Do freshwater growth opportunities and migration barriers limit recovery of imperiled anadromous *Oncorhynchus mykiss* populations?

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Abstract

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Steelhead (*Oncorhynchus mykiss*) populations in the Puget Sound Distinct Population Segment are far below their historic abundance and are currently listed as Threatened under the Endangered Species Act. Despite the declines in steelhead abundance, resident *O. mykiss* populations appear to be relatively abundant and little is known about how resident *O. mykiss* above anadromous barriers interact with steelhead downstream. This study evaluated the dispersal and gene flow of above-barrier *Oncorhynchus mykiss* in the Duckabush and Hamma Hamma Rivers, Washington into below-barrier reaches that were accessible to anadromous salmonids. As well as the abundance, sex ratio and annual survival rate of *O. mykiss* above and below the barriers, and their influence on gene flow. We also evaluated the annual and summer growth potential of *Oncorhynchus mykiss* at various life stages (age 1-2, age 2-3 and age 3-4) above and below anadromous barriers in rivers to determine whether growth was limited by environmental conditions within the watershed, and if so, whether the greatest influence over growth was the availability of food or the thermal regime. Although a three year mark-recapture study did not detect any dispersal from the above the barrier populations into the below-barrier populations, microsatellite DNA analysis detected gene flow from the above-barrier populations into the below barrier populations. Gene flow was more pronounced in the Duckabush River, where 90% of the below-barrier fish were assigned to the above-barrier population. The Hamma Hamma River populations were more distinct, with 41% of the below-barrier population assigning to the above-barrier population. Abundance was estimated for *O. mykiss* $\geq$130 mm FL in the Duckabush River, with 150 trout per km in the lower below-barrier reach, 907 per km in the middle reach below the barrier and 1,165 per km above the barrier. Catch per unit effort in the Duckabush River above the barrier was 5.4 fish per angler hour compared to 2.0 in the Hamma Hamma River above the barrier, suggesting much lower relative abundance in the Hamma Hamma River. These results indicate that the gene flow from the above-barrier populations into the below-barrier population is strongly tied to abundance. Annual survival rates were low ranging from 5.7% to 21.4%, with 2013-2014 generally having better survival than 2014-2015. Survival declined with age in the middle (below-barrier) and above-barrier reaches, but improved with age in the lower reaches.

Bioenergetic modeling was used with empirical data on growth, diet, and thermal experience to estimate the feeding rate, growth trajectory and the amount of food required to attain the growth observed between life stages in the, middle and lower reaches below the barrier and above the barrier in both watersheds. Model outputs indicated that consumption rates were low throughout the watersheds, ranging from 20% (age-2) to 32% (age-4) of their maximum consumption rate (%Cmax) annually. Food was insufficient to support adequate growth for age-2 and older *O. mykiss* in the middle and upper reaches in the Duckabush and Hamma Hamma Rivers. Food availability and quality appeared to limit growth of juvenile *O. mykiss* in the middle and upper reaches in the Duckabush and Hamma Hamma Rivers with little scope for growth beyond age-2, making these populations vulnerable to density-dependent effects on growth and production. Prey supply and quality were less limiting in the lower reaches below the barriers, largely due to marine subsidies from spawning salmon; however it appears that other factors might limit the populations in these reaches. These results suggest that high mortality rates in freshwater caused by delayed smoltification due to low growth rates may be significant factors limiting these steelhead populations. With steelhead facing declines across their range, this study highlights the importance of identifying factors limiting growth and survival during critical life stages in freshwater.

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Chapter 1: Review of the life history patterns, freshwater growth limitations, status and management of wild Puget Sound steelhead and rainbow trout (*Oncorhynchus mykiss*).

The salmonid *Oncorhynchus mykiss* displays highly diverse, complex and plastic life history patterns across their native range from the Kamchatka Peninsula in Russia to Southern California in the USA. Compared with other species of the genus *Oncorhynchus*, *O. mykiss* displays a much wider variety of life history diversity, which ranges from stream resident (rainbow trout) to fully anadromous (steelhead). This life history variation serves as an evolutionary adaptation to exploit available resources and acts as a buffer to environmental conditions and natural disasters, providing increased stability to the overall population. However, tradeoffs associated with different life history pathways can influence the growth, survival and reproductive success of individuals. For rainbow trout, the chances of survival to maturity are much higher, although growth in streams may be limited by space, temperature, or prey, resulting in lower fecundity. In contrast, steelhead which enter marine waters as smolts typically have much lower survival rates, but significantly higher growth and fecundity.

Life History Patterns of *Oncorhynchus mykiss* in the Puget Sound Region

In the Puget Sound region *O. mykiss* represent a Distinct Population Segment (DPS) of the species based on genetic structure, geography, and to some degree, life history patterns (Hard et al. 2007). *O. mykiss* in the region exhibit rainbow trout as well as winter- and summer-run steelhead life histories. Puget Sound steelhead spend between one and three years at sea (Scott and Gill 2008) during which time they may travel as far east as the coast of Kamchatka, Russia before returning to their natal river to spawn (Burgner et al. 1992, Quinn 2005). The winter-run life history predominates in Puget Sound steelhead and adults typically return to freshwater between December and April (Hard et al. 2007) and are ready to spawn shortly after entering freshwater. In contrast, the summer populations enter freshwater between May and October as sexually immature adults, using higher flows to access headwater reaches, where they spawn the next year (PSSTRT 2013a). Like all *O. mykiss*, the Puget Sound populations are iteroparous spring spawning fish, although spawn timing may vary from December to early July depending on life history and water temperatures with summer steelhead typically spawning earlier (peak February) than winter steelhead (peak April) (Leider et al. 1984, Behnke 2002, PSSTRT 2013a). Rainbow trout and steelhead throughout the region are often sympatric and often interbreed, although in some cases rainbow trout spawn slightly later (Van Doornik et al. 2013, McMillan et al. 2007). Female *O. mykiss* typically dig a one or two redds (Berejikian et al. 2005, Gallagher and Gallagher 2005, Kuligowski et al. 2005), but unlike other species of Pacific salmon they do not guard their redds, and instead migrate back to marine waters as kelts (steelhead) or to their feeding territory (rainbow trout) after spawning (Burgner et al. 1992). Males typically remain on spawning ground longer, fighting for mates and seeking additional opportunities to spawn, and thus have much lower post-spawning survival rates as kelts when compared to the females (Withler 1966, McGregor 1986, McMillan et al. 2007, PSSTRT 2013a). Overall, repeat spawning steelhead only represent 19% or less of all spawners in Puget Sound populations (Scott and Gill 2008), but may gain significant additional success when spawning for the second time (Seamons and Quinn 2010).

