al. 2013). Similarly, K was greater in rematuring reconditioned repeat spawning Atlantic salmon from 18-34 weeks after spawning (Johnston et al. 1987). As spawning approached for consecutive spawning fish, ML levels tended to decrease (though not significantly), and ultimately became significantly lower than ML levels in skip spawning fish at week 50. This likely reflects mobilization of muscle lipids for incorporation into the ovary and reduced appetite.

Unlike TG and ML levels, K did not decrease significantly in consecutive spawners over the period immediately before spawning. This was likely due to the presence of fully developed ovaries in the body cavity of the consecutive spawners. The much higher K at repeat versus maiden spawning in consecutive spawners is both because the mass of the eggs was not included in somatic mass at maiden spawning, and because feeding and somatic growth continued through the fall in consecutive spawners, unlike in maidens. Similar results were observed in reconditioned Atlantic salmon kelts that were rematuring in consecutive years; these fish experienced a minor decline in K following spawning but remained well above maiden spawning levels (Johnston et al. 1987).

At maiden spawning, no differences in lipid reserve metrics were detected between subsequently rematuring and non-rematuring fish, providing no evidence for a determinative role of lipid reserves at spawning in the rematuration decision. This does not necessarily imply that the critical period hypothesis of salmonid maturation is incorrect, as the critical decision period may occur before spawning. It is also possible that the lipid reserve metrics employed in this study did not capture the relevant physiological signals, or lipid reserves are not the relevant measure of energy reserves in rematuring steelhead. Signaling factors associated with energy reserves, rather than energy reserves directly, presumably directly regulate neuroendocrine mechanisms underlying the maturation decision (Wootton and Smith 2015). Future study measuring signaling factors associated with energy reserves and growth is required to elucidate mechanisms underlying maturation decisions in steelhead.

*Year before repeat spawning.—*Over the year prior to repeat spawning, TG levels were suppressed in consecutive versus skip spawners at weeks 0 and 10, which can be attributed to the costs of fasting, migration, spawning and recovery in consecutive spawners. A comparable effect was seen in juvenile rainbow trout, whereby TG levels decreased during fasting in juvenile rainbow trout, and this decrease was exacerbated by swimming (Simpkins et al. 2003).

Over the summer before repeat spawning, ML levels continued to increase in skip spawners, and remained higher than in consecutive spawners, indicating greater energy reserves in the skip spawners due to the much longer time for recovery from maiden spawning. K increased in both consecutive and skip spawners. Although the increase was steeper in consecutive spawners, this was not sufficient to surpass the much higher K levels in skip spawners. As K is a measure of body shape, this likely reflects increased size and greater energy stores in a variety of tissues and organs in the skip spawners, including muscle tissue, visceral lipids, and the developing ovaries.

Growth

*Year after maiden spawning.—*MSGR diverged between rematuring and non-rematuring fish during the first 10 weeks after maiden spawning and remained elevated in rematuring versus non-rematuring fish through the summer growing season, although these differences were not always significant. Growth in length was generally similar to mass over the summer growing period, aside from the negative length growth over the first 10 weeks discussed later. Rematuring fish gained mass whereas non-rematuring fish lost mass over the first 10 weeks after spawning. As discussed above, this is almost certainly due to greater food intake and assimilation in rematuring versus non-rematuring fish. These results are consistent with the greater spring to fall growth found in rematuring reconditioned wild Yakima River female steelhead kelts compared with non-rematuring fish from spring to fall (Pierce et al. 2017), and with post-spawning mass gain in rematuring rainbow trout (Caldwell et al. 2013).

Growth in both mass and length decreased compared to earlier time periods during the final 10 weeks approaching repeat spawning for consecutive spawners. This is likely at least in part due to seasonal growth patterns dictated by water temperature and photoperiod (Burgner et al. 1992). However, decreases in growth as spawn timing approached were also reported in both maturing and non-maturing Atlantic salmon. And, related to what was observed in this study, decreases were more dramatic in maturing fish (Kadri et al. 1996). Additionally, Stead et al. (1999) found a correlation between decreased growth, increased levels of plasma sex steroids, and decreased food consumption during later stages of maturation in Atlantic salmon (Stead et al. 1999). Negative mass growth (e.g., weight loss) in rematuring fish during the 10 weeks before spawning may reflect both reduced food consumption and the energetic cost of ovarian growth.

*Year before repeat spawning.—*MSGR was greater in skip spawners than in consecutive spawners over weeks 0-10 during the year before repeat spawning. This difference can be attributed to the impact of prolonged fasting, migration, and maiden spawning on consecutive spawners. The gut is atrophied in post-spawning summer run steelhead, and degenerative changes are found in the liver (Penney and Moffitt 2014a). The gut-somatic index decreases linearly over time in fasted juvenile rainbow trout, with a loss of ~40% of the relative mass of the gut over 147 days of fasting (Simpkins et al. 2003; Zaldua and Naya 2014). In Atlantic salmon fasted for 50 days, restoration of the gut upon refeeding required at least one week, during which feed intake was reduced (Krogdahl and Bakke-McKellep 2005). The duration of fasting and energetic demands of migration and ovarian development were substantially greater in the DNFH steelhead kelts used in the present study than in the Atlantic salmon refeeding study, as indicated by a proportional lipid depletion of 93-98% from upstream to kelt migration (Penney and Moffitt 2014b), and less than 1% wet muscle lipid mass at spawning (this study). Thus, restoration of digestive function, feeding motivation, and feed intake would be expected to take at least several weeks in post-spawning steelhead.

Length decrease over the immediate 10 weeks after maiden spawning was observed for both rematuring and non-rematuring fish. Over the initial 10 weeks of the year before repeat spawning, consecutive spawners decreased in length whereas skip spawners increased. This length decrease (approximately 1cm) may be due to recession of the kype, a secondary sexual characteristic consisting of elongation of the lower jaw. Although kype development is more pronounced in male salmonids, it also occurs over the period before spawning in females (Vandenberghe and Gross 1989; Willson 1997). Consistent with this possibility, length increase in rematuring fish exceeded that of non-rematuring fish over the 10 weeks preceding spawning.

Estradiol-17β

*Year after maiden spawning.—*E2 decreased following spawning regardless of rematuration trajectory, increased to peak at 40 weeks post-spawning in rematuring fish before decreasing at repeat spawning, and diverged between rematuration trajectories at 20 weeks after maiden spawning. Post-ovulatory decreases in E2 over the month after spawning have been described in rainbow trout and Atlantic salmon (De Mones et al. 1989; Andersson et al. 2013; Caldwell et al. 2014). This post-ovulatory decrease may be physiologically significant in that gonadal steroids and other gonadal factors suppress plasma levels of follicle-stimulating hormone (FSH) in post-ovulatory rainbow trout (Breton et al. 1998; Chyb et al. 1999). Thus, it is possible that clearance of these factors may be necessary before FSH stimulation of ovarian development can occur. The decrease in E2 late in oogenesis is consistent with previous studies in salmonids (Fostier et al. 1978; Whitehead et al. 1983; Nagler et al. 2012; Andersson et al. 2013), and likely reflects a steroidogenic shift from E2 to the maturation inducing steroid 17a,20b-DP induced by luteinizing hormone (LH) (Nagahama 1994; Bobe et al. 2006). The divergence of E2 levels 20 weeks after maiden spawning indicates that the rematuration decision was made prior to this time point. At approximately 5 months after spawning, this was somewhat slower than in reconditioned wild-origin Yakima River steelhead (approximately 3 months) (Pierce et al. 2017), and slower than in feed-restricted versus fully fed post-spawning rainbow trout (10 weeks) (Caldwell et al. 2014). The differences in divergence timing may be due to fish origin and variation in metabolic rate. DNFH hatchery-origin steelhead are larger than Yakima River steelhead, and much larger than rainbow trout, and were held in colder water than in the previous studies. Both the size and temperature differences would be expected to result in a lower metabolic rate in the DNFH fish. The time between natural spawning and collection for reconditioning, as well as potential differences between natural and artificial spawning, could result in the apparent more rapid development in the Yakima River fish.

*Year before repeat spawning.—*E2 was low through the winter in skip spawners, increased from week 0 (+1 year)-week 10 to approximately 10-fold higher in skip spawners than consecutive spawners, comparable to levels in consecutive spawners at week 20. This indicates that the initiation of rematuration occurred before week 10 in skip spawners and suggests that reproductive development was accelerated in skip spawners versus consecutive spawners. Consistent with this idea, spawning was later in consecutive versus skip spawning DNFH steelhead (Chapter 1), as in consecutive versus maiden spawning Atlantic salmon (Pankhurst et al. 2011). E2 levels remained greater in skip versus consecutive spawners as oogenesis proceeded through the summer growing season and levels increased. Similarly, E2 levels were lower in repeat spawning Atlantic salmon versus maidens at sampling time points approximately 10 to 20 weeks after spawning (Pankhurst et al. 2011). The higher E2 levels in skip versus consecutive spawners may have resulted in the 14% greater size-adjusted total egg mass found in these fish (Chapter 1). Both the delay in initiation of maturation in consecutive spawners and the greater investment in skip spawners can be attributed to the effects of recovery from maiden spawning on consecutive spawners. These effects were likely largely mediated by energetic status, as discussed above. However, in addition, reproductive development in consecutive spawners may have been directly affected by recovery from spawning, due to time required for clearance of steroids and other gonadal factors, continued steroid production by postovulatory follicles, and tissue resorption and remodeling of the postovulatory ovary (De Mones et al. 1989; Chyb et al. 1999; Caldwell et al. 2014). The impact of maiden spawning on reproductive development in consecutive spawners illustrates the benefits of having recovery from maiden spawning occur before the early stages of oogenesis.

Conclusions

Within the first 10 weeks after maiden spawning, growth and TG levels were greater in consecutive spawners than in skip spawners. This implies that the rematuration decision takes place prior to 10 weeks after spawning in consecutive spawners. During the rematuration year, E2 levels increased by 10 weeks in skip spawners, suggesting that the rematuration decision occurs prior to 10 weeks +1 year after spawning in skip spawners. The increase in E2 was delayed by 10 weeks in consecutive spawners compared to skip spawners, suggesting that reproductive development was delayed due to the effects of maiden spawning. After maiden spawning, rematuring fish recovered more quickly than non-rematuring fish, consistent with stimulation of feeding by rematuration. Furthermore, while consecutive spawners sustained greater growth rates during oogenesis, skip spawners accumulated higher levels of energy reserves and E2, which may be causally related to the 14% greater reproductive investment found in skip spawners (Chapter 1).

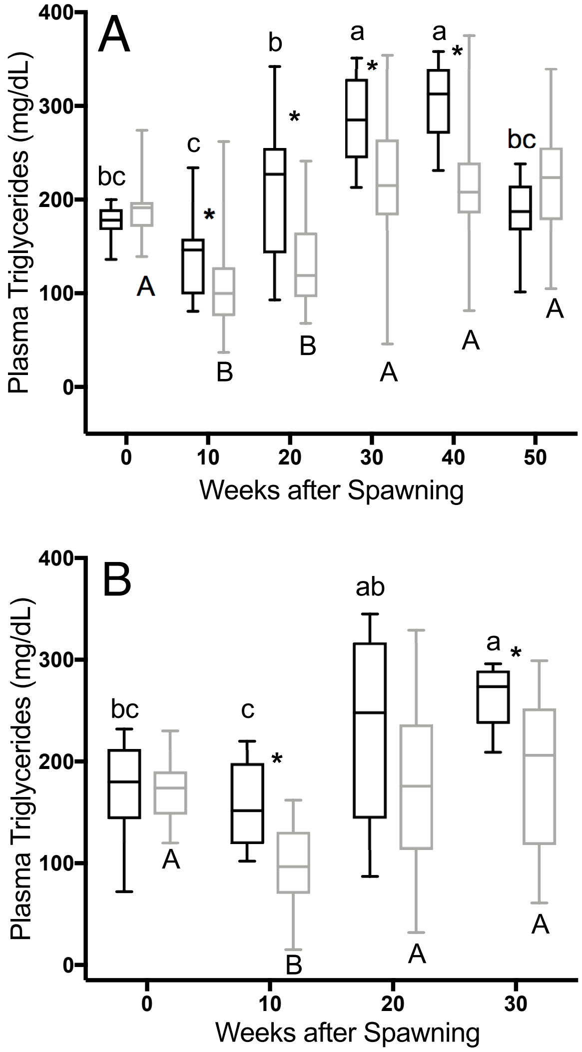

FIGURE 2.1. Plasma triglyceride concentrations in female steelhead from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Females were grouped as rematuring (black: 2015, N=13; 2016, N=12) or non-rematuring (grey: 2015, N=29; 2016, N=18). Box heights indicate interquartile range, horizontal lines within indicate the median, and whiskers show the data range. Time points within a rematuration group sharing the same letter do not differ significantly (One-Way ANOVA followed by Tukey’s Multiple Comparison Test, $P<0.05$). Asterisks indicate significant differences between rematuration groups at each time point (T-test, $P<0.05$).

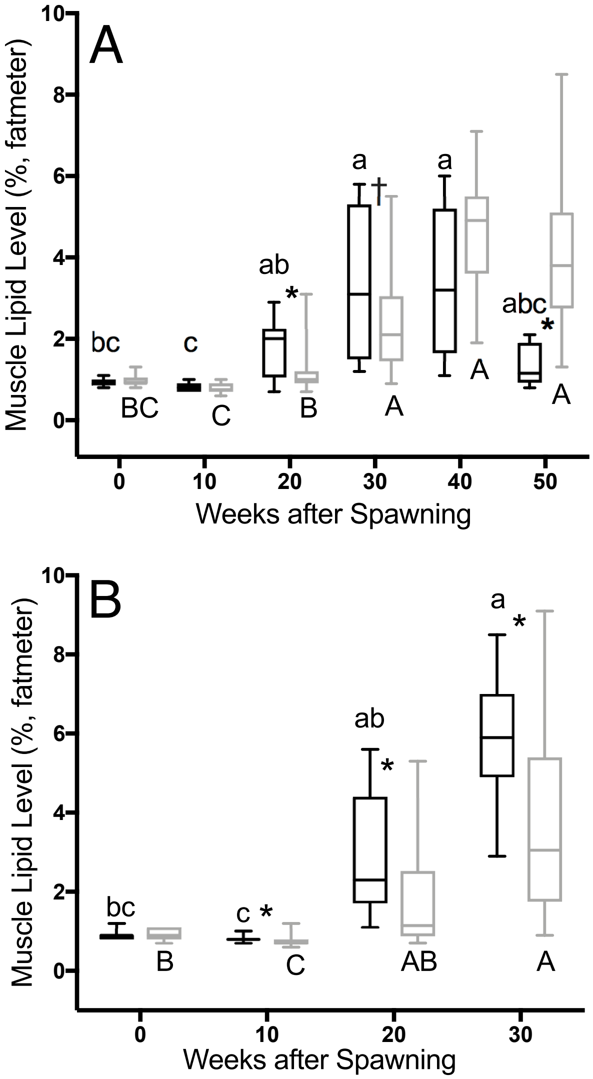

FIGURE 2.2. Muscle lipid levels in female steelhead from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Maturation groups, box and whisker plots, and significance indication are as in Figure 2.1. Due to truncation of the data, non-parametric statistical tests were used (time points: Kruskal-Wallis followed by Dunn’s Multiple Comparison Test, maturation groups: Mann-Whitney Test, P<0.05). †Marginal non-significance at week 30 in 2015, Mann-Whitney Test: U = 199, P=0.0585.