Emergence times for fry depend on water temperature for *O. mykiss*, which require between 115 days to hatch at 2°C to 28 days at 11°C (Quinn 2005). Newly emerged fish are typically 25-30 mm fork length (FL) (Deschutes River, OR) (Zimmerman and Reeves 2000), and these young-of-year fish initially rear along the stream margins, feeding primarily on small drifting invertebrates (Quinn 2005). During their first few years of life, rainbow trout and juvenile steelhead occupy the same habitat, and establish territories and feed on a variety of aquatic insects and small fish. In most sympatric steelhead and rainbow trout populations, the rainbow trout component is composed primarily of males (Quinn 2005). The rainbow trout males generally reach maturity at age two, although some may mature earlier, while rainbow trout females typically mature at age three. While rainbow trout spend their entire life cycle in freshwater, steelhead spend their first one to three years in freshwater before migrating to the ocean with the majority migrating as age-2smolts. The triggers that lead to the adoption of a particular life history strategy are a combination of genetic and environmental factors (Kendall et al. 2015). Berejikian et al. (2013) showed that the presence or absence of rainbow trout populations strongly influenced the degree of anadromy in the Hood Canal populations. Additionally, some offspring of both anadromous and rainbow trout parents adopted the opposite life history of their parents (Berejikian et al. 2014). Those juveniles that smolt generally out-migrate in the spring with peak out-migration in the Puget Sound occurring between April and Mid-May (Scott and Gill 2008). Once smolts enter the estuary, they move through the Puget Sound relatively quickly, with mean travel times from their natal river mouths to the Strait of Juan de Fuca of 6 to 18 days (Moore et al. 2015).

Factors Affecting Growth and Survival of Juvenile O. mykiss in Freshwater

Growth is affected by many factors, but temperature and prey availability are two of the primary factors. In ectothermic species like fish, temperature is particularly important as it directly affects metabolic processes and growth (Jobling 1994). Stream environments in particular are strongly affected by seasonal shifts, with the highest production of aquatic invertebrates typically occurring during spring, while inputs of terrestrial invertebrates often dominate during the summer months (Nakano and Murakami 2001, Wipfli and Baxter 2010, Richardson and Sato 2015). Additionally influxes of food from migratory species, such as salmon, may seasonally create periods of high food abundance that can account for a significant portion of annual growth (Jaecks and Quinn 2014). However, these seasonal shifts in prey abundance and flow can also create bottlenecks in which density-dependent effects limit growth and survival. Life history variation typically allows species to buffer against the impacts of intraspecific competition by more efficiently utilizing the diversity of available habitat and resources. A common example of this life history variation is seen in sub-arctic lakes, where multiple morphs of Arctic char arise, each utilizing a different food source (Knudsen et al. 2006). However, niche partitioning via life history variation does not completely eliminate density-dependent effects which may result in displacement and increased migration, lower growth rates and increased mortality and lower reproduction. As a result many species establish and defend territories to ensure access to adequate resources (Rossell et al. 1998, de Kort et al. 2008). In fish populations, the faster growing individuals typically have a survival advantage over their smaller conspecifics (Healey 1982, Post and Prankevicius 1987, Ward and Slaney 1988, Sogard 1997, Thompson and Beauchamp 2014). As such, density-dependent effects may have a disproportionate impact on the survival of slower growing sub-dominant individuals.

The growth experienced by juvenile steelhead during their early rearing phase, which typically lasts one to three years, is crucial to their long-term survival, as increased early growth may allow them to avoid some sources of size-selective mortality (Ward et al. 1989, Thompson and Beauchamp 2014). This extended period of freshwater rearing makes steelhead particularly sensitive to seasonal changes in temperature, prey quantity and quality in the freshwater environment (McCarthy et al. 2009, Doctor et al. 2014); moreover, rainbow trout and steelhead overlap with habitat and food resources during this period. During summer, stream temperatures are highest and inputs of terrestrial invertebrates may be a substantial contributor to the overall prey available in the drift. These terrestrial invertebrates are a high energy food source when compared with the immature forms of aquatic invertebrates, and can influence the overall growth for juvenile *O. mykiss* (McCarthy et al. 2009). Increased energetic quality of prey can improve growth and compensate for reduced food availability or buffer against extreme temperatures by broadening the range of temperatures at which optimum growth is achieved. As such, summer may be a particularly important period for juvenile *O. mykiss*, accounting for the majority of the annual growth.

Density-dependence may contribute to growth and survival limitations during the first few years of life, when rainbow trout and steelhead life history forms co-mingle in freshwater. Like other lotic salmonid species, competitive behavior is common in *O. mykiss*, and individuals often establish and defend territories when resources are limited (Li and Brocksen 1977, Keeley and McPhail 1998, Keeley 2001, Imre et al. 2004). Territories are defended by threat displays, chases and nipping of the fins (Abbott and Dill 1985). These aggressive behaviors may cause shifts in selection, with larger or more aggressive individuals having a competitive advantage. Abbott et al. (1985) showed that juvenile steelhead could assert dominance over conspecifics when they had a 5% or greater weight advantage. When similar-sized dominant and sub-dominant juvenile steelhead were fed equal rations, the dominant fish grew 12.3% faster than sub-dominant individuals (Abbott and Dill 1989). This behavior generally leads to high variability in size and the amount of food consumed. However, when food is relatively abundant, feeding hierarchies tend to break down and overall variation in size of individuals in the population decreases (McCarthy et al. 1992). Slaney and Northcote (1974) demonstrated that at higher prey levels, juvenile rainbow trout defended smaller territories and exhibited lower frequency of aggressive behaviors.

Steelhead have larger eggs and offspring at emergence, and therefore should have a size based competitive advantage over the offspring of rainbow trout females in sympatry (Quinn 2005). Additionally, rainbow trout typically spawn later in the season than steelhead (Zimmerman and Reeves 2000, McMillan et al. 2007), creating an even larger gap in the size difference between the offspring of steelhead and rainbow trout. However, habitat only accessible to rainbow trout above anadromous barriers acts as a refugia for rainbow trout, and while they are smaller at age, rainbow trout that migrate downstream of such barriers at older ages would be expected to have a competitive advantage over younger offspring of steelhead. This would be especially true if the rainbow trout are large, as they would be able to competitively dominate the smaller steelhead and would require larger territories (Keeley 2003). In such cases competition could potentially pose a significant impact to already imperiled anadromous populations.

The relationship between the abundance of rainbow trout and the growth, survival and abundance of steelhead is currently poorly understood. Resource limitations may be a particularly important factor in steelhead populations, as slower growing individuals are more likely to adopt an anadromous life history, while faster growing fish, typically males, may either head to sea at a younger age or mature early and remain in freshwater their entire lives (Quinn 2005). However, if the fish are not able to reach a sufficiently large size prior to smolting, they may experience higher mortality during their ocean phase (Ward et al. 1989, Thompson and Beauchamp 2014). In rainbow trout populations, as population density increases, growth rates, growth efficiencies and fat content of individuals decline, while metabolism increases (Li and Brocksen 1977); these relationships may also hold true for steelhead. While the average size at smoltification is fairly constant among years and populations (Kendall et al. 2015), slower growth and delayed smoltification results in a much greater risk of mortality before achieving the smolt size threshold. Additionally, as rainbow trout density increases so does the likelihood that density-dependent effects will become a limiting factor to imperiled steelhead populations.

The Status of *Oncorhynchus mykiss* in the Puget Sound Region

Over the past century *O. mykiss* have shown population declines across much of their native range in North America. This decline has been the most significant for the anadromous form (steelhead), and out of the fifteen distinct population segments (DPS) identified by NOAA fisheries in lower 48 states, only three are considered to be healthy (NMFS 2016). In the Puget Sound region, steelhead populations experienced a substantial decline in the mid-1980s to early 1990s and populations have remained low since (Hard et al. 2007, Ford 2011, WDFW SCoRE 2015). In response to the declining population trends and loss of overall abundance, the Puget Sound steelhead DPS was listed as Threatened under the Endangered Species Act in 2007 and re-affirmed in 2016 (NMFS 2007, NMFS 2016). The run-size of winter steelhead in the Puget Sound in 1895 was estimated at approximately 622,000 spawners, while the average run-size between 1980 and 2004 was 22,000 or just 1-4% of the historical abundance (Gayeski 2011), and declined by 53% between 1980 and 2013 (Kendall et al. in press). Among the distinct independent populations (DIPs) identified in the Puget Sound Steelhead DPS, those in the Hood Canal basin have experienced some of the most significant declines and were a driver of the 2007 listing. By the late 1990s returns to some Hood Canal populations fell to <10 spawners in some years. Little data are currently available on the population trends of rainbow trout, although their populations are believed to be relatively stable throughout much of the Puget Sound region, and as such, rainbow trout populations were not included in the 2007 listing (Hard et al. 2007, NMFS 2007).

The decline in steelhead abundance across the Puget Sound region has been attributed to a number of both natural and anthropogenic factors. Four primary factors have been identified as reducing the viability of Puget Sound steelhead including loss of: abundance, productivity (including freshwater water rearing), life history diversity, and spatial structure (PSSTRT 2013b), all of which have been affected by both natural and anthropogenic factors. Natural causes attributed to the decline of steelhead in Puget Sound include poor marine survival trends (Moore 2010, Moore 2015, Kendall et al. in press), disease and predation (Good et al. 2007, Berejikian et al. 2016), while anthropogenic factors such as over-harvest, habitat degradation and interactions with hatchery fish have also been implicated in decline (Hard et al. 2007, Scott and Gill 2008). Poor marine survival is considered one of the most significant factors, because smolt-to-adult returns (SARs) declined significantly in the late 1980's and early 1990's in the Puget Sound region and have shown little sign of improvement since (Kendall et al. in prep.).