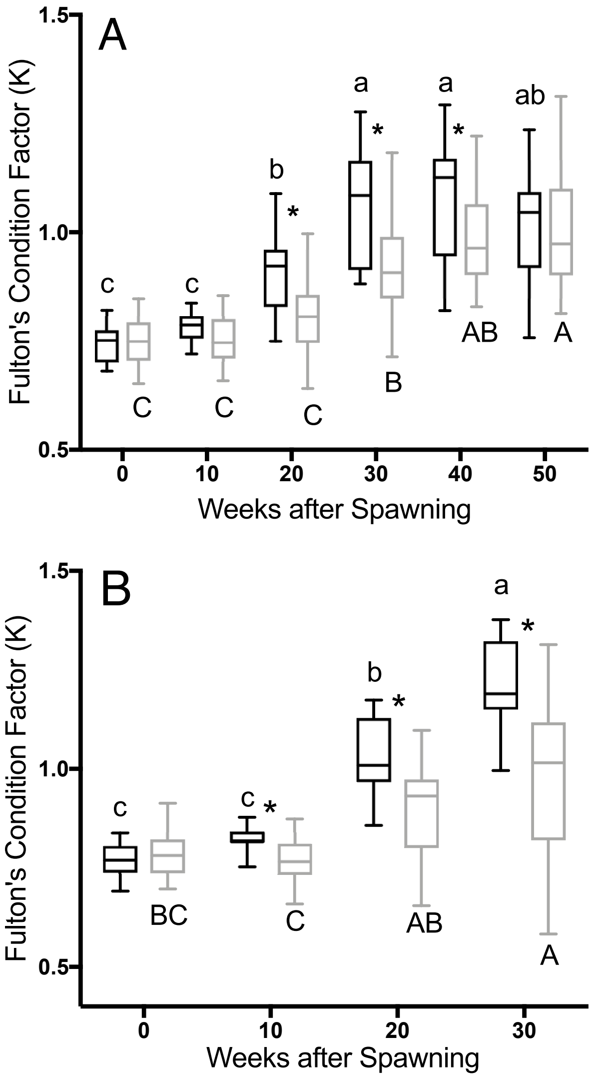

FIGURE 2.3. Fulton's condition factor (K) in female steelhead from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

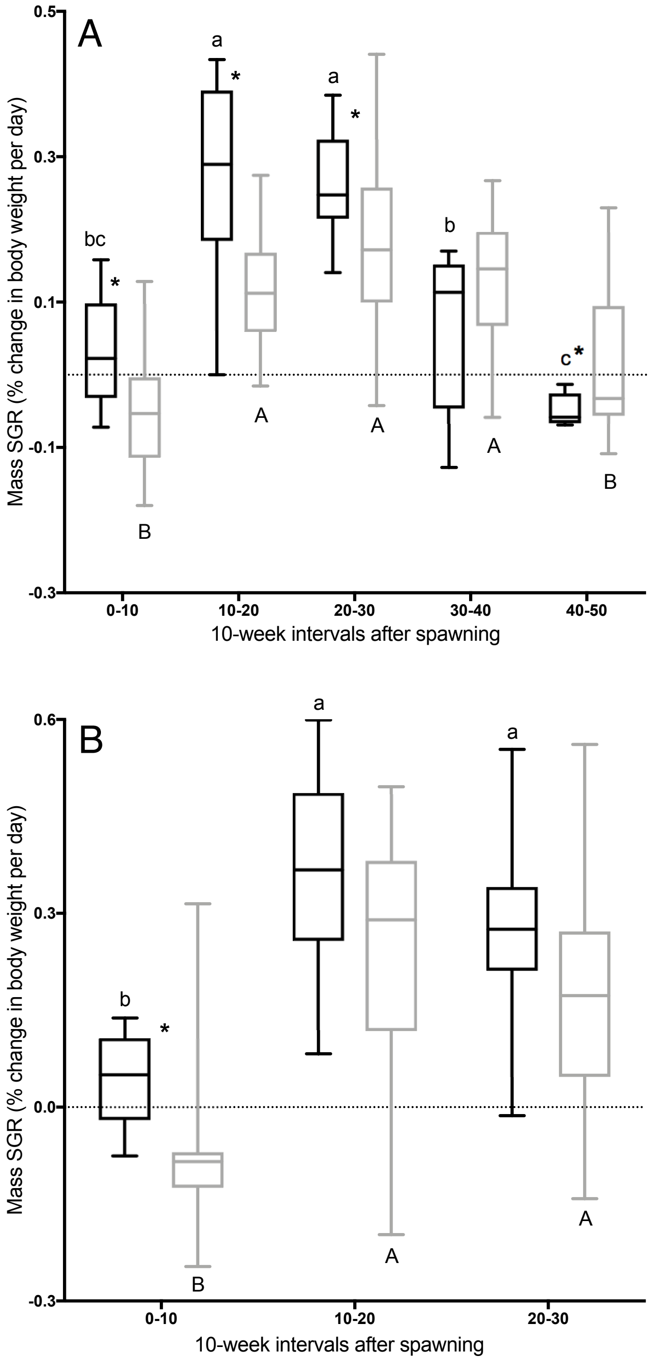

FIGURE 2.4. Mass specific growth rate (Mass SGR, % change in body weight per day) in female steelhead from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) calculated over 10-week intervals following spawning. Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

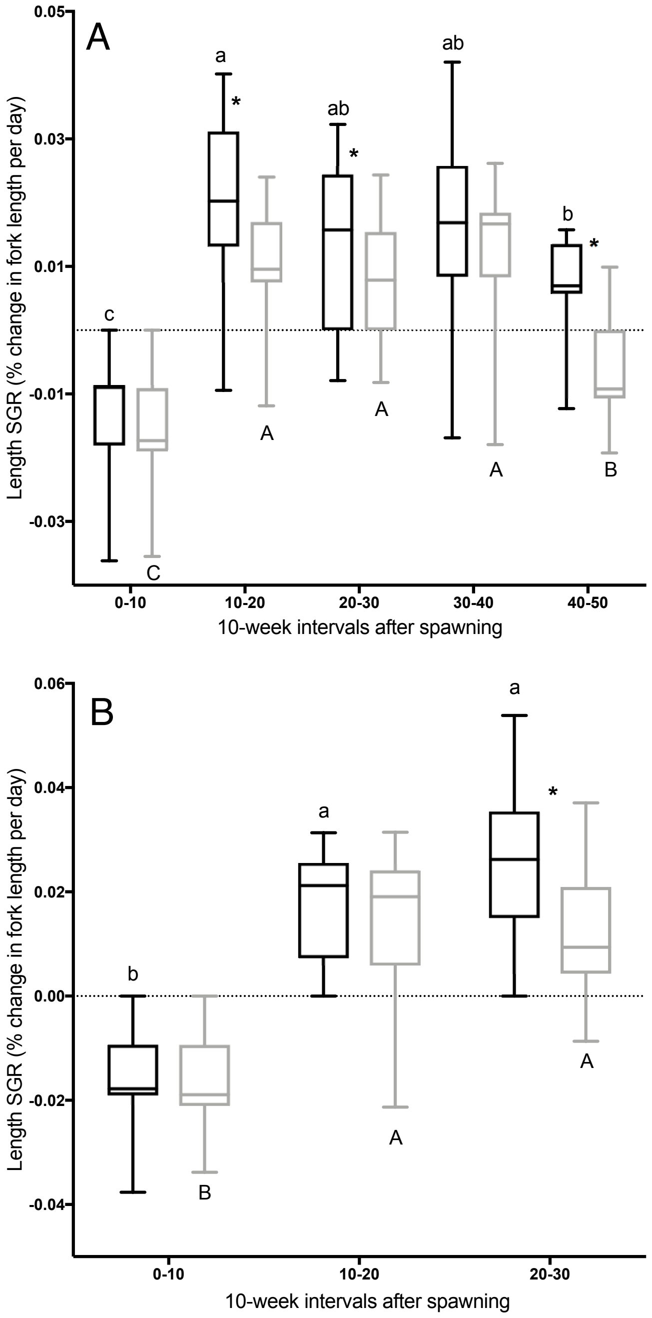

FIGURE 2.5. Length specific growth rate (Length SGR, % change in fork length per day) in female steelhead from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following spawning. Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1. In 2016, N=11 for rematuring fish.

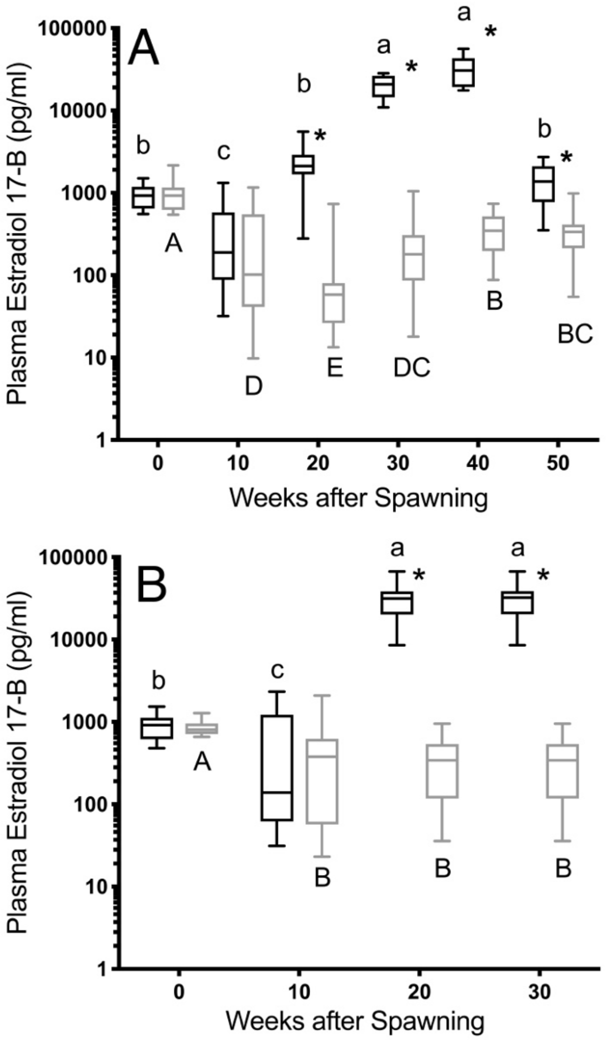

FIGURE 2.6. Plasma estradiol-17*β* concentrations in female steelhead from the Clearwater River, Idaho, sampled in 2015 (A) and 2016 (B) at 10-week intervals following maiden spawning. Females were grouped as rematuring (red: 2015, N=13; 2016, N=12) or non-rematuring (blue: 2015, N=29; 2016, N=18). Maturation groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

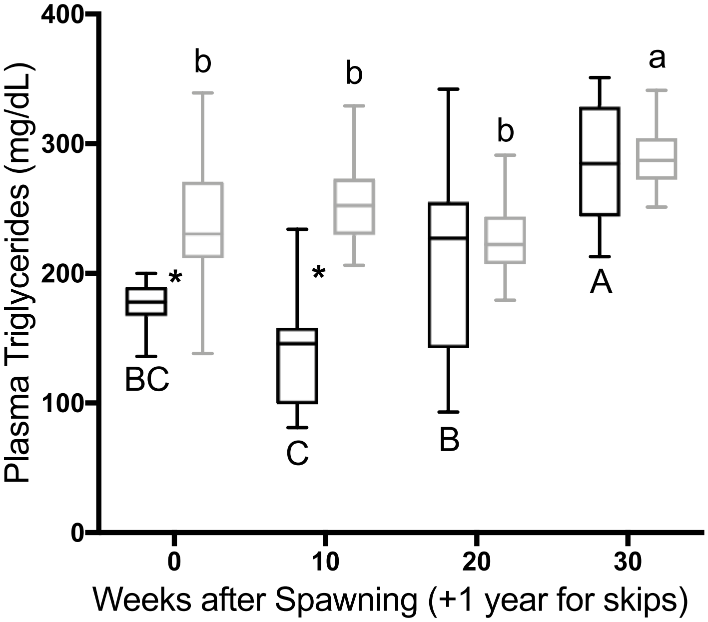

FIGURE 2.7. Plasma triglyceride concentrations during maturation for repeat spawning in female steelhead from the Clearwater River, Idaho. Fish from the 2015 cohort year were sampled in 2015 (black, N=13, rematured in year 1 as consecutive spawners) and in 2016 (grey, N=18, rematured in year 2 as skip spawners) at 10-week intervals. Box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

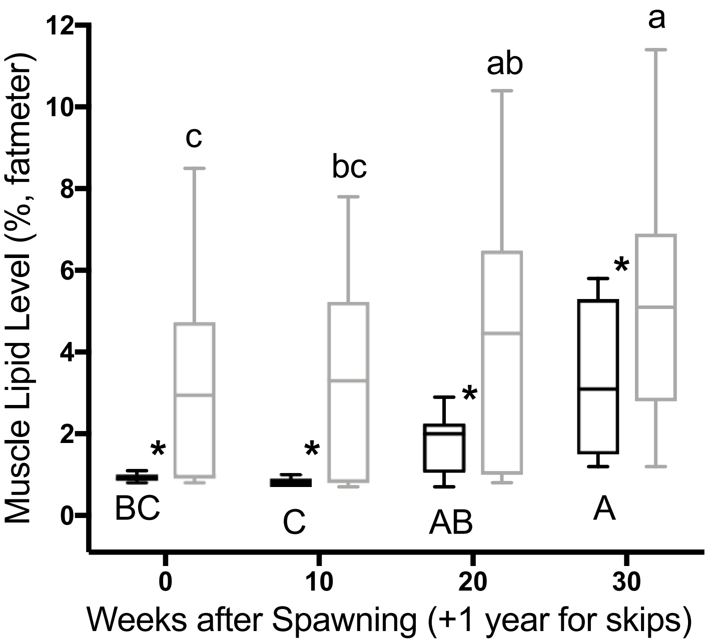

FIGURE 2.8. Muscle lipid levels during maturation for repeat spawning in female steelhead from the Clearwater River, Idaho. Spawning groups, box and whisker plots, and significance indication are as in Figure 2.1. Due to truncation of the data, non-parametric statistical tests were used (time points: Kruskal-Wallis followed by Dunn's Multiple Comparison Test, maturation groups: Mann-Whitney Test, $P<0.05$).

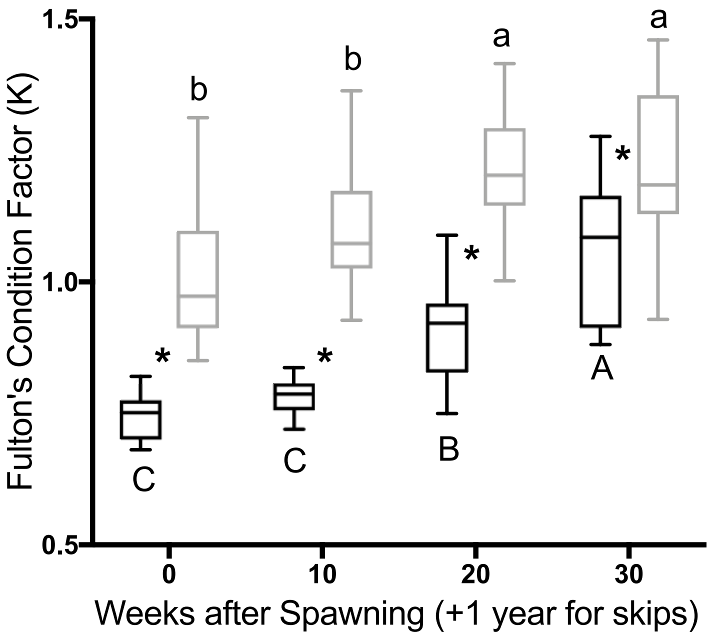

FIGURE 2.9. Fulton's condition factor (K) during maturation for repeat spawning in female steelhead from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