Management of Oncorhynchus mykiss in the Hood Canal Basins

Due to their complex life history and challenging environmental conditions, *O. mykiss* are difficult to manage and monitor compared to other species of Pacific salmon (Behnke 2002; Kendall et al. 2015; Pavlov et al. 2008; Quinn 2005). Historically rainbow trout and steelhead were managed separately in the Hood Canal region; however, as understanding of the connectivity between these life history variants has increased (Zimmerman and Reeves 2000, McMillan et al. 2007), management practices have shifted to address *O. mykiss* as a whole. In 2008, the Washington Department of Fish and Wildlife (WDFW) adopted a Statewide Steelhead Management Plan (SSMP), which sets a framework for managing natural production, hatchery production, habitat protection and restoration, and fisheries management.

Land Use

Compared to other areas in the Puget Sound, habitat for Hood Canal steelhead is relatively intact, with headwaters of most of the streams on the Westside of the Hood Canal originating within Olympic National Park, Olympic National Forest or Wilderness areas. However the lower reaches of the watersheds have been impacted by land use practices associated with transportation, timber harvest, and agriculture and residential development. Impacts from transportation have reduced connectivity of estuary habitat and created potential barrier effects (Correa 2003). The Hood Canal floating bridge delays the migration of juvenile steelhead and might create a bottleneck aggregating juvenile steelhead, resulting in a higher rate of predation (Moore et al. 2013). Many watersheds in the Hood Canal have also been impacted by past timber practices, which have increased the amount of fine sediments and reduced riparian habitat and recruitment of large wood debris. High road densities and improper construction and maintenance or decommissioning of forest roads has been associated with increased runoff, mass wasting events and high sediment loads throughout the Hood Canal region. Agricultural and residential development have resulted in stream channelization and lost riparian habitat. These impacts reduce overwintering and rearing habitat associated with complex stream channels and off channel habitat (Correa 2003). Juvenile steelhead might be especially vulnerable to these impacts, because they remain one or more years longer in freshwater than other anadromous salmonids. To address habitat limitations in the watersheds, a number of restoration projects have been implemented to restore channel complexity and access to off channel habitat, as well as increase the amount of large wood debris and decommission forest roads.

Fisheries Management

The streams of the Hood Canal have a long history of hatchery supplementation. Starting in the 1930s steelhead were planted in Hood Canal streams to support recreational sport fisheries, with the early-timed Chambers Creek stock becoming the dominant stock by the 1980s (WDFW Hatchery Plants Database 2017). However, with the decline of wild steelhead in the Hood Canal region, low catch rates and poor smolt to adult survival of hatchery fish (mean 0.07% for 1995-2002); stocking for harvest supplementation was discontinued in 2002. Similarly, hatchery rainbow trout were stocked in many watersheds for harvest supplementation, but based on the SSMP, hatchery rainbow trout are no longer stocked anywhere with access to anadromous waters (WDFW 2008). While hatchery plants were considered relatively unsuccessful at reproducing in the wild, their impact on the natural populations in the region are unclear.

Today Hood Canal Steelhead are co-managed between the State of Washington and the local treaty tribes. According to *US v Washington* (1974) the treaty tribes are entitled to 50% of the harvestable fish. Each year WDFW and the four Hood Canal treaty tribes (Jamestown S'Klallam, Port Gamble S'Klallam, Skokomish and Lower Elwha Klallam) jointly develop a harvest management plan. Due to the depressed status of steelhead populations in the Hood Canal watershed, current targeted steelhead fisheries are limited to tribal ceremonial and subsistence fisheries, with an expected harvest rate of <2% (Jamestown S'Klallam Tribe et al. 2015). Additionally, recreational fisheries for trout in the anadromous reaches of all of the systems have been set as catch and release only from June through August to protect listed adult and juvenile steelhead.

The Hood Canal Steelhead Supplementation Project and Monitoring

The decline in natural abundance resulted in the initiation of the Hood Canal Steelhead Supplementation project in 1998 with the Hamma Hamma River Winter Steelhead Project. During this first phase of the project, the Hamma Hamma River was supplemented with winter steelhead, while the Duckabush, Tahuya and Dewatto Rivers acted as non-supplemented control streams, and baseline data were collected on other Hood Canal populations to determine population trends. The program was operated as a conservation hatchery by collecting naturally spawned eyed-eggs via hydraulically pumping redds. These embryos were then incubated at Quilcene National Fish Hatchery, and juveniles were reared to either two year old smolts released during April and May or four to six year old mature adults released ready to spawn in the wild. The Hamma Hamma segment of the project was completed in 2008. Results of the program showed that the abundance of redds increased on the Hamma Hamma River as a result of supplementation, while the control streams remained stable or declined during the same period (Berejikian et al. 2008). Since the last returns from the supplementation program in 2008, returns to the Hamma Hamma River have declined from the supplemented level, but have remained stable at a higher level than the pre-supplementation numbers (WDFW SCoRE 2016).

With the completion of supplementation on Hamma Hamma River, the program was expanded in 2007 to a sixteen year before-after impact study. This study is focusing on the Dewatto, South Fork Skokomish and Duckabush Rivers as supplementation streams, and Big Beef Creek, the

Tahuya, Dosewallips, and Little Quilcene Rivers as non-supplemented control streams within the Hood Canal watershed. Supplementation occurred for the first eight years, followed by an additional eight years post-supplementation monitoring to determine whether supplementation increased abundance and productivity while maintaining the demographic, life-history and genetic characteristics of the wild steelhead populations. Monitoring for the project includes redd and snorkel surveys to determine timing and abundance of spawners on eight streams. Smolt traps operate on six streams during the peak out migration window. These traps are used to determine the timing, abundance, age, size distribution and parentage of out-migrating smolts in the Hood Canal (Weinheimer 2014).

Summer parr sampling is used to assess life history patterns and the size at age during summer low flows as well as the reproductive success of supplementation fish. This sampling has revealed significant interactions between rainbow trout and steelhead in watersheds with migration barriers (Berejikian et al. 2013, Van Doornik et al. 2013). Outstanding questions that may influence the success of the supplementation project include how growth opportunities in freshwater and interactions with rainbow trout are likely to affect juvenile steelhead rearing in the watersheds.

Research Questions and Objectives

There were two primary components of this study: 1) a mark-recapture study to determine migration downstream over the barrier falls, and 2) an assessment of the growth of *O. mykiss* above and below the barriers in the watersheds. We hypothesized that *O. mykiss* from above the barriers disperse downstream below barriers where they interact with steelhead. The primary objective of this study was two-fold: to determine the rate of dispersal of above-barrier *O. mykiss* downstream into anadromous reaches and to determine whether gene flow occurred from above-barrier populations to below-barrier populations. Our secondary hypothesis was that the effects of a combination of reach-specific growth potential, density-dependent reduction in growth, or survival, influences the tendency of above-barrier fish to migrate downstream. To investigate this, our objective was to determine the abundance, survival rate and size and age structure of *O. mykiss* in the Duckabush and Hamma Hamma Rivers both above and below anadromous barriers and their relationship to dispersal of above-barrier fish. Secondly, I used a bioenergetics-modeling approach to evaluate the factors affecting growth performance of juvenile *O. mykiss* in the Duckabush and Hamma Hamma Rivers, Washington. We hypothesized that seasonal food quantity and quality, mediated by temperature limit the growth performance of *O. mykiss* in the watersheds. The objectives of the growth assessment were to: 1) determine the growth performance of *O. mykiss* and 2) assess whether food resources growth by analyzing temperature, diet composition, and invertebrate drift between watersheds and among reaches above and below anadromous barriers. To answer these questions, age 1-5 *O. mykiss* were sampled and tagged in the above- and below-barrier reaches of the Duckabush and Hamma Hamma Rivers during the summer (June-October) of 2013 to 2015. Drift abundance and seasonal and reach scale variations in diet were compared to determine whether the populations appeared to be near their carrying capacity.

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Chapter 2: Abundance patterns, dispersal below a barrier and gene flow between sympatric life history forms of *Oncorhynchus mykiss* in the Duckabush and Hamma Hamma Rivers, WA

Abstract

Steelhead (*Oncorhynchus mykiss*) populations in the Puget Sound Distinct Population Segment are far below their historic abundance and are currently listed as Threatened under the Endangered Species Act. Despite the declines in steelhead abundance, resident *O. mykiss* populations appear to be relatively abundant and little is known about how resident *O. mykiss* above anadromous barriers interact with steelhead downstream. This study evaluated the dispersal and gene flow of above-barrier *Oncorhynchus mykiss* in the Duckabush and Hamma Hamma Rivers, Washington into below-barrier reaches that were accessible to anadromous salmonids. Additionally we investigated the abundance, sex ratio and annual survival rate of *O. mykiss* above and below the barriers, and their influence on gene flow. Although a three year mark-recapture study did not detect any dispersal from the above- to below-barrier populations, microsatellite DNA analysis detected gene flow from the above-barrier populations into the below barrier populations. Gene flow was more pronounced in the Duckabush River, where 90% of the below-barrier fish were assigned to the above-barrier population. The Hamma Hamma River populations were more distinct, with 41% of the below-barrier population assigning to the above-barrier population. The sex ratio above the barrier became increasingly more biased towards females at older age, whereas the opposite pattern was observed below the barrier. Despite a male sex bias below the barrier, approximately 70% of juveniles sampled in the smolt trap were females, indicating that anadromy may be female-biased. These results suggest that above-barrier males may be dispersing over the barriers at a higher rate than females or experiencing higher post-spawning mortality. Abundance was estimated for *O. mykiss* ≥130 mm FL in the Duckabush River, with 150 trout per km in the lower below-barrier reach, 907 per km in the middle reach below the barrier and 1,165 per km above the barrier. Where abundance could not be accurately estimated, catch per unit effort (CPUE) was used to compare relative abundance. The

lowest observed CPUE was in the lowest reaches of the watersheds and in the Hamma Hamma River immediately above the barrier. CPUE in the Duckabush River above the barrier was 5.4 fish per angler hour compared to 2.0 in the Hamma Hamma River above the barrier. The lower below-barrier reaches on these rivers may be either unsuitable as steelhead rearing habitat, or under-seeded by depressed steelhead populations. These results indicate that the gene flow from the above-barrier populations into the below-barrier population is strongly tied to abundance.

Introduction

The salmonid *Oncorhynchus mykiss* displays a complex suite of life history strategies, ranging from non-migratory stream resident rainbow trout, to anadromous steelhead. Historically, resident and anadromous *O. mykiss* have been managed separately; however, recent research indicates that resident and anadromous *O. mykiss* often form a single population, with gene flow occurring between the life history forms (Christie et al. 2011; Van Doornik et al. 2013). Additionally, parental life history does not necessarily indicate the life history strategy adopted by the offspring (Courter et al. 2013; Pascual et al. 2001; Sloat and Reeves 2014). Adopting either life history pathway involves trade-offs, as resident *O. mykiss* typically experience higher survival, but experience fecundity and growth compared to anadromous individuals. The factors controlling life history expression in *O. mykiss* are not fully understood, but both genetics (Martinez et al. 2011) and the environment play a role (Quinn et al. 2000). Specific factors that have been identified include maternal origin (Berejikian et al. 2014), sex, growth and population density (Kendall et al. 2015). This life history diversity and plasticity allows *O. mykiss* to adapt to and more fully utilize their environment while stabilizing overall abundance through a portfolio effect (Greene et al. 2010). When the two life history forms exist in sympatry, this likely indicates that neither form consistently experiences higher fitness than the other. However, over the past several decades steelhead populations have declined while rainbow trout populations have remained relatively abundant.