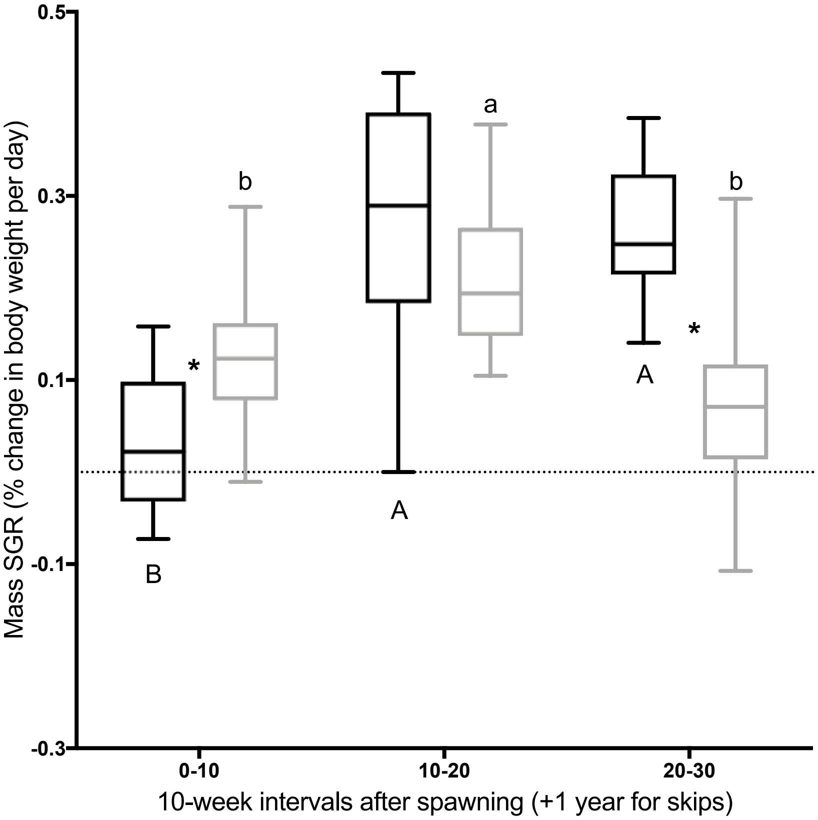

FIGURE 2.10. Mass specific growth rate (Mass SGR, % change in body weight per day) during maturation for repeat spawning in female steelhead from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

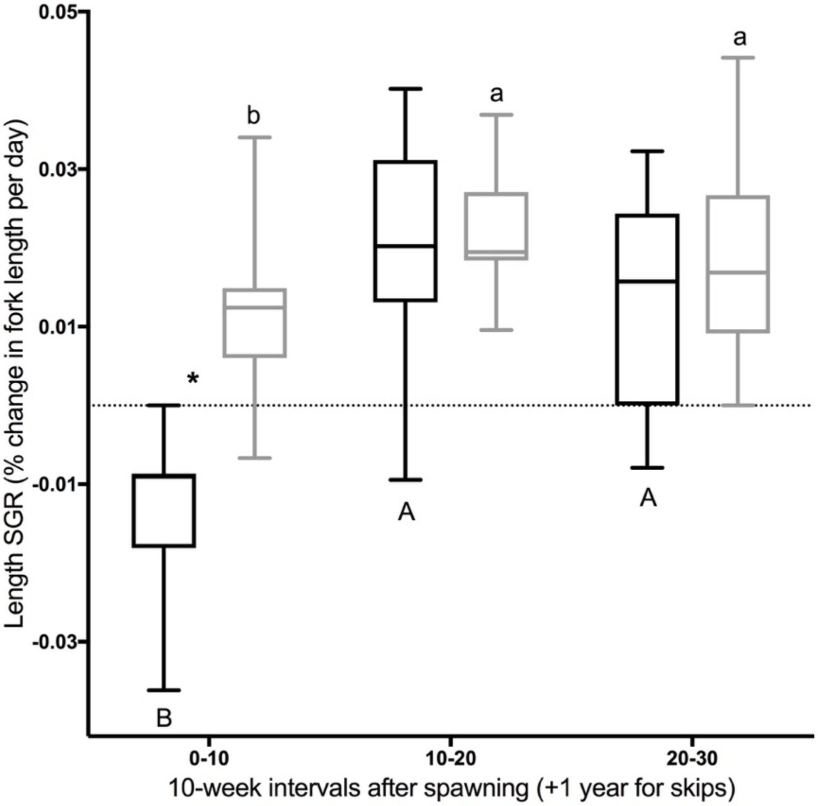

FIGURE 2.11. Length specific growth rate (Length SGR, % change in fork length per day) during maturation for repeat spawning in female steelhead from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

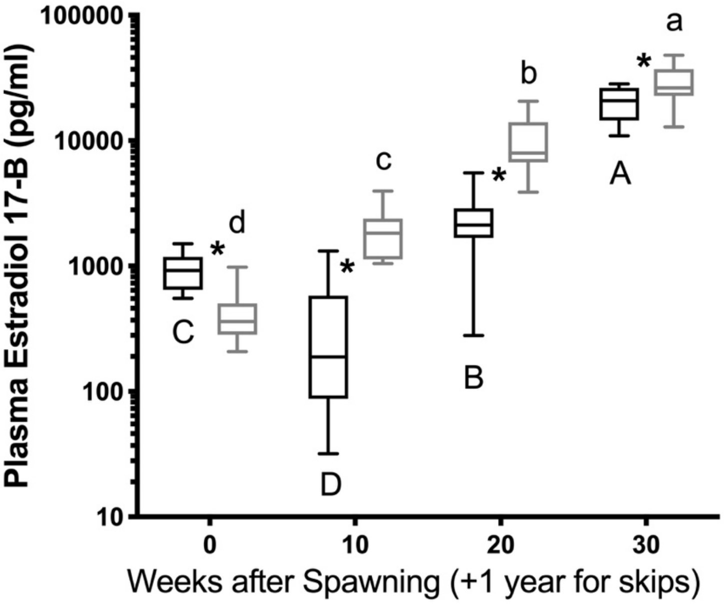

FIGURE 2.12. Plasma estradiol-17*β* concentrations during maturation for repeat spawning in female steelhead from the Clearwater River, Idaho. Spawning groups, box and whisker plots, significance indication, and statistical analyses are as in Figure 2.1.

TABLE 2.1. Test statistics for 2-way ANOVAs of each dependent variable and time during the year following maiden spawning for consecutive and skip spawning fish from 2015-2016.

Measure	Year	Source of Variation	F (DF_n, DF_d)	P-value
TG	2015	Time	F (5, 225) = 33.60	P<0.0001
		Maturation	F (1, 225) = 25.35	P<0.0001
		Interaction	F (5, 225) = 7.950	P<0.0001
	2016	Time	F (3, 108) = 11.74	P<0.0001
		Maturation	F (1, 108) = 4.119	P=0.0449
		Interaction	F (3, 108) = 9.089	P<0.0001
ML	2015	Time	F (5, 223) = 73.25	P<0.0001
		Maturation	F (1, 223) = 6.214	P=0.0134
		Interaction	F (5, 223) = 19.13	P<0.0001
	2016	Time	F (3, 106) = 72.39	P<0.0001
		Maturation	F (1, 106) = 12.36	P=0.0006
		Interaction	F (3, 106) = 4.239	P=0.0072
K	2015	Time	F (5, 227) = 57.09	P<0.0001
		Maturation	F (1, 227) = 18.77	P<0.0001
		Interaction	F (5, 227) = 2.835	P=0.0167
	2016	Time	F (3, 109) = 52.12	P<0.0001
		Maturation	F (1, 109) = 25.52	P<0.0001
		Interaction	F (3, 109) = 6.739	P=0.0003
MSGR	2015	Time	F (4, 183) = 47.53	P<0.0001
		Maturation	F (1, 183) = 5.751	P=0.0175
		Interaction	F (4, 183) = 9.478	P<0.0001
	2016	Time	F (2, 80) = 31.98	P<0.0001
		Maturation	F (1, 80) = 12.92	P=0.0006
		Interaction	**F (2, 80) = 0.0370**	**P=0.9637**
LSGR	2015	Time	F (4, 185) = 49.26	P<0.0001
		Maturation	F (1, 185) = 20.62	P<0.0001
		Interaction	**F (4, 185) = 1.811**	**P=0.1285**
	2016	Time	F (2, 79) = 63.17	P<0.0001
		Maturation	F (1, 79) = 4.35	P=0.0402
		Interaction	**F (2, 79) = 1.369**	**P=0.2603**
E2	2015	Time	F (5, 225) = 52.18	P<0.0001
		Maturation	F (1, 225) = 434.8	P<0.0001
		Interaction	F (5, 225) = 53.7	P<0.0001
	2016	Time	F (3, 109) = 38.61	P<0.0001
		Maturation	F (1, 109) = 156.5	P<0.0001
		Interaction	F (3, 109) = 54.76	P<0.0001

TABLE 2.2. Test statistics for 2-way ANOVAs for each dependent variable and time during the rematuration year for 2015 maiden spawn year fish.

Measure	Source of Variation	F (DFn, DFd)	P-value
TG	Time	F (3, 107) = 26.02	P<0.0001
	Maturation	F (1, 107) = 38.48	P<0.0001
	Interaction	F (3, 107) = 8.622	P<0.0001
ML	Time	F (1, 223) = 6.214	P = 0.0134
	Maturation	F (5, 223) = 73.25	P<0.0001
	Interaction	F (5, 223) = 19.13	P<0.0001
K	Time	F (3, 116) = 33.57	P<0.0001
	Maturation	F (1, 116) = 175.8	P<0.0001
	Interaction	F (3, 116) = 3.112	P=0.0290
MSGR	Time	F (2, 86) = 27.75	P<0.0001
	Maturation	F (1, 86) = 8.781	P=0.0039
	Interaction	F (2, 86) = 19.94	P<0.0001
LSGR	Time	F (2, 87) = 35.19	P<0.0001
	Maturation	F (1, 87) = 17.26	P<0.0001
	Interaction	F (2, 87) = 10.14	P=0.0001
E2	Time	F (3, 112) = 332.9	P<0.0001
	Maturation	F (1, 112) = 62.71	P<0.0001
	Interaction	F (3, 112) = 45.65	P<0.0001

CHAPTER 3

Condition-dependent survival, rematuration, and reproductive performance in reconditioned female steelhead

Introduction

Physiological condition is thought to be a key predictor of many outcomes in life histories in fishes, including survival, migration, size and age at initial maturation, and reproductive mode and performance. Physiological condition has been defined as "…the relative capacity to maintain optimal functionality of all vital systems within the body…" (Hill 2011), which encompasses the energy reserves and health of an individual. However, how this should be quantified is not clear and the relevant aspects of physiological condition may vary in different situations. Survival is intuitively condition-dependent. An individual in positive energy balance and health is likely to survive, and an individual in negative energy balance and failing health is not. Migratory decisions have also been hypothesized to be condition-dependent, whereby a minimum condition threshold is required for initiating migration. Cyprinids with greater body condition were able to migrate away from areas of greater feed availability to escape areas of high predator density sooner than their feed restricted counterparts (Brodersen et al. 2008). In the case of anadromous juvenile rainbow trout, larger individuals were more likely to migrate to locations with greater feed availability (Caisman 2015). Repeat spawning adult Atlantic salmon migrated upstream earlier in the season than their maiden spawning counterparts (Niemelä et al. 2006), which was attributed to differing diets and the nearby locale of estuary feeding grounds. In many salmonid populations, the age at the onset of initial maturation (puberty) varies, and is regulated by aspects of individual condition such as growth rate, lipid stores, and feeding, as well as environmental factors such as temperature, photoperiod, and social cues (Taranger et al. 2010). The initiation of maturation in Atlantic salmon and other salmonids has been hypothesized to depend on energy stores during a critical period approximately one year before spawning associated with early stages of oogenesis (Thorpe 1994; Thorpe 2007). Consistent with this hypothesis, feed restriction early in oogenesis reduced the probability of completion of maturation in lake charr (*Salvelinus namaycush*) (Henderson and Wong 1998). In Atlantic salmon parr, males that reached a minimum lipid threshold at a younger age than their siblings matured precociously at smaller sizes than their siblings (Rowe and Thorpe 1990). Female salmonids reaching first maturity at younger ages than their siblings generally mature at smaller sizes and with reduced reproductive effort due to the positive correlations between fecundity and egg size with fork length (Crespi and Teo 2002; Quinn et al. 2011).

Reproductive mode is also widely found to be condition-dependent. Alternative mating phenotypes of male dung beetles, bees, and fish, such as sneaking, female mimicry, and satellite males are subject to diversifying selection, with the alternative tactic resulting from a genotypic polymorphism associated with reduced diet, size, and growth (Gross 1996). The selected tactic within the flexible strategy is expected to yield greater fitness (greater reproductive performance balanced with survival), but at some switch-point in condition either tactic is expected to yield equal fitness. Where reproductive mode is fixed, reproductive performance has been observed to be condition-dependent. For example, physiological condition impacted reproductive performance in both capital and income breeders (*i.e.*, species that “fund” reproduction using energy stores (capital) gained beforehand versus using feed continually accessed during vitellogenesis (income) (McBride et al. 2015). Egg number and egg size were positively related to body condition in European plaice (*Pleuronectes platessa*; an income breeder) based on feed restriction during late vitellogenesis, indicating condition-dependent reproductive performance attributed to a relationship between feed availability and the rate of vitellogenesis (Kennedy et al. 2008). In rainbow trout and lake charr (capital breeders), feed restriction reduced reproductive performance, but only for feed restriction during early oogenesis (Bromage et al. 1992; Henderson and Wong 1998). Condition impacted reproduction at different times during oogenesis for the two reproductive modes, likely due to the difference in funding strategies. These observations highlight how condition, reproductive mode, and reproductive performance interact.

In iteroparous species, individuals can reproduce either at the shortest interval of the reproductive cycle for that species, referred to as consecutive spawning for seasonally breeding iteroparous fishes that spawn at 1-year intervals, or at reduced frequencies, referred to as skip spawning for fishes that omit 1 or more reproductive cycles, spawning at intervals of 2 or more years (Bull and Shine 1979; Rideout et al. 2005; Rideout and Tomkiewicz 2011). Skip spawning is particularly prevalent in capital breeders, due to the necessity to migrate to feeding locations following spawning for recovery of energy reserves (McBride et al. 2015), suggesting that rematuration depends on condition. However, the effect of condition on rematuration has been much less studied than the effect of condition on initial maturation (puberty). Mechanisms linking condition with rematuration are likely to be similar to those in initial maturation, but also influenced by energetic costs associated with maiden spawning. The decision to remature has been framed as condition-dependent, as in winter flounder (*Pleuronectes americanus*), where individuals in better condition rematured despite feed restriction (Burton 1994), and in Atlantic salmon, where both condition factor and rematuration rate were greater after a year of reproductive inactivity than in the year immediately following spawning (Crim et al. 1992). Repeat maturation was also deferred as a result of poor condition in long distance migrating female albatross (*Thalassarche melanophris*) (Crossin et al. 2012). Repeat maturation has alternately been framed in relation to energy availability in the environment and energy expenditure, presumably mediated by individual condition. Consecutive spawning Atlantic salmon were distributed in areas with high prey availability near river mouths (Chaput and Benoit 2012), whereas skip spawners travelled farther to feed. Finally, the effect of energy reserves on rematuration is not necessarily always positive. In Atlantic cod (*Gadus morhua*), skipping was associated with too little energy for rematuration, but also with abundant energy and growth opportunities, due to the potential increase in reproductive performance (Jorgensen et al. 2006).

Despite extensive theory surrounding "condition-dependent" impacts on survival, behavioral, and reproductive outcomes, definitions of physiological condition are often vague. Measures used to capture condition range from body size (often one dimensional, such as body length), to condition indices incorporating mass and body size such as K, to measures of stored energy such as muscle and other tissue lipid levels. However, though these measures of condition indicate energetic status, they may not give a complete picture of an organism's physiological condition, and they do not directly interact with the physiological systems that regulate life history decisions. Integrated measures of the ability to maintain homeostasis, such as circulating levels of metabolic fuels, may be more relevant indicators of physiological condition by indicating the organism's ability to function, and may more directly interact with physiological systems that regulate life history decisions and outcomes. For example, K did not differ between survivors and mortalities of post-spawning Atlantic salmon, but upon follow-up investigation of mortalities, extensive evidence of parasites was discovered (Crim et al. 1992). As lipids are a major energy source and are primarily stored as triglycerides in poikilotherms (Sheridan 1994), circulating triglyceride level is a reasonable measure of the ability to maintain homeostasis by circulating of readily available energy. Growth rates in both length and mass are integrated measures of energy balance over time. However, measures of energy circulation and balance alone may fail to capture key aspects of the health of an individual, essential to the organism's ability to function. For fish, active maintenance of plasma solute concentration within a narrow range is required for the functioning of physiological systems, and osmoregulatory failure has been identified as an earlier indicator of senescence in sockeye salmon (*Oncorhynchus nerka*) (Jeffries et al. 2011). Thus, plasma osmolality shows promise as an integrated indicator of the ability of an individual to maintain homeostasis. Individuals with weakened immune systems are subject to rapid colonization by ubiquitous pathogens. Quantification of parasite load can be used both as a proxy to measure immune system function and as an indicator of parasitic drain on energy reserves. Finally, quantification of reproductive investment at maiden spawning (total egg mass, E2) should provide insight into the relationship between reproductive investment and physiological condition. Reproductive investment has been theorized to tradeoff with survival (Abrahamse and Murdoch 2014; Christie et al. 2018) and future reproductive decisions through effects on condition (Stearns 1992). Limited energy reserves resulting in poor physiological condition leaves fewer resources (energy, materials) available for allocation to physiological processes. Evaluation of relationships between maiden reproductive investment and condition, survival, rematuration, and reproductive investment at repeat spawning, are required to determine whether these tradeoffs occur, and the magnitude of any effects in our experimental system.

An experimental model system of post-spawning female steelhead as described in Chapter 1 was used to study intersections between maiden condition, maiden reproductive investment, and aspects of repeat spawning (survival, timing, and effort) in this study. The objectives of this study were to determine whether and how post-spawning survival or rematuration were related to maiden reproductive investment or condition and whether reproductive investment at repeat spawning was condition-dependent during reconditioning, based on physiological condition measured early following maiden spawning. Within the first objective, there were four aims. Aim 1 was to determine whether post-spawning survival or the consecutive rematuration decision were condition-dependent. Aim 2 was to evaluate measures of physiological condition that indicated either the energetic status or the functional ability of an organism and to determine which were most relevant to outcomes in our study system. The third aim was to determine whether maiden reproductive investment was related to physiological condition at spawning, as reproductive life history trade-offs between maiden reproductive investment and post-spawning survival or the consecutive rematuration decision are hypothesized to be mediated by physiological condition. The fourth aim was to directly assess whether there was a reproductive life history trade-off between maiden reproductive investment and post-spawning survival or the consecutive rematuration decision, since the measures of condition used here may not capture the relevant aspects of physiological condition. It was hypothesized that (a) post-spawning survival, the consecutive rematuration decision, and reproductive investment at consecutive spawning would vary positively with condition, due to being condition-dependent traits, and (b) condition at maiden spawning, post-spawning survival, and the decision to remature in consecutive years would vary negatively with maiden reproductive investment, due to a trade-off resulting from limited energy reserves.

Materials and Methods

*Fish.—*Female steelhead originating from DNFH were captured in February through April in 2015 and 2016 and were held, spawned, selected for the study, and individually tagged as described in Chapter 1. Fish husbandry was as described in Chapter 1.

Sampling.—Fish were sampled for FL and wet body mass, ML, and blood at spawning and at 10-week intervals thereafter, as described in Chapters 1-2. Fish were sampled for parasitic gill copepods *Salmincola californiensis* counted on the left gill (PL).

Morphometric Analysis.—K, MSGR, and LSGR were calculated as in Chapter 2. Wet body mass and K were adjusted to account for eggs retained from maiden spawning in the body cavity, as described in Chapter 1, to represent somatic measures of mass and K.

Reproductive Performance Measures.—Repeat spawning individual egg mass (IEM), total egg mass (TEM), and fecundity, were quantified gravimetrically and standardized based on fish mass, as described in Chapter 1.

Assays.—E2 concentration and TG level were measured as described in Chapter 2. Plasma osmolality (mmol/kg, OS) was measured using a VAPRO Vapor Pressure Osmometer 5520 (WESCOR, Elitech, Puteaux, France).

Ethics.—Fish care and sampling were conducted in accordance with a protocol reviewed and approved by the University of Idaho Animal Care and Use Committee.

Statistical Analysis

Survival.—The probability of survival post-spawning in female steelhead was assessed for associations with potential explanatory variables using univariate and multiple logistic regression. Binomial response variables were defined as survival to 70 days (1=Survival) versus mortality prior to 70 days (0=Mortality).

Seven continuous predictor variables measured at maiden spawning included K, ML (arc-sine square-root transformed), TG, OS, PL (number of copepods), TEM, and E2 ($\log_{10}$-transformed). Predictor variables were standardized in order to compare effect sizes ([x-mean]/SD) (Keefer et al. 2017).

Years (2015, 2016) were analyzed separately, as well as with the data combined. When data from both years were combined, year was included as an effect. Because preliminary univariate tests saw a year effect (in the survival analysis only, P=0.00393), a model was tested with all first order interactions with year. No evidence supported an interaction with year, so the interactions were dropped.

Survival Duration.—To further explore potential predictors of survival, multiple linear regression was used to identify predictors of time survived for mortalities within the first ten weeks following maiden spawning, with the continuous response variable "days survived". The same 7 standardized predictor variables listed above were included (8 with year when data from both years were combined). Predictor variables were tested first in univariate models. The whole model including all predictor variables was then tested. When data from both years were combined, interactions with year were explored as described above. Significant interactions with year were included in the whole model where identified.

Rematuration.—The likelihood of rematuration as a consecutive repeat spawner (vs. deferring rematuration for a future year) was assessed for associations with the potential explanatory variables listed above, using univariate and multiple logistic regression models, as described above for the survival analysis. Maturation trajectory was determined using E2 at 30 weeks post-spawning as described in Chapter 2. The same 7 standardized explanatory variables described above were examined in models. Year was included as an effect in models with data from both years combined. Year was not a significant univariate predictor of maturation (P = 0.38819). A model was tested with all first order interactions with year. No evidence was found for a significant interaction between year and any predictor, so the interactions were dropped.

Reproductive Performance.—To identify potential sensitive periods for condition-dependent repeat reproductive performance, simple linear regression was used to test for relationships between 6 potential predictor variables, measured at time points starting at maiden spawning, and 3 dependent variables of reproductive performance at repeat spawning. Potential predictor variables were: MSGR, LSGR, TG, E2, ML (arcsine square root-transformed), and K. Dependent variables were: repeat spawning IEM, TEM, and fecundity. Consecutive spawners (N=12) from the 2015 spawn year were included in the analysis as the most complete dataset was available for these fish. Coefficients of determination (R) and an associated P value were calculated for each predictor at each time period (spawning, and at each 10-week sampling period or interval thereafter).

Results

Patterns of Survival

Survival declined steeply during the first 70 days (10 weeks) following maiden spawning and remained approximately constant between 10-30 weeks (Figure 3.1). Survival was lower in 2016 than in 2015 (Log-rank test, $\chi^2 = 9.335$, $P = 0.0022$). Mortality decreased after 70 days, which coincided with the first sampling event. Survival to 10 weeks after spawning was 53% (80/150) in 2015 and 38% (62/164) in 2016, averaging 45% (142/314) in the two years combined (Table 3.1).

Predictors of Survival

In the survival dataset (N=314), several parameters were significantly but not strongly intercorrelated ($R \leq 0.469$; Table 3.5). TG was weakly positively correlated with ML, K, OS, and E2 ($r^2=0.148$, 0.019, 0.151, and 0.220, respectively; $P<0.0001$, $P=0.0141$, $P<0.0001$, and

P<0.0001, respectively), with relationships explaining less than 25% of the variability for each. ML was weakly positively correlated with K, OS, and E2 (r^2=0.013, 0.019, and 0.106; P = 0.0489, P = 0.0154, and P<0.0001). TEM was weakly negatively correlated with TG, ML, K and OS (r^2 = 0.041, 0.018, 0.060, and 0.049; P=0.0004, P=0.0202, P<0.0001, and P<0.0001, respectively), and weakly positively correlated with PL (r^2 = 0.046, P = 0.0001).

Significant univariate predictors of survival were found in 2015, 2016, and in years combined (Table 3.6). OS was a significant univariate predictor of survival in 2015, 2016, and in years combined (Figure 3.2A), (χ^2 = 4.40, 15.29, 23.90; P=0.0359, P<0.0001, P<0.0001, respectively). TG was a significant univariate predictor of survival in 2016 (χ^2 = 12.63, P=0.0004), in years combined (χ^2 = 20.88, P<0.0001) (Figure 3.2B) and was nearly significant in 2015 (χ^2 = 2.89, P=0.0891).

In 2016, K was a significant univariate predictor of survival (χ^2 = 3.89, P=0.0486) (Table 3.6). E2 was nearly significant in 2016 (χ^2 = 3.24, P=0.0720). TEM was a significant negative predictor of survival in years combined (χ^2 = 6.97, P=0.0083, OR=0.396033), and was nearly significant in 2016 (χ^2 = 2.88, P=0.0897), but not in 2015 (χ^2 = 0.28, P=0.5954). ML was a nearly significant predictor of survival in 2016 and in years combined (χ^2 = 1.83, χ^2 = 3.39; P=0.1763, P=0.0654 respectively), but as a slightly positive predictor in 2016 and a slightly negative predictor in years combined.

In the whole model results, where data from years were combined and year was included as an effect (Table 3.2), OS and TG were both strongly and significantly related to survival (χ^2= 10.94, 7.20; P=0.0009, 0.0073, respectively). The 95% confidence intervals of all other standardized coefficients overlapped zero and are thus without significant effect by comparison (Figure 3.3).

Predictors of Survival Duration

For mortalities within the first 10 weeks after spawning (N=172), parameters were not strongly intercorrelated (Table 3.7). TG was weakly positively correlated with ML, OS, and E2 ($r^2 = 0.093$, 0.169, and 0.236, respectively; P<0.0001 for each), with relationships explaining less than 25% of the variability for each. TEM was weakly negatively correlated with TG, K and OS ($r^2 = 0.078$, 0.109, and 0.035; P=0.0002, P<0.0001, and P=0.0156, respectively), and weakly positively correlated with parasite load ($r^2 = 0.035$ and P=0.154).

Significant univariate predictors of survival duration (days) were found in 2015, 2016, and in both years combined (Table 3.8). TG was a significant univariate predictor in 2015, 2016, and in both years combined (t-ratio=3.35, 2.31, and 4.62; P=0.00133, P=0.0230, and P<0.0001, respectively) (Figure 3.4A). OS was a significant univariate predictor in 2016 and in both years combined (t-ratio=3.73, 4.06; P=0.0003, P<0.0001, respectively) (Figure 3.4B).

E2 and TEM were nearly significant univariate predictors of survival in 2015 and in years combined (E2: t-ratio = 1.31 and 1.86, P = 0.19395 and 0.06450; TEM: t-ratio = -1.16 and -1.96, P = 0.25086 and 0.0517) (Table 3.8). PL was nearly significant in 2016 and in years combined (t-ratio=1.50 and 1.43, P=0.1360 and 0.1558).

In the whole model (Table 3.3), when data from both years were combined, two interactions with year were significant, resulting in a whole model: "Survival days = Intercept + Year + TG + ML + K + OS + PL + TEM + E2 + TG*Year + OS*Year", in which plasma triglycerides (TG, P=0.0059), plasma osmolality (OS, P=0.0312) and the interactions of TG and OS with year (TG*Year, P=0.0255; OS*Year, P=0.0364) were significant. Overall, 22.7% of the variation in survival duration was explained by the predictors.

Patterns of Rematuration

The proportion of fish that survived and rematured in the year following spawning was 9% (13/150) in 2015 and 7% (12/164) in 2016, averaging 8% average in years combined (Table 3.1). Of the fish that survived to 30 weeks, 30% (13/43) rematured in 2015 and 40% (12/30) rematured in 2016 (34% rematuration in years combined).

Predictors of Rematuration

Of fish that survived to 30 weeks after maiden spawning (N=73), parameters were not strongly intercorrelated (Table 3.9). TG was weakly positively correlated with ML (r^2=0.302, P<0.0001), OS (r^2=0.063, P=0.0435), and E2 (r^2=0.155, P=0.0009). ML was also weakly positively correlated with E2 (r^2=0.266, P<0.0001). TEM and K were weakly negatively correlated (r^2=0.077, P=0.0206). TEM was also weakly negatively correlated with TG, ML, and OS, and positively correlated with PL, but unlike in the complete (survival) dataset (N=314), these relationships were not significant (P=0.3776, 0.3411, 0.2986, and 0.3958). Also unlike the complete dataset, TG and PL were not negatively related, though this was not previously a significant correlation.

Significant univariate predictors of maturation were found when data from both years were combined (Table 3.10). TEM was a significant positive predictor of maturation in both years combined (χ^2 = 4.07, P=0.0437), and nearly significant in 2015 and 2016 (χ^2 = 2.36, χ^2 = 1.52; P=0.1241, P=0.2172, respectively) (Figure 3.5). TG was a nearly significant negative predictor of maturation in 2015 (χ^2 = 1.83, P=0.1756), as was ML in years combined (χ^2 = 1.80, P=0.1795) (Table 3.10).

In the whole model results, where data from years were combined and year was included as an effect (Table 3.3), TEM was a significant positive predictor of maturation (χ^2=

5.02; P=0.0250). The 95% confidence intervals of all other standardized coefficients overlapped zero and are thus without significant effect by comparison (Figure 3.6).

Patterns of Reproductive Performance

Consecutive spawners of the maiden spawn year 2015 (N=12) included in this analysis had an average 96.1mg (SD=13.5) IEM, 721.9g (SD=132.8) TEM, and 7,505 eggs (SD=1,456) fecundity, as reported in Chapter 1.

Predictors of Reproductive Performance

Relationships were found between reproductive performance at repeat spawning (IEM, TEM, and fecundity) and growth, available energy, and condition at early time points (0-10 weeks and 10-20 weeks) during the year prior to repeat spawning (Table 3.11, Figure 3.7). Relationships between IEM and TG, MSGR, and LSGR peaked in strength at 10 weeks post-spawning (r^2=0.5593, 0.5247, and 0.4758, respectively (Figure 3.7 A, D and G; Figure 3.8 A, C, and E). Relationships between TEM and MSGR, K, and TG peaked in strength at 20 weeks post-spawning (r^2=0.5382, 0.4768, and 0.4595, respectively (Figure 3.7 B, E, and H, Figure 3.8 B, D, and F). Relationships between fecundity and MSGR, TG, and LSGR peaked in strength at 20 weeks post-spawning (r^2=0.41725, 0.56586, and 0.54093, respectively (Figure 3.7 C, F, and I), but these relationships were not quite significant (P=0.1772, 0.0551, 0.0693, respectively).

Discussion

Evidence from this study indicates condition-dependent post-spawning survival and reproductive performance in repeat spawning female steelhead. Evidence herein is also consistent with a condition-dependent rematuration decision occurring prior to maiden spawning. Post-spawning survival was condition-dependent based on functional measures of physiological condition: fish with greater TG and OS at maiden spawning had an increased probability of survival. Reproductive performance at consecutive repeat spawning was condition-dependent during the period immediately after spawning: IEM was most strongly correlated with growth and TG during the first 10 weeks after spawning and TEM was most strongly correlated with growth, TG and K during the second 10-week interval after maiden spawning. The consecutive rematuration decision was positively related to maiden reproductive effort, consistent with the possibility that both reproductive investment at maiden spawning and consecutive rematuration depend on condition at time points before maiden spawning. Maiden reproductive performance (TEM) and measures of physiological condition at maiden spawning were statistically significant but weakly negatively correlated ($r^2<0.06$), providing little evidence for a biologically significant tradeoff between maiden reproductive investment and condition. Consistent with this, maiden reproductive performance was not negatively related to post-spawning survival nor to consecutive rematuration. Finally, measures of physiological condition indicating the organism's functional abilities, such as the ability to mobilize stored lipids as TG, maintain OS in an optimal range, and growth rates, were better predictors of survival and reproductive performance than physiological condition measures indicating energetic status, such as ML and Fulton's K.

Condition-Dependent Survival

Post-spawn mortality averaged >50% in both years, with the number of surviving females leveling off by 2.5 months. Post-spawning mortality is potentially due to a variety of factors:

handling stress, injuries during migration or spawning, difficulty reactivating the digestive tract, inability to switch off the reproductive mode, diseases and parasites, and energetic costs associated with an approximately 6 months fasting freshwater spawning migration and ovarian development. Mortality in the present study was higher than that reported for Atlantic salmon kelts reconditioned in freshwater or brackish salt water (14-38%) (Johnston et al. 1987; Dumas et al. 1991; Crim et al. 1992), but was in the range reported for other programs for steelhead and Atlantic salmon kelts reconditioned in freshwater (38-80%) (Moffett et al. 1996; Hatch et al. 2013). Surviving spawning and the concurrent trauma requires accessing stored energy and the ability to maintain homeostasis. This study employed condition measures indicating energy reserves (K, ML), as well as those more directly reflecting the ability to maintain homeostasis: plasma triglyceride (TG) level, osmoregulatory function measured as plasma osmolality (OS), and immune system function at the time of spawning as the ability to resist parasites (PL).