Eleven of the 15 recognized Distinct Population Segments (DPSs) of steelhead in the lower 48 states are currently listed as Threatened or Endangered under the Endangered Species Act (ESA). Despite the relationships between rainbow trout and steelhead life history forms, the listing only pertains to "naturally spawned anadromous winter-run and summer-run *O. mykiss* (steelhead) populations, below natural or man-made migration barriers" (NMFS 2007 FR 72FR26722). While above-barrier fish are not included in the listing, they have the potential to interact with anadromous fish below barriers if they disperse downstream. Recent research indicates that rainbow trout populations above migration barriers have the potential to produce anadromous offspring (Hayes et al. 2012, Van Doornik et al. 2013). Yet the rate of dispersal and gene flow from above- to below-barrier populations and the factors influencing this process or its implications on listed steelhead populations are not well understood.

Above-barrier populations may affect below-barrier populations in several ways if they disperse into below-barrier reaches. If above-barrier fish retain the ability to produce smolts, they may provide a genetic reservoir for below-barrier populations (Thrower and Joyce. 2004b, Hayes et al. 2013) and provide needed genetic diversity by raising the effective population size for imperiled steelhead populations (Araki et al. 2007). Alternatively, resident fish from above the barriers may have a genetic propensity to remain in freshwater (Pearse et al. 2009; Martinez et al. 2011) and may pass this to their offspring if they interbreed with steelhead, potentially reducing the proportion of anadromous individuals in the population (Thrower et al. 2004a). Additionally, there may be a survival disadvantage for anadromous offspring of above-barrier rainbow trout parents, for which survival rates can be four to five times lower than those from steelhead parents (Thrower and Joyce 2004b). Another concern is that rainbow trout dispersing from above-barrier populations may increase density-dependent effects on juvenile steelhead if abundances are high and rearing habitat or resources are limiting.

Steelhead populations in the Duckabush and Hamma Hamma Rivers, Washington declined sharply in the 1990's. In response, a supplementation project was implemented to increase the abundance of the anadromous life history for of *O. mykiss*. However, the abundance of *O. mykiss* above and below anadromous barriers in the rivers and survival trends during freshwater rearing remain unknown. Estimating survival and abundance is crucial to conserving steelhead populations as it may suggest which portions of the watershed are near their rearing capacity, which portions may be susceptible to density-dependent effects, and where rearing habitat may be potentially underutilized. Such information could help prioritize restoration or supplementation efforts within specific reaches of the river to improve and fully seed the habitat. Abundance of resident *O. mykiss* above-barriers may also provide insights into factors controlling gene flow between above- and below-barrier populations and for assessing how resident and anadromous fish interact during their freshwater rearing. Additionally comparison of overall abundance and smolt abundance may provide insights into whether rainbow trout or steelhead life histories predominate in the watersheds. Understanding freshwater juvenile abundance and survival patterns is essential for evaluating the success of conservation hatchery programs such as the Hood Canal Steelhead Supplementation Project.

In this study we investigated the abundance, migration patterns and genetic origin of resident rainbow trout and anadromous steelhead in the Duckabush and Hamma Hamma Rivers, Washington State, where both life history forms are present. We also examined the sex ratio of *O. mykiss* above and below migration barriers. Previous work indicated that the above- and below-barrier populations in the Duckabush and Hamma Hamma Rivers are distinct and above-barrier fish may produce anadromous offspring (Van Doornik et al. 2013). We hypothesized that *O. mykiss* from above the barriers disperse downstream below barriers where they interact with steelhead. The primary objective of this study was two-fold: to determine the rate of dispersal of above-barrier *O. mykiss* downstream into anadromous reaches and to determine whether gene flow occurred from above-barrier populations to below-barrier populations. Our secondary hypothesis was that the effects of a combination of reach-specific growth potential, density-dependent reduction in growth, or survival, influences the tendency of above-barrier fish to migrate downstream. To investigate this, our objective was to determine the abundance, survival rate and size and age structure of *O. mykiss* in the Duckabush and Hamma Hamma Rivers both above and below anadromous barriers and their relationship to dispersal of above-barrier fish.

Methods

Study Area: The Duckabush and Hamma Hamma Rivers, Washington are snow-rain dominated watersheds that drain the eastern slopes of the Olympic Mountains into the Hood Canal (Figure 1). The Duckabush watershed has a drainage area of 194 km^2, with the headwaters in Olympic National Park and National Forest. The Hamma Hamma River has a drainage area of 220 km^2, with headwaters in Olympic National Park, Olympic National Forest, and the Brothers and Mount Skokomish Wilderness Areas. Primary land use in the lower watersheds is timber harvest, recreation, residential and agricultural. The both watersheds support populations of Chinook *O. tshawytscha*, Coho *O. kitsuch*, Chum *O. keta* and Pink salmon *O. gorbuscha*, as well as Coastal Cutthroat *O. clarki clarki* and Rainbow/Steelhead trout *O. mykiss*.

Waterfalls at river km (Rkm) 12.1 on the Duckabush and Rkm 4.0 on the Hamma Hamma block all anadromous access. Rainbow trout are considered native to both rivers above the barriers. Brook trout *Salvelinus fontinalis* and Cutthroat trout *O. clarki. spp.* have been introduced and are present at low abundance in the Hamma Hamma River above the barrier. The Hamma Hamma River above the barrier received extensive hatchery releases of McCloud River, California stock rainbow trout from 1979 until 1996, while the Duckabush River experienced minimal hatchery stocking above the barrier between the 1930's and 1940's (WDFW Hatchery Plants Data 2017). Additional partial anadromous barriers occur at Rkm 7.2 on the Duckabush and Rkm 3.2 on the Hamma Hamma that block access to all anadromous species except *O. mykiss*.