Significant relationships with survival were not found with the more traditional, energy reserve indices of condition (K, ML), nor was a negative relationship found with PL. In the present study, we measured condition factor at spawning as “somatic” mass excluding eggs, such that it was independent from total egg mass, one of our measures of reproductive investment. Similarly, in a reconditioning study using Atlantic salmon, K was not found to be predictive of post-spawning survival (Crim et al. 1992). Upon later investigation, Crim et al. (1992) discovered parasites in many of the mortalities. It is possible that parasite load was not a significant predictor of survival in the present study because all study subjects were given an effective treatment for copepods at the time of spawning (see Materials and Methods, Chapter 1 and Chapter 2). The treatment, however, would only interfere with the impact of the copepod parasites following its application. Any impact of the copepods on individuals prior to spawning would remain as a potential impact on post-spawning mortality. The lack of a relationship with post-spawning mortality suggests that the copepods at the infestation levels observed did not substantially impact the health of these fish. Up to 120 adult *S. californiensis* per kg of fish were reported as a heavy but sublethal infestation in a captive population of rainbow trout (Roberts et al. 2004), suggesting that levels observed in the present study (approximately 1-11 adult *S. californiensis* beneath one opercula) would not be expected to have lethal effects unless further exacerbated by interacting stressors. ML was very low in all fish spawned, averaging ~0.9-1%. However, levels of 0.5% or lower were measured in study fish, so this is not the minimum muscle lipid level detectable for the instrument used. In order to use muscle lipid stores to support metabolism, post spawning kelts must be able to mobilize these stores from the muscle into the circulation. Many mortalities during the first 70 days after spawning were observed with muscle lipid levels above the minimum detectable with the instrument used (data not shown). Thus, the ability to mobilize stored lipids from body stores (e.g., liver) may be a better indicator of physiological condition than a measure of muscle lipid reserves. This is supported by our finding of a significant relationship between TG and post-spawning survival.

Plasma Triglycerides.–Survival and TG level were positively related. For mortalities during the first 70 days after maiden spawning, the relationship between survival duration and TG level appeared consistent over the range of the data, without any apparent threshold effects. TG on average was greater in survivors to 70 days than in mortalities within each year. The positive relationship between TG level and survival is most likely because fish with higher TG level showed a stronger ability to mobilize stored energy to meet the demands required to survive spawning (resume feeding, re-generate the digestive system, transition out of the reproductive mode, fight parasites, infection, stress, etc.) than fish with lower TG levels. TG levels on average for both survivors and mortalities were consistent with non-fasted, sedentary juvenile rainbow trout (Simpkins et al. 2003), and were greater than post-spawning TG levels in brown trout (Gauthey et al. 2015). Lipids measured in the plasma prior to vitellogenesis in rainbow trout were found to be used for energy (plasma triacylglycerols or triglycerides), as opposed to lipoproteins for vitellogenin synthesis, consistent with a relationship between circulating TG and survival (Bon et al. 1997).

Plasma Osmolality.–OS was positively associated with survival and with survival duration. This association was particularly strong over the low range of the data, such that fish with low OS were highly subject to mortality in the early days after maiden spawning. This suggests that there may be a threshold below which low OS indicates impending mortality. The body fluids of fish have greater solute concentration than found in freshwater, so they must take up ions through active transport mechanisms in the gill and excrete excess water through the kidney to maintain OS in a narrow range, approximately 308.5 +/- 3.9 mOsm/kg for rainbow trout in freshwater (Oguri and Ooshima 1977). Low plasma osmolality in individual fish indicates failure of these systems, which explains the association with mortality. Slightly lower values than the normal biological range were observed in the present study: 295-303mmol/kg. The discrepancy in units is due to previous reporting of osmolality in different units: mOsm/kg. Standard International units of osmolality (mmol/kg) were adopted for increased accuracy as early as 1995, as mOsm/kg implies a linear relationship between solute concentration and dissolution, which is misleading due to residual attraction between dissolved ionic compounds (*i.e.*, NaCl) (WESCOR manual, Elitech, Puteaux, France). Consistent with our study, reduced osmoregulatory ability, measured as decreased OS, was associated with post-spawning senescence in sockeye salmon in freshwater (~20% reduction, ranging from 315- 235mOsm/kg, with fish becoming moribund below 280mOsm/kg) (Jeffries et al. 2011). Also consistent with our study, Jeffries et al. (2011) observed a time effect, whereby osmolality declined days in advance of changes in other variables associated with post-spawn mortality, suggesting that failure of the osmoregulatory system may be an early indicator of impending mortality. Similarly, plasma osmolality was used as a measure of physiological recovery in post-capture survival of gill-netted coho salmon (*Oncorhynchus kisutch*) in saltwater, and survival was positively associated with decreases in plasma osmolality (Farrell et al. 2001).

Life history trade-off.–This study also provides an examination of whether a reproductive life history trade-off occurred between energy reserves spent on maiden reproductive performance (TEM, E2) and post-spawning survival. Post-spawning survival was not significantly negatively correlated with TEM in the whole model, so a life history trade-off between reproductive performance and survival is not supported by the data. Although TEM was a significant predictor of post-spawning survival in the univariate model with years combined, this effect was largely driven by the ~15% greater TEM in 2016 versus 2015 in combination with higher survival in 2015. In the AICc comparison of the univariate model (TEM as a predictor of survival) with the whole model, there was no support for the univariate model (ΔAIC=48) [Analysis not shown]. Our data were not consistent with a reproductive life history trade-off between maiden reproductive investment and survival to future spawning that was proposed for a population of winter-run steelhead from the Hood River, Oregon, based on analysis of adult to adult returns (Christie et al. 2018). However, this study did not directly measure maiden reproductive investment, and there may be alternate explanations for the adult to adult return result. E2 level at spawning did not predict subsequent survival. During the year before repeat spawning, E2 levels increased to a peak 10 weeks before spawning, and then declined (Chapter 2). It is possible that E2 levels at an earlier time point could indicate reproductive investment better than levels at spawning. However, E2 levels over the year before repeat spawning were not significantly correlated with any measure of reproductive performance at repeat spawning (Table 3.11), suggesting that E2 levels may not be a good proxy for reproductive investment.

Relationship between Maiden Reproductive Performance and Rematuration

Consecutive rematuration was 30% for fish surviving to 30 weeks post-spawning (40% in 2016). Similarly, rematuration was estimated at 38% in 2002-2004 for wild Snake River steelhead surviving to make a consecutive repeat spawning migration after being tagged as out-migrating kelts at LGR (Keefer et al. 2008). Consecutive maturation averaging near 60% is typical of wild kelt reconditioning projects in the Columbia River Basin, somewhat higher than proportions found in DNFH-origin kelts (Hatch et al. 2017).

The present study did not find any significant relationships between measures of physiological condition and consecutive rematuration, suggesting that the reproductive decision is not condition-dependent at time of spawning. There was also no support for a life history trade-off between consecutive rematuration and reproductive investment (TEM) at maiden spawning. Interestingly, consecutive rematuration was positively related to maiden TEM, consistent with the possibility that both TEM and consecutive maturation were positively regulated by condition at time points before spawning. Individual fish in better condition would both invest more in TEM and be more likely to have initiated rematuration as a consecutive spawner before maiden spawning. These fish would then have begun investment of energy into the second batch of developing oocytes before maiden spawning, which would differentially reduce energy stores versus skip trajectory fish, potentially resulting in no measurable difference in condition at spawning. Evidence of the growth of a second batch of developing oocytes prior to ovulation of the imminent batch has been found in rainbow trout and steelhead (De Mones et al. 1989; Penney and Moffitt 2014a). Support for part of this model is provided by the positive relationships found between energy reserves early in the year before repeat spawning and reproductive performance at repeat spawning (next section). In contrast to our results, large scale analysis seeking to explain the evolution of senescence in Pacific salmonids found increased reproductive investment and egg size to be correlated with a decreased likelihood of repeat breeding (Crespi and Teo 2002).

Condition-Dependent Reproductive Performance at Repeat Spawning

In Chapter 2, measures of homeostatic ability (TG and growth) were greater in skip than consecutive fish during the first 0-20 weeks of the year prior to repeat spawning, whereas measures of energetic reserves (ML, K) were greater in skip spawners over the entire maturation year. Skip spawners made substantially greater reproductive investment (TEM) than consecutive spawners, as detailed in Chapter 1. Taken together, these results indicate that physiological condition during the year prior to repeat spawning is related to reproductive investment at repeat spawning. These findings are supported by the present analysis of condition-dependent reproductive performance at consecutive repeat spawning. IEM was most strongly positively correlated with growth rates (mass, FL) and TG at 10 weeks after maiden spawning, TEM was most strongly positively correlated with mass growth rate, TG, and K at 20 weeks after maiden spawning, and fecundity was most strongly positively correlated with growth rates (mass, FL) and TG at 20 weeks after maiden spawning, although the correlations with fecundity were not quite significant. These findings suggest that, among fish on the consecutive spawning trajectory, egg size is set first based on condition at approximately 10 weeks after spawning, and then fecundity (and consequently TEM) is set based on condition at approximately 20 weeks after spawning. A study in rainbow trout found that reproductive effort was impacted by feed restrictions in the period close to a year prior to spawning, but not at time periods approaching repeat spawning (Bromage et al. 1992). Similarly, feed restriction implemented prior to the theorized critical period for maturation negatively impacted reproductive investment in lake charr in terms of both ovarian lipid density and fecundity (Henderson and Wong 1998), which was not the case for feed restriction implemented later during oogenesis. The present data suggest that not only is reproductive investment plastic based on physiological condition, but that physiological condition during early- to mid- oogenesis will impact the maternal and offspring generations differently through microevolutionary, intergenerational trade-offs (Stearns 1992). Restricted energy during early oogenesis will reduce egg size, potentially detrimental to both offspring survival and maternal fitness, yet restricted energy during mid-oogenesis will reduce offspring number, potentially detrimental to maternal fitness, but beneficial for the survival of individual offspring via reduced competition. In contrast to the present results, condition at breeding in female albatross was associated with rematuration decision but not reproductive performance (Crossin et al. 2012), likely due in part to different physiological mechanisms regulating energy allocation between reproductive and somatic processes than in the present model.

Management Implications

The ability to access energy and maintain osmotic balance, measured as TG and OS in steelhead in the weeks prior to spawning, are particularly important to post-spawning survival. Conditions in the river prior to spawning, as well as hatchery holding practices, can be expected to favor post-spawning survival and consecutive rematuration. The relationships between physiological condition, post-spawning survival, and rematuration may differ between summer-run steelhead, which hold in freshwater for many months prior to spawning, as compared to winter-run steelhead, which enter freshwater often just prior to spawning. Commonly utilized condition measures that serve to indicate energetic reserves, such as condition factor and muscle lipids, may not reliably predict post-spawning survival in various taxa as well as functional measures indicating the ability of the individual to access energy and/or maintain homeostasis, based on the present results. Increasing our understanding of condition-dependent traits, and relevant measures of condition, may provide clues as to how populations will react to a changing environment (Hutchings 2011).

No evidence was observed for a trade-off between maiden reproductive investment and post-spawning survival (Stearns 1992), providing no support for the idea that iteroparity selects for fish with poorer maiden reproductive investment, as was hypothesized for winter-run steelhead in Hood River (Christie et al. 2018). Thus, kelt reconditioning would not be expected to select for fish with poorer reproductive investment, a concern which has been raised about reconditioning programs (Abrahamse and Murdoch 2014). Females with greater maiden reproductive investment were more likely to mature as consecutive spawners, suggesting that both reproductive investment and consecutive spawning may be condition-dependent traits, and raising the key question of when condition influences these outcomes. Effectively, kelt reconditioning as a recovery effort, and/or managing for increased rates of iteroparity, should not be expected to select for high nor low reproductive investment, nor either life history, but rather should increase life history diversity and increase population stability during bottleneck years (Moore et al. 2014).

This study provides evidence of modulation of reproductive investment, and modulation of energy allocation within reproduction, based on physiological condition early during oogenesis in steelhead. This provides a mechanism linking ocean and other environmental conditions with reproductive performance in salmonids and potentially other species of management interest. As with plasticity in maturation schedules (*i.e.*, consecutive and skip spawning), plasticity in reproductive effort (egg size, egg number) related to physiological condition during oogenesis should be incorporated into recruitment models, particularly in a changing environment.

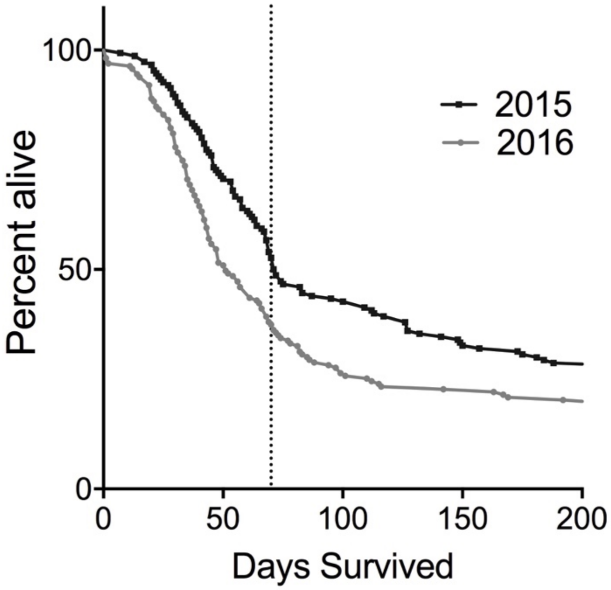

FIGURE 3.1. Survival of female steelhead (N=314) following maiden spawning in 2015 and 2016.

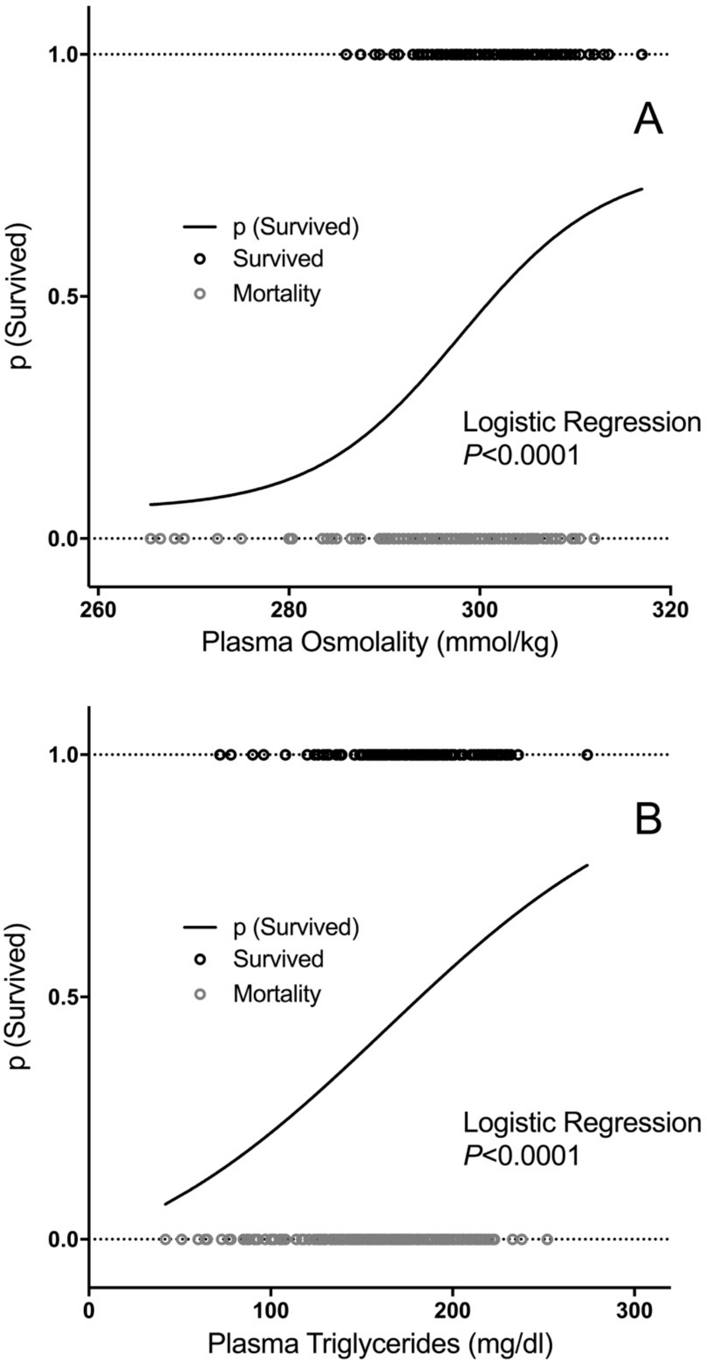

FIGURE 3.2. Plasma osmolality and plasma triglycerides as univariate predictors of survival to 10 weeks after manual spawning in female steelhead at Dworshak National Fish Hatchery in Ahsahka, Idaho, 2015 and 2016 combined data.