The watersheds were separated into two reaches below the barrier and one (Upper reach) above the barrier in each watershed. The Duckabush was divided into two sample reaches below the barrier: Lower (Rkm 0.0 to 4.8); and Middle (Rkm 7.2 above the partial barrier to 12.6). The Hamma Hamma River below the barrier comprised a single reach (Lower) (Rkm 0.0 to 4.4). Above the complete anadromous barrier, the Upper Duckabush River was sampled from Rkm 12.6 to 15.3 and the Upper Hamma Hamma River was sampled from Rkm 4.4 to 24.3. Smolt traps and PIT tag antennas were located in the lower watersheds at Rkm 0.5 (~0.3 km above tidal influence) on the Duckabush and Rkm 0.8 (~0.7 km above tidal influence) on the Hamma Hamma (Figure 1).

Fish Sampling and Tagging: To determine dispersal patterns and population abundance within the watersheds, a mark-recapture study was implemented between 2013 and 2015 targeting fish >70 mm FL to examine dispersal patterns, and fish >130 mm FL for abundance estimates. Fish were collected via hook and line angling and electrofishing in the Hamma Hamma and Duckabush Rivers from June to October of each year. Our goal was to annually catch 400 fish from above each of the barriers and 400 fish from below each of the barriers. The sample size was selected based on previous work by Hayes et al. (2012) analyzing downstream barrier dispersal in Scott Creek, California. Drought conditions in 2015 resulted in significantly fewer fish collected, despite a similar amount of effort as in previous years. Collected fish were anesthetized in a buffered MS-222 solution, counted, measured for fork length (FL nearest mm) and weighed (W nearest g). A clip was removed from the caudal fin and preserved in 95% ethanol for DNA extraction. A scale sample was collected from the preferred area below and just posterior of the dorsal fin to determine age. During 2013 and 2014 all *O. mykiss* ≥ 70 mm captured by hook and line angling were implanted with a PIT Tag (12.5 mm long, 2.1 mm in diameter; Prentice et al. 1990). All fish were checked for the presence of a PIT tag prior to tagging or release throughout the study. During 2014 four specific reaches on the Hamma Hamma and six on the Duckabush River were sampled multiple times to compute abundance estimates within each reach (Figure 1). All sampled *O. mykiss* were allowed to recover in a holding area until they regained equilibrium prior to release. The mark-recapture effort targeted ages 1-5 within a size range of 130-550mm FL (Table 1). Each reach sampled for abundance estimates included a substantial buffer distance between it and the next reach upstream, so the number of tags within each discrete reach could be determined more clearly. Restrictive terrain and flow conditions barred access to the area nearest the complete anadromous barriers and we were unable to get within 0.7 km (Hamma Hamma 2013-2015) and 0.8 km (Duckabush 2013-2015). Age-0 fish in the Duckabush and Hamma Hamma Rivers typically emerge in early July (Katy Doctor NOAA Fisheries personal communication) and were not encountered with hook and line sampling until late September. Therefore, backpack electrofishing was used in late August 2015 in the Hamma Hamma River above the barrier to collect information about age-0 fish (FL, W and age). The average size of age-0 fish was 67mm FL, which was below the PIT tag threshold size, consequently age-0 fish were excluded from abundance and survival estimates. All activities involving the capture and handling of fish for this study conformed to the University of Washington's IACUC protocol 3286-21. *O. mykiss* is listed as a threatened species in the Puget Sound under the ESA and the necessary research permit (NMFS 4d Research Permit #'s 17604, 18327, 19222) and ESA consultation requirements were met.

Fish Recaptures, Detections and Movements: Tagged fish were recaptured opportunistically during hook and line sampling from June through September or at smolt traps operated from January through June. Smolt traps were operated on the Duckabush River throughout the entire study and during the first two years of the study on the Hamma Hamma River. All *O. mykiss* >130mm FL that were captured in the smolt traps were considered smolts, while those ≤ 130mm FL were considered parr. Production estimates were not available from smolt traps on the Hamma Hamma during the study due to low catches and capture efficiencies. Total smolt production was estimated by WDFW staff on the Duckabush River for 2014-2016 using a single partial-capture trap design (Volkhardt et al. 2007): data were stratified by week in order to account for temporal changes in trap efficiency. Trap efficiencies were determined by hatchery releases in 2014-2015 and natural-origin migrants in 2016. Stationary multiplex PIT tag transceivers were installed in March 2015 and operated through September of 2015. The antennas consisted of four eight-foot sections, which spanned the entire wet width of the river during normal flow conditions. However, high flows and equipment failure rendered the PIT Tag readers inoperable during periods of the study and as such we were unable to determine efficiencies for the arrays.

Mark-recapture analysis and survival estimates: Different mark-recapture models were employed for each river in order to deal with the unique challenges of estimating reach-scale abundance in each system (Table 1). Estimates were conducted during the summer low flow period for fish >130 mm FL, as fish <130 mm FL were underrepresented in catches. Insufficient recaptures were collected in the Hamma Hamma River, thus catch per unit effort (CPUE) was used as a surrogate to compare with abundance estimate. CPUE was reported as catch per angling hours for each sampled reach and was standardized by using data from a subset of anglers with a relatively consistent skill level. All recoveries for the mark-recapture analysis displayed strong site fidelity and were recaptured within the same reach as they were tagged. Based on the short window between sampling events (1-2 months) and strong site fidelity of recaptures, mortality and emigration was expected to be negligible. The Chapman estimator (Chapman 1948, Seber 1982) provided the least biased and most precise abundance estimate for the Duckabush River above the barrier, which was based on two sampling events. Two below-barrier reaches (lower and middle) on the Duckabush River were sampled multiple times throughout the season to provide more robust abundance estimates (Table 1).