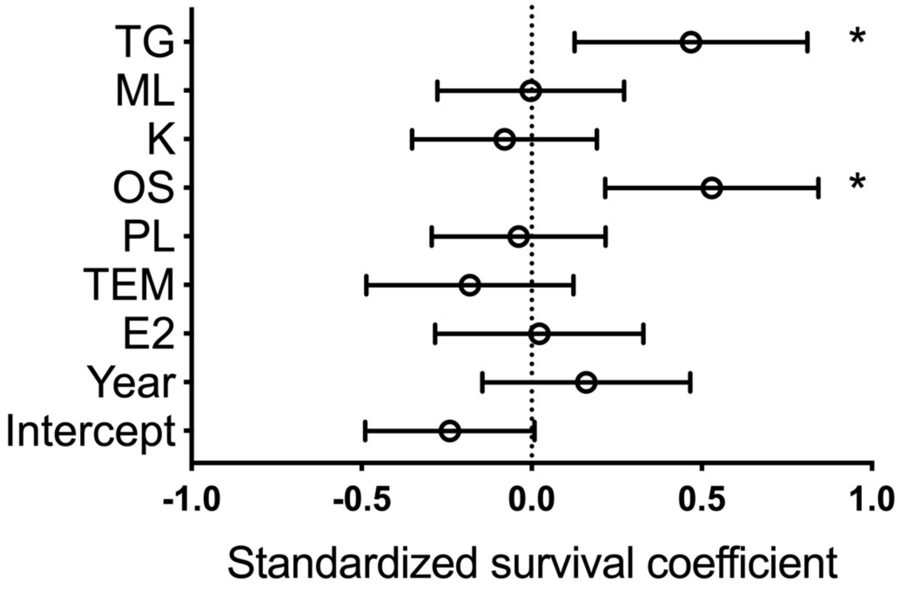

FIGURE 3.3. Standardized coefficients ([x-mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of survival of female steelhead to 10 weeks after manual spawning. Positive estimates indicate greater probability of post-spawning survival. TG: plasma triglycerides (mg/dL), ML: muscle lipid level (%), K: Fulton's condition factor, OS: plasma osmolality (mm/kg), PL: parasite load (# copepods), TEM: mass standardized total egg mass (g), E2: plasma estradiol (ng/mL). Coefficients with confidence intervals that do not overlap zero were statistically significant ($P < 0.05$) and are marked with an asterisk. The intercept is nearly significant ($P = 0.0578$). N=291 of 314 due to missing values (N = 7) and removal of outliers (N = 16) based on the Rout Outlier Test (Q=1%). Survival analysis was conducted on combined data from 2015 and 2016.

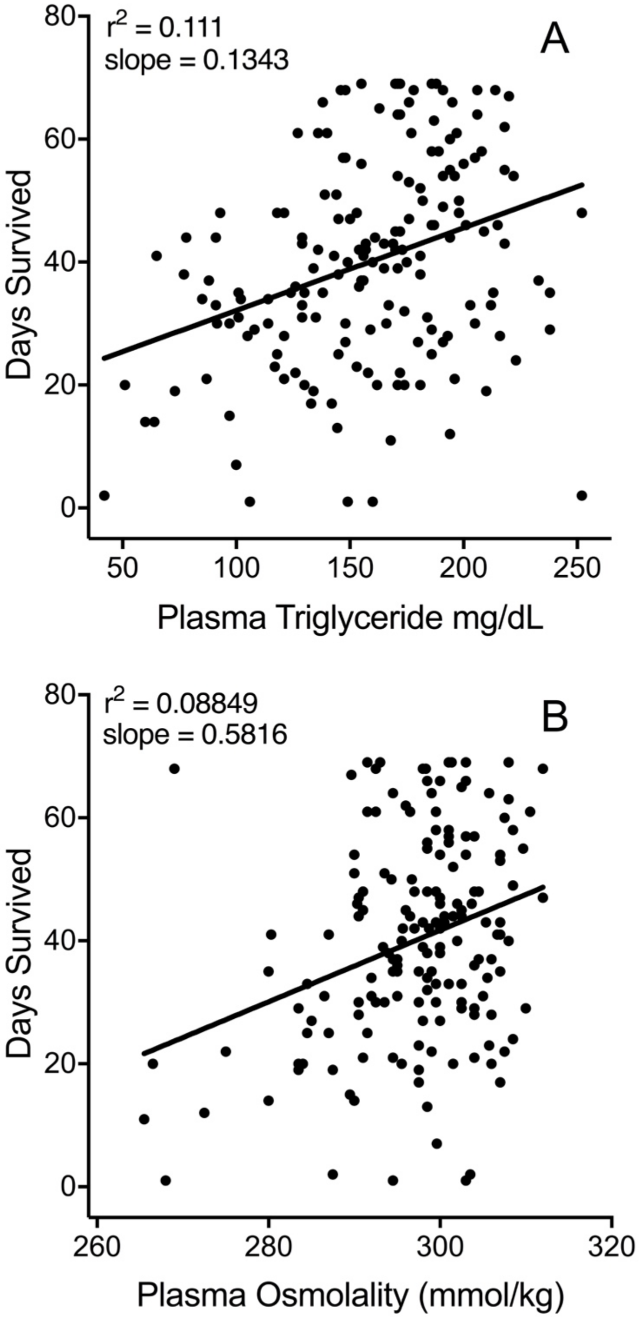

FIGURE 3.4. Plasma triglyceride level and plasma osmolality at spawning as univariate predictors of survival duration in female steelhead during the first 10 weeks following maiden spawning at Dworshak National Fish Hatchery in 2015 and 2016. Regressions are significant (P<0.0001).

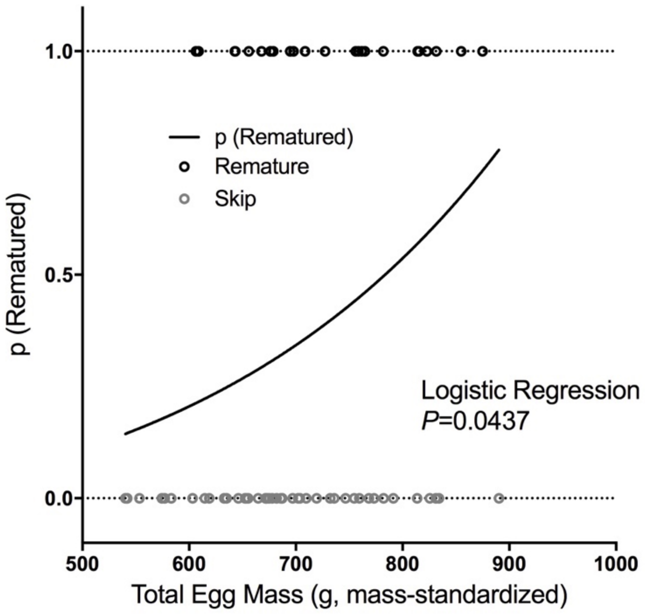

FIGURE 3.5. Total egg mass (g, mass-standardized) from maiden spawning as a univariate predictor of consecutive rematuration in female steelhead at Dworshak National Fish Hatchery in Ahsahka, Idaho, 2015 and 2016 combined data.

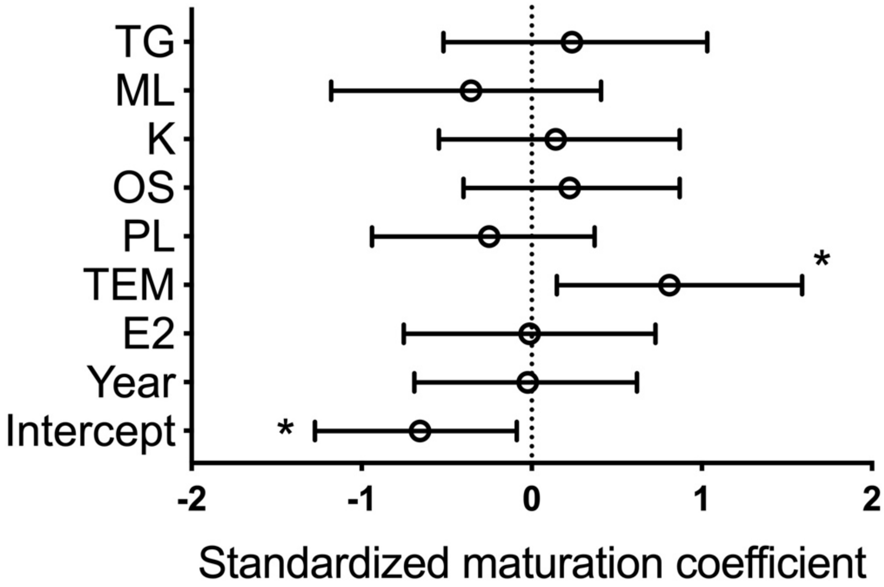

FIGURE 3.6. Standardized coefficients ([x-mean]/SD) with 95% confidence intervals of potential predictors in a multiple logistic regression model of consecutive rematuration of female steelhead after maiden spawning. Positive estimates indicate greater probability of consecutive rematuration. TG: plasma triglycerides (mg/dL), ML: muscle lipid level (%), K: Fulton's condition factor, OS: plasma osmolality (mm/kg), PL: parasite load (# copepods), TEM: mass standardized total egg mass (g), E2: plasma estradiol (ng/mL). Coefficients with confidence intervals that do not overlap zero were statistically significant ($P < 0.05$) and are marked with an asterisk. N = 60 of 72 due to missing values and removal of outliers based on the Rout Outlier Test (Q=1%). Consecutive rematuration analysis was conducted on combined data from 2015 and 2016.

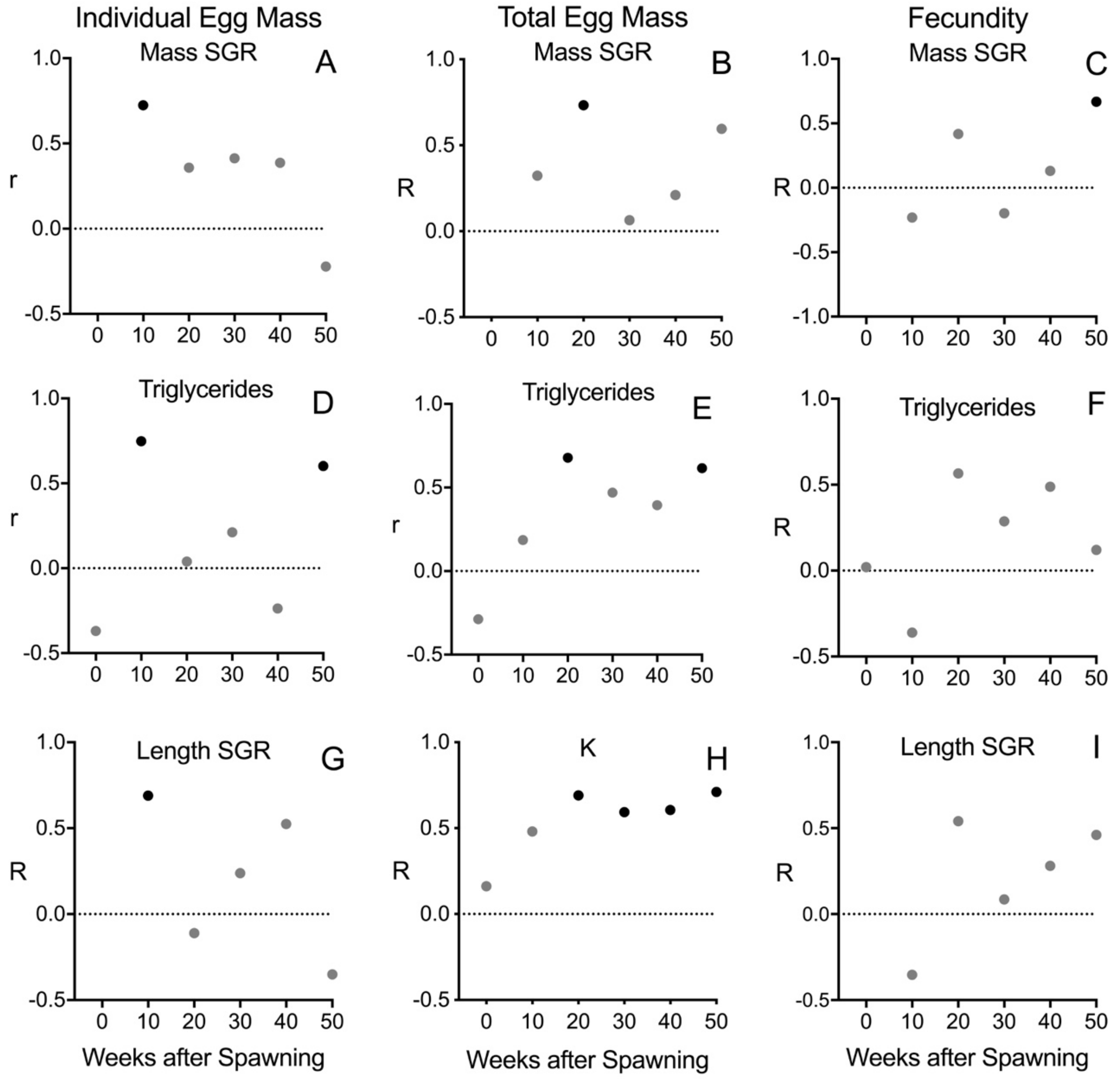

FIGURE 3.7. Correlation coefficients (R) over weeks after maiden spawning for relationships between individual egg mass (A, D, G), total egg mass (B, E, H), and fecundity (C, F, I), with specific growth rate in mass (mass SGR), specific growth rate in fork length (length SGR), plasma triglycerides, and Fulton's condition factor (K) in consecutive repeat spawning female steelhead. Specific growth rates were calculated over the preceding 10-week period. Black points indicate statistically significant linear regressions ($P < 0.05$), and grey points indicate non-statistically significant linear regressions. Fecundity was correlated with mass SGR, plasma triglycerides, and length SGR at 20 weeks after maiden spawning, but these relationships were not quite significant (C, F, and I; $P = 0.1772$, 0.0551, and 0.0693).

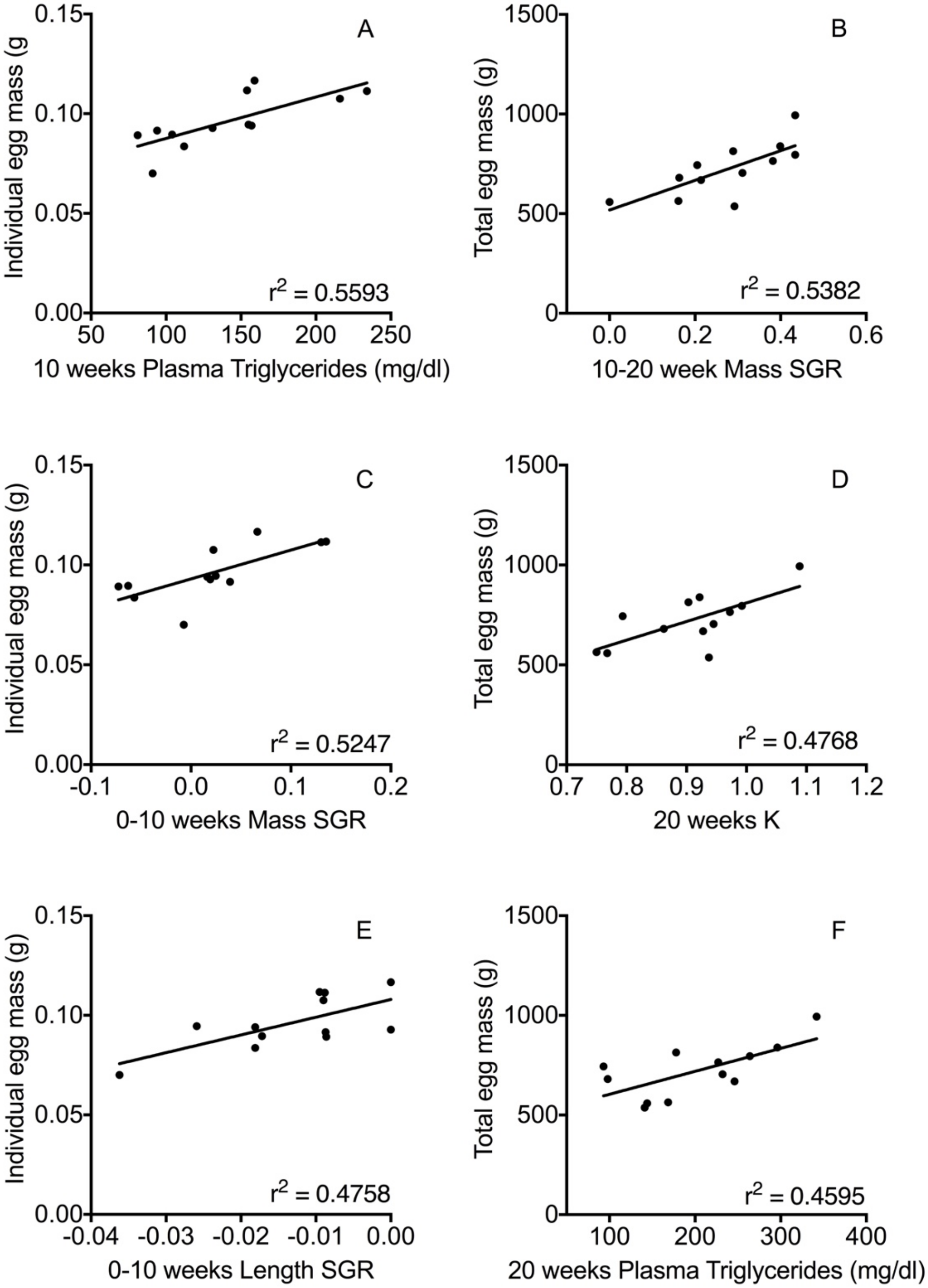

FIGURE 3.8. Relationships between repeat spawning individual egg mass (A, C, E) and total egg mass (B, D, F) with plasma triglycerides (A, F), specific growth rate in mass (B, C), specific growth rate in length (E), or K (D) in consecutive repeat spawning female steelhead at time points when the relationships were significant ($P < 0.05$) and first peaked in strength when compared over time.

TABLE 3.1. Metrics indicating energetic status, homeostatic ability, and reproductive performance at maiden spawning for all female steelhead collected for study from 2015 to 2016. N (%) is provided with mean (SD) values of all metrics for each outcome (survived to 10 weeks versus mortality, matured versus skipped based on E2 at 30 weeks).

			Energetic Status		Homeostatic Ability			Reproductive Performance	
		N (%)	Muscle Lipid Level (%)	Condition K	Plasma Triglycerides (mg/dL)	Plasma Osmolality (mmol/kg)	Parasite Load (# copepods)	Total Egg Mass (g)	Plasma Estradiol-17b (ng/mL)
2015	Survived	80 (53)	0.96 (0.12)	0.758 (0.05)	189.6 (28.4)	303.1 (5.7)	4.0 (2.7)	688.8 (97)	984.8 (327.6)
	Mortality	70 (47)	0.95 (0.13)	0.763 (0.05)	180.8 (33.4)	301.0 (6.1)	4.1 (2.7)	698.0 (116)	958.0 (292.6)
	Matured	13 (9)	0.92 (0.10)	0.743 (0.04)	176.8 (17.4)	303.2 (5.6)	4.0 (2.6)	723.7 (89)	940.4 (312.9)
	Skipped	30 (20)	0.96 (0.13)	0.750 (0.05)	185.8 (19.4)	303.3 (4.8)	3.7 (2.4)	679.9 (80)	976.5 (380.9)
	All	150	0.96 (0.12)	0.760 (0.05)	185.5 (31.0)	302.1 (6.0)	4.0 (2.7)	693.0 (106)	972.4 (311.1)
2016	Survived	62 (38)	0.93 (0.14)	0.779 (0.05)	167.7 (36.1)	300.3 (5.6)	3.9 (2.8)	776 (129)	956.4 (382.5)
	Mortality	102 (62)	0.90 (0.11)	0.763 (0.05)	143.5 (40.2)	294.6 (9.2)	4.3 (2.5)	811.3 (127)	851.1 (307.5)
	Matured	12 (7)	0.90 (0.11)	0.770 (0.04)	183.5 (33.3)	299.7 (5.5)	3.7 (1.8)	743.7 (68)	902.8 (318.8)
	Skipped	18 (11)	0.94 (0.14)	0.777 (0.06)	171.5 (32.0)	299.3 (6.4)	4.6 (3.7)	701.6 (92)	853.4 (181.2)
	All	164	0.91 (0.11)	0.769 (0.05)	152.5 (40.3)	296.7 (8.4)	4.2 (2.6)	798.0 (129)	891.7 (341.1)
Both Years	Survived	142 (45)	0.95 (0.12)	0.767 (0.05)	180.0 (33.6)	301.8 (5.8)	4.0 (2.7)	726.6 (119)	972.4 (351.6)
	Mortality	172 (55)	0.92 (0.12)	0.763 (0.05)	158.5 (41.7)	297.2 (8.6)	4.2 (2.6)	765.6 (135)	895.0 (305.2)
	Matured	25 (8)	0.91 (0.11)	0.756 (0.05)	180.0 (25.8)	301.5 (5.7)	3.8 (2.2)	732.4 (80)	921.6 (309.5)
	Skipped	48 (15)	0.95 (0.13)	0.760 (0.05)	180.5 (25.5)	301.7 (5.8)	4.0 (3.0)	687.9 (84)	932.0 (326.3)
	All	314	0.93 (0.12)	0.765 (0.05)	168.2 (39.7)	299.3 (7.8)	4.1 (2.7)	747.8 (129)	930.5 (329.0)

TABLE 3.2. Whole model results for multiple logistic regression analysis of post-spawning survival in female steelhead in years combined. Fish that died before the first sampling point at 70 days were classified as mortalities (coded as 0). Prior to analysis, covariates were standardized ([x-mean]/SD) in order to assess the relative effects of each parameter. N = 291 due to missing values (N = 7) and outlier removal (N = 16). Significant effects ($P < 0.05$) are bolded.

Effect	Parameter Estimate	Standard Error	χ^2	P-value
Intercept [Survival]	-0.24086217	0.1269621	3.60	0.0578
Year	0.1606668	0.1558857	1.06	0.3027
Triglycerides	0.4687915	0.1746524	7.20	**0.0073**
Muscle lipid	-0.00279237	0.1400080	0.00	0.9841
Condition K	-0.07991895	0.1383801	0.33	0.5636
Osmolality	0.5293059	0.1600113	10.94	**0.0009**
Parasite Load	-0.03816411	0.1305209	0.09	0.7700
Total Egg Mass	-0.18191859	0.1554757	1.37	0.2420
E2	0.0223342	0.1560571	0.02	0.8862

TABLE 3.3. Whole model results for multiple linear regression analysis of survival duration for female steelhead in years combined. Days lived was regressed against predictor variables for fish that died before the first sampling point at 70 days. Prior to analysis, covariates were standardized as in Table 2. N=156 due to missing values (N = 13) and outlier removal (N = 3). Significant effects are bolded.

Effect	Parameter Estimate	Standard Error	t-ratio	Prob>|t|
Intercept [Days Survived]	40.224895	1.412952	28.47	**<0.0001**
Year	1.4297038	1.588125	0.90	0.3695
Triglycerides	4.4385188	1.586608	2.80	**0.0059**
Muscle lipid	2.4025687	1.289991	1.86	0.0646
Condition K	-0.590319	1.369398	-0.43	0.6671
Osmolality	3.1601919	1.452456	2.18	**0.0312**
Parasite Load	1.4043316	1.222431	1.15	0.2525
Total Egg Mass	-0.930838	1.472516	-0.63	0.5283
E2	-0.78957	1.455299	-0.54	0.5883
Year*Triglycerides	-3.2989	1.461476	-2.26	**0.0255**
Year*Osmolality	3.2818172	1.553802	2.11	**0.0364**

TABLE 3.4. Whole model results for multiple logistic regression analysis of maturation for female steelhead in years combined. Maturation status was assigned based on plasma estradiol level at 30 weeks (maturing coded as 1). Prior to analysis, covariates were standardized as in Table 2. N = 60 due to missing values (N = 5) and outlier removal (N = 7). Significant effects are bolded.

Effect	Parameter Estimate	Standard Error	χ^2	P-value
Intercept [Maturation]	-0.6567599	0.2992295	4.82	**0.0282**
Year	-0.0226766	0.3296143	0.00	0.9452
Triglycerides	0.23741929	0.3886998	0.37	0.5413
Muscle lipid	-0.3564938	0.3990727	0.80	0.3717
Condition K	0.14084619	0.3543409	0.16	0.6910
Osmolality	0.22438056	0.3191371	0.49	0.4820
Parasite Load	-0.2498512	0.3266694	0.58	0.4444
Total Egg Mass	0.80928209	0.3610577	5.02	**0.0250**
E2	-0.0132513	0.3716522	0.00	0.9716

TABLE 3.5. Correlation coefficients (R) (below diagonal) for all female steelhead collected for study from 2015 to 2016 (n = 314). Correlations were based on transformed data (ML = arcsine-square root; E2 = $\log_{10}$). P-values for correlations are above diagonal. Significant correlations ($P < 0.05$) are bolded.

	Triglycerides (mg/dL)	Muscle Lipid Level (%)	Condition K	Osmolality (mmol/kg)	Parasite Load (number of copepods)	Total Egg Mass (g)	E2 (ng/mL)
Triglycerides	x	**<0.0001**	**0.0141**	**<0.0001**	0.5933	**0.0004**	**<0.0001**
Muscle Lipid Level	**0.384**	x	**0.0489**	**0.0154**	0.7491	**0.0202**	**<0.0001**
Condition K	**0.139**	**0.113**	x	0.2556	0.1960	**<0.0001**	0.1808
Osmolality	**0.389**	**0.140**	0.065	x	0.2903	**<0.0001**	**0.0005**
Parasite Load	-0.031	0.018	-0.074	-0.061	x	**0.0001**	0.1402
Total Egg Mass	**-0.201**	**-0.132**	**-0.244**	**-0.220**	**0.214**	x	0.1647
E2	**0.469**	**0.326**	0.076	**0.198**	-0.085	0.079	x

TABLE 3.6. Results of univariate logistic regression models tested to predict female steelhead survival to 10 weeks after maiden spawning. Survival was coded as 1. Univariate models are shown when $P < 0.25$ for a univariate relationship in a given year and then for that same effect in each other year (or years combined) for comparison. Odds ratio values <1 represent “negative odds” or likelihood of non-rematuration as the effect increases in value. Significant effects ($P < 0.05$) are bolded.

	Effect	df	χ^2	*P*	Odds ratio	95% CI
2015	Osmolality	1	4.40	**0.0359**	3.449132	0.5369-22.15779
	Triglycerides	1	2.89	0.0891	4.218233	0.385319-46.17863
	Condition K	1	0.43	0.5100	0.34912	0.041309-2.95056
	E2	1	0.15	0.6962	1.302279	0.132607-12.78914
	Total Egg Mass	1	0.28	0.5954	0.456986	0.055577-3.757627
	Muscle Lipid	1	0.29	0.5926	1.093447	0.186193-6.421422
2016	Osmolality	1	15.29	**<0.0001**	45.10924	3.138067-648.4385
	Triglycerides	1	12.63	**0.0004**	23.83648	1.856865-305.9875
	Condition K	1	3.89	**0.0486**	1.40327	0.133512-14.74894
	E2	1	3.24	0.0720	0.785226	0.036113-17.07343
	Total Egg Mass	1	2.88	0.0897	0.377656	0.05058-2.819756
	Muscle Lipid	1	1.83	0.1763	1.110909	0.200052-6.169004
Both Years	Osmolality	1	23.90	**<0.0001**	32.39213	4.125691-254.3211
	Triglycerides	1	20.88	**<0.0001**	15.4575	2.093281-114.1434
	Condition K	1	0.12	0.7279	0.621488	0.123711-3.12218
	E2	1	0.75	0.3878	1.180187	0.122063-11.41088
	Total Egg Mass	1	6.97	**0.0083**	0.396033	0.083927-1.868789
	Muscle Lipid	1	3.39	0.0654	0.984414	0.210247-4.609196
	Year	1	8.21	**0.0042**	1.3787	0.748598-2.539165

TABLE 3.7. Correlation coefficients (R) (below diagonal) for mortalities within 10 weeks after spawning in female steelhead (n = 172) are in the lower left of table. Correlations were based on transformed data (ML = asin-sqrt; E2 = log10). P-values for correlations are above diagonal. Significant correlations ($P < 0.05$) are bolded.

	Triglycerides (mg/dL)	Muscle Lipid Level (%)	Condition K	Osmolality (mmol/kg)	Parasite Load (number of copepods)	Total Egg Mass (g)	E2 (ng/mL)
Triglycerides	x	**<0.0001**	0.0590	**<0.0001**	0.7142	**0.0002**	**<0.0001**
Muscle Lipid Level	**0.304**	x	0.1943	0.2715	0.3574	0.0549	**0.0102**
Somatic K	0.146	0.102	x	0.4566	0.2532	**<0.0001**	0.7932
Osmolality	**0.410**	0.086	0.058	x	0.6495	**0.0156**	**0.0007**
Parasite Load	-0.028	-0.072	-0.089	-0.035	x	**0.0154**	0.2357
Total Egg Mass	**-0.280**	-0.151	**-0.329**	**-0.187**	**0.188**	x	0.7530
E2	**0.485**	**0.201**	-0.021	**0.261**	-0.093	-0.025	x

TABLE 3.8. Results of univariate linear regression models to predict survival duration (days) for female steelhead that died during the first 10 weeks after maiden spawning. Univariate models are shown when $P < 0.25$ for a univariate relationship in a given year and then for that same effect in each other year (or years combined) for comparison. Significant effects ($P < 0.05$) are bolded.

	Effect	df	t-ratio	*P*
2015	Triglycerides	68	3.35	**0.00133**
	Osmolality	68	0.21	0.83810
	Total Egg Mass	68	-1.16	0.25086
	E2	68	1.31	0.19395
	Parasite Load	67	0.71	0.48170
2016	Triglycerides	100	2.31	**0.0230**
	Osmolality	99	3.73	**0.0003**
	Total Egg Mass	97	-0.30	0.7680
	E2	97	0.82	0.4157
	Parasite Load	99	1.50	0.1360
Both Years	Triglycerides	169	4.62	**<0.0001**
	Osmolality	168	4.06	**<0.0001**
	Total Egg Mass	166	-1.96	0.0517
	E2	166	1.86	0.0645
	Parasite Load	167	1.43	0.1558
	Triglycerides	169	-2.71	**0.0075**

TABLE 3.9. Correlation coefficients (R) (below diagonal) for female steelhead that survived to week 30 (n = 73) in lower left half of table. Correlations were based on transformed data (ML = asin-sqrt; E2 = log10). P-values for correlations are above diagonal. Significant correlations ($P < 0.05$) are bolded.

	Triglycerides (mg/dL)	Muscle Lipid Level (%)	Condition K	Osmolality (mmol/kg)	Parasite Load (number of copepods)	Total Egg Mass (g)	E2 (ng/mL)
Triglycerides	x	**<0.0001**	0.5740	**0.0435**	0.5184	0.3776	**0.0009**
Muscle Lipid Level	**0.549**	x	0.4451	0.1166	0.5686	0.3411	**<0.0001**
Condition K	0.069	0.091	x	0.5153	0.2704	**0.0206**	0.4126
Osmolality	**0.251**	0.192	0.080	x	0.1999	0.2986	0.4662
Parasite Load	0.080	0.068	-0.132	-0.159	x	0.3958	0.8113
Total Egg Mass	-0.110	-0.116	**-0.278**	-0.132	0.105	x	0.1402
E2	**0.393**	**0.516**	0.099	0.090	-0.029	0.1820	x

TABLE 3.10. Results of univariate logistic regression models to predict consecutive rematuration of female steelhead after maiden spawning. Univariate models are shown when $P < 0.25$ for a univariate relationship in a given year and then for that same effect in each other year (or years combined) for comparison. Odds ratio values <1 represent "negative odds" or likelihood of non-rematuration as the effect increases in value. Significant effects ($P < 0.05$) are bolded.

	Effect	df	χ^2	*P*	Odds ratio	95% CI
2015	Total Egg Mass	1	2.36	0.1241	117.2602	1.077105-12765.65
	Muscle Lipid	1	0.96	0.3278	2.498202	0.019924-313.2479
	Triglycerides	1	1.83	0.1756	0.078502	0.000461-13.37439
2016	Total Egg Mass	1	1.52	0.2172	121.5049	0.272936-54091.28
	Muscle Lipid	1	0.63	0.4258	0.050838	0.000191-13.50347
	Triglycerides	1	0.92	0.3382	19.54283	0.059273-6443.432
Both Years	Total Egg Mass	1	4.07	**0.0437**	28.29456	1.521568-526.1559
	Muscle Lipid	1	1.80	0.1795	0.180527	0.00422-7.7122035
	Triglycerides	1	0.01	0.9340	2.851269	0.098832-82.2579

TABLE 3.11. Correlation coefficient (R) of relationships between measures at each time point with individual egg mass, total egg mass, and fecundity measured at repeat spawning in female steelhead. Bolded R values are significant ($P < 0.05$). Data shown for SGR is over 10-week ranges (*i.e.*, growth from weeks 0-10). N=12 consecutive repeat spawners from spawn year 2015.

Repeat Reproductive Performance	Sampling Point	MSGR	LSGR	TG	E2	ML	K
Individual Egg Mass	Spawning	-	-	-0.36919	0.04996	-0.49224	0.06760
	10 Weeks	**0.72436**	**0.68978**	**0.74786**	0.00072	0.35128	0.32573
	20 Weeks	0.35805	-0.11077	0.03912	0.26900	0.56018	0.47413
	30 Weeks	0.41316	0.23780	0.21166	0.15970	0.45935	0.57009
	40 Weeks	0.38704	0.52412	-0.23688	0.00200	0.49487	**0.58395**
	50 Weeks	-0.22154	-0.35100	**0.60166**	0.08780	0.52163	0.54065
Total Egg Mass	Spawning	-	-	-0.28775	0.00050	-0.51643	0.16214
	10 Weeks	0.32265	0.12227	0.18569	-0.48146	-0.10630	0.48000
	20 Weeks	**0.73362**	0.52602	**0.67786**	0.47021	0.56921	**0.69051**
	30 Weeks	0.06447	0.28773	0.46936	0.09101	0.38105	**0.59287**
	40 Weeks	0.45365	**0.73000**	0.39484	-0.07677	0.46271	**0.60605**
	50 Weeks	0.59565	0.22213	**0.61563**	0.43440	0.34322	**0.71099**
Fecundity	Spawning	-	-	0.02072	-0.13364	-0.11082	0.17516
	10 Weeks	-0.23117	-0.35285	-0.36111	-0.41122	-0.30188	0.15713
	20 Weeks	0.41725	0.54093	0.56586	0.06789	0.12526	0.26753
	30 Weeks	-0.19703	0.08621	0.28660	-0.18363	0.04873	0.12853
	40 Weeks	0.13119	0.28100	0.48765	-0.03984	0.09320	0.13004
	50 Weeks	0.66746	0.46033	0.12112	0.20698	-0.03676	0.24862

CONCLUSIONS

Physiological trade-offs

Egg size was negatively correlated with egg number in consecutive and maiden spawners. A similar, insignificant trend was observed in skip spawners (Chapter 1). Consecutive spawners were more energy restricted than skip spawners during the first 0-20 weeks of the rematuration year (Chapter 2). This suggests that energy allocation between offspring is condition-dependent during a sensitive period almost a year before spawning, and results in a trade-off between egg size and egg number at repeat spawning. This is further supported by the positive correlations found between egg size and total reproductive effort at repeat spawning with TG and growth at 10 and 20 weeks after maiden spawning (Chapter 3). These results from steelhead suggest that the effect of energy balance during oogenesis on egg size and fecundity may outweigh previously hypothesized mechanisms influencing egg size and fecundity; the adjustment of fecundity to meet an optimum egg size (Sargent et al. 1987) and the egg-size fecundity relationship resulting from maternal experience in the tributary as a juvenile (Thorpe et al. 1984; Morita et al. 1999).

The adaptive nature of condition-dependent egg size and the trade-off between egg size and egg number depends on ecological and environmental context (e.g., competition, resource availability, substrate size) and generational perspective. Individuals of the parental generation benefit from having a greater number of offspring, whereas individuals of the offspring generation benefit more from starting life with more resources and fewer competitors (Stearns 1992). The ability to reduce investment into each offspring when resources are scarce without having to reduce offspring number is advantageous for individuals that survive to spawn. There are lower and upper limits for egg size, outside of which are maladaptive in given environments. For example, in benthic spawning fishes, modulation of investment resulting in eggs too small or too large for the substrate could be maladaptive. Reduction of egg size below a lower limit, such that offspring are not competitive with conspecifics or able to acquire resources from the environment, would be maladaptive. The ability to make larger eggs when resources are available during early oogenesis, resulting in offspring that are highly competitive with larger buccal gapes for feeding and greater swimming ability, would be likely considered adaptive. The benefits of large egg size are found to increase in environments of decreasing quality (Rollinson and Hutchings 2013). Further study is required to investigate whether and how condition-dependent determination of egg size early in oogenesis is adaptive, or alternatively whether this is a constraint imposed by the underlying physiology.

Neither physiological condition at the time of maiden spawning, post-spawning maturation, nor the rematuration decision were observed to trade-off with maiden reproductive performance, despite energy depletion in this population at maiden spawning (Chapter 3). Results presented here are inconsistent with theoretical suggestions that iteroparity selects for reduced maiden reproductive investment (Abrahamse and Murdoch 2014; Christie et al. 2018).

Condition-dependent strategies

Survival after maiden spawning was positively related to TG levels and osmoregulatory ability (*i.e.*, OS), but not to K or ML levels (Chapter 3). TG and OS likely serve as better indicators of physiological condition and predictors of survival because they provide information on the ability of the individual to access stored energy and to maintain osmotic homeostasis, rather than simply providing a measure of the energy stores of the individual.

Consecutive maturation following maiden spawning was not directly observed to be condition-dependent based at maiden spawning but was positively related to maiden reproductive investment (Chapter 3). This result suggests that both maiden reproductive investment and consecutive maturation were positively regulated by a common factor. Although the identity of this common factor is not known, it is reasonable to speculate that condition at time points prior to maiden spawning positively regulates both maiden reproductive investment and consecutive spawning, consistent with a condition-dependent maturation decision occurring prior to maiden spawning. Additionally, consecutive spawners had greater TG and growth during early oogenesis (10 weeks after maiden spawning), though this is likely a consequence, rather than a cause, of the maturation decision (Chapter 2).

Finally, aspects of reproductive performance at consecutive repeat spawning were most strongly positively correlated with TG and growth during the first 10 weeks after maiden spawning (IEM), and during the second 10 weeks after maiden spawning (TEM) (Chapter 3). Likely, TG and growth were again predictors of condition-dependent outcomes due to the insight they provide into functional aspects of condition: both TG levels and mass growth provide integrated indicators of the energy balance of individual fish.

Critical period hypothesis of maturation

Consecutive rematuring fish had greater growth in mass during the first 0-10 weeks after maiden spawning, and greater circulating TG at 10 weeks after maiden spawning than did skip spawners. This indicates faster recovery for the consecutive spawning life history trajectory and that the rematuration decision must be made prior to 10 weeks after maiden spawning (Chapter 2). No relationship was observed between the rematuration decision and physiological condition at the time of maiden spawning. However, the probability of rematuration increased with greater maiden reproductive investment, suggesting that both maiden reproductive investment and consecutive rematuration are condition-dependent based on condition at a time point prior to maiden spawning (Chapter 3). Despite slower recovery from maiden spawning, skip spawning females had substantially greater overall energy reserves during rematuration (Chapter 2), which increased reproductive investment and shifted allocation toward the production of larger eggs (Chapter 1). Skip spawning females also upregulated plasma E2 10 weeks earlier than consecutive spawners (at 10 weeks into the rematuration year, rather than at 20 weeks), indicating a greater burden of maiden spawning on rematuration in consecutive spawners. It is probable that rematuration is initiated even earlier than during the first 10 weeks after maiden spawning (Chapter 2), consistent with results from Chapter 3, which suggest that the maturation decision occurs prior to one year before spawning.

Energy allocation

Mass-standardized egg size was smaller and fecundity greater in consecutive spawners compared to maiden spawners, likely due to comparatively greater energy restriction in consecutive spawners during early oogenesis (Chapter 1). Skip spawners had the greatest reproductive investment (absolute and mass-standardized total egg mass) and the greatest individual egg mass (Chapter 1), presumably due to having the greatest quantity of energy reserves during early oogenesis (Chapter 2). Based on these results, the first 10-week interval after maiden spawning was identified as a potential sensitive period for determining egg size based on energy availability. Further, egg size in consecutive spawners was most strongly correlated with growth and TG during the first 10-week interval after maiden spawning, and total reproductive investment was most strongly correlated with TG and growth during following 10 weeks interval (Chapter 3). This is further evidence that egg size and reproductive investment are phenotypically plastic and related to measures of physiological condition. These results are consistent with findings in steelhead and Atlantic salmon, where egg size differs across life history types (Quinn et al. 2011; Reid and Chaput 2012), which presumably differ in schedules of energy acquisition. However, previous studies have not indicated the timing of sensitive periods for determination of energy allocation for reproduction, or the egg size versus fecundity relationship, which were identified as key questions in salmonid biology (Bromage et al. 1992; Campbell et al. 2006a). Figure 4.1 illustrates a conceptual model for the rematuration and energy allocation sensitive periods incorporating evidence presented in this dissertation.

Management Implications

Repeat spawners are important to steelhead populations (Moore et al. 2014), increasing stability during years when environmental events (e.g., drought) cause reduced populations, as having multiple cohorts available to interbreed spreads the risks associated with poor ocean or river conditions. Releasing captive-reconditioned wild steelhead, particularly with more and larger eggs, and greater total egg mass per fish, will increase the productivity of steelhead populations. Larger eggs are increasingly beneficial in variable and poor-quality environments and more eggs would be expected to increase the numbers of adult offspring. Assuming reproductive effort is allocated appropriately between fecundity and egg size, the increase in total egg mass provides a means to quantify the productivity increase of consecutive and skip spawners (1.28- and 1.52-fold over that of maiden spawners, respectively, in this study). Spawn timing was not substantially changed by reconditioning, suggesting that after release, reconditioned steelhead will be able to find mates and spawn at a time that is appropriate for the population. Optimal feeding conditions during the period after capture would be expected to result in the maximal reproductive performance of kelts released to spawn. Conversely, continued feeding of the fish into the fall does not appear to translate into increased reproductive performance, but it could be used to increase the somatic energy stores required for migration.

This dissertation provides the first mechanistic look at the physiological factors involved in steelhead rematuration. Mass growth and available energy, measured as triglyceride levels in the plasma, and the timing of when these factors diverge between females of differing post-spawning life history decisions, occurred by 10 weeks after maiden spawning. Measures of physiological condition are shown here to relate to survival as well as reproductive performance by providing insight into their ability to maintain homeostasis. In this study, the ability to access energy reserves and osmoregulate were measured using samples taken from the blood plasma. Traditional measures of physiological condition that indicate energy status (e.g., K and ML) were not able to predict post-spawning survival or reproductive effort. Finally, plasticity in reproductive effort (e.g., egg size, egg number) related to physiological condition during oogenesis should be incorporated into recruitment estimates in population models used for management.

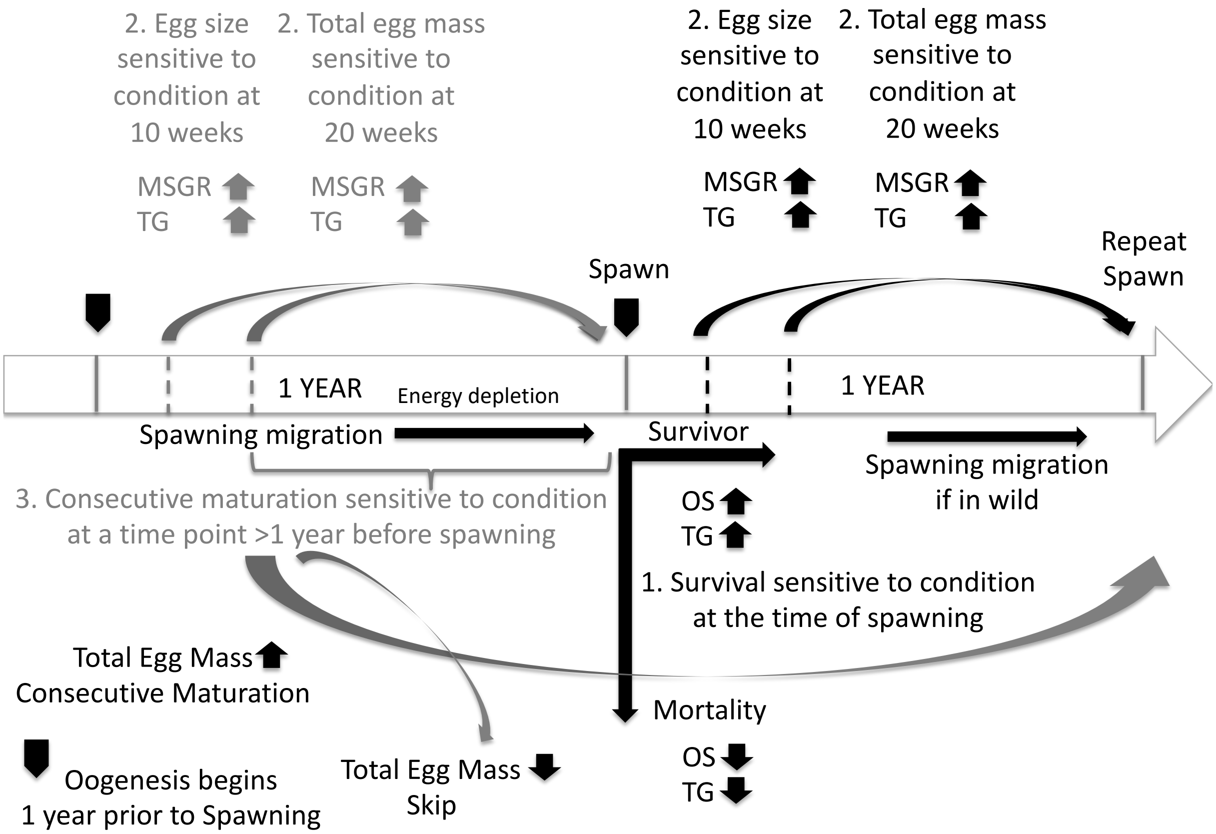

Figure 4.1. A conceptual model for the regulation of survival, rematuration, and reproductive performance by condition during sensitive periods derived from female steelhead: (1) survival, positively related to plasma osmolality (OS) and plasma triglycerides (TG) at the time of maiden spawning, (2) mass specific growth rate (MSGR) and TG, positively related to reproductive performance measures at repeat spawning, and (3) consecutive spawning, positively related to total egg mass at maiden spawning, which is hypothesized to be sensitive to physiological condition 30 weeks prior to maiden spawning. Symbols and terms in black indicate relationships directly supported by data presented herein, and symbols and terms in grey indicate deduced or hypothesized relationships.

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