For multiple sampling period estimates, abundance was estimated using the Closed Captures formulation of the Lincoln-Peterson model in the program MARK (White and Burham 1999). The best fit model included heterogeneous capture probability that varied over time and was selected based on AIC_c scores. Survival between sampling years was estimated with a catch-curve analysis (Miranda and Bettoli 2007), using cohort based 2013-2015 length frequency data for age two to age-4 fish, which were fully recruited to hook and line sampling. Survival was estimated for age 2-3 and age 3-4 between the years 2013-2014 and 2014-2015 within each sample reach. We were unable to estimate survival in some cases, where insufficient age-4 fish were available. Additional overall survival estimates were provided for the age 2-4 2013-2015 cohorts where sufficient data was available.

Age Structure: Age was determined from scale samples. Scales were taken from the preferred region of the fish above the lateral line between the dorsal and adipose fins (DeVries and Frie 1996). Scales were mounted on a gummed card in the field and were pressed into heated acetate impressions for analysis. All scale samples were aged by the Washington Department of Fish and Wildlife (WDFW) aging laboratory and annuli were identified as a region of the scale where circuli spacing is constricted, indicating a period of slow growth.

Genetic Analysis: Microsatellite DNA analysis was used to determine whether the populations above- and below-barriers in each watershed were unique, or represented a single homogenous population. A subsample of 90 fish below-barrier and 30 above-barrier samples were genotyped each year from 2013 to 2014, as well as 172 samples from smolt traps during 2007-2011. Smolt trap samples were collected from fish >125mm between April and June and were predominately age-2 with an average size of 172mm (SD = 21 mm). Fish >200 mm collected during summer sampling were considered residents as they would be above the observed size threshold for smolts during the subsequent spring migration window. Fish <200mm were considered parr, as it was unknown whether they represented resident or anadromous individuals. All samples were genotyped for 15 microsatellite DNA loci following the methods of Van Doornik et al. (2013). To determine whether the sex ratio deviated from 1:1 in any of the groups, all samples were genotyped for the male specific locus OmyY1 (Brunelli et al. 2008).

Genetic analyses were conducted by Don Van Doornik at the NOAA Fisheries, Manchester Research Station. Genetic similarity between the groups (above- and below-barrier and among parr, residents, and smolts) was compared using the program STRUCTURE (Pritchard et al. 2000), which uses a Bayesian clustering analysis to determine the number of (K) populations in the sample without first defining the populations, and to infer the proportion of ancestry of each individual in each population by estimating the percent membership of each individual in each of the populations. The STRUCTURE analysis done by Van Doornik et al. (2013) showed that the greatest ΔK (rate of change between the mean log probability value ($L(K)$) for each population (K) value and successive K values), for both the Duckabush and Hamma Hamma Rivers was obtained when K = 2. Therefore, the average percent membership of individuals was estimated for each of two populations.

Results

Fish Sampling, age and size distribution: A total of 1,907 *O. mykiss* were captured from the sampled reaches in the Duckabush River and 1,678 from the Hamma Hamma River during 2013-2015. In the Hamma Hamma River during 2014 when abundance estimates were conducted, size at age was typically greater below the barrier, although age-2 fish were similar above the barrier at 162mm FL (2SE = ±2.9mm) versus 159mm FL (2SE = ±3.3mm) below the barrier (Figure 2). Below the barrier in the Duckabush River, size at age was similar in the middle and lower reaches for age-2 and age-3. However age-1 fish were significantly smaller in the lower reach 124mm FL (2SE = ±3.9mm) than in the middle reach 133mm FL (2SE = ±3.4mm). The sizes at age were similar between the above-barrier and the middle below-barrier reach on the Duckabush River for most age classes, although age-2 fish were slightly larger in the Duckabush above the barrier (175mm FL, 2SE = ±4.5mm) than in the middle below-barrier reach in the Duckabush (170mm FL, SE = ±2.6mm) (Figure 2). Lower contributions of age-1 than age-2 fish in length frequency distributions indicated that hook and line samples were biased towards fish >130mm FL based on visual inspection of frequency distributions. As such, age-1 fish were not fully recruited to this sampling method. Age-0 fish were only encountered by hook and line in the Duckabush River above the barrier during late-September 2014 at a mean size of 83 mm FL (N=2). Age-0 fish captured during electrofishing in the Hamma Hamma River above the barrier during early-September 2015 had a mean size of 68mm FL (N = 27). During September 2015 17 *O. mykiss* between 300-550 mm FL were caught in the reach 1 of the Hamma Hamma River and 8 in reach 1 of the lower Duckabush. Whether these fish were resident or anadromous individuals is unknown as they represented an intermediate size class between typical age-3and older resident fish and adult steelhead which are typically >550.

Migration Patterns and Barrier Dispersal: Tagged fish showed strong site inter-annual fidelity with all but one recapture recovered in the same sample reach or the one immediately above or below it between years. Between 2013 and 2014, 761 *O. mykiss* were tagged and released above the barriers in the Duckabush River and 804 in the Hamma Hamma Rivers. A total of fish 27 tagged above the barriers were recaptured the following year; however, no above-barrier tags were recovered below the barriers in any year. Below the barriers, 631 fish were tagged in 2013 and 757 in 2014, with a total of 17 recaptures the follow the following year. No fish tagged during 2013 were recovered in 2015. The one recapture that migrated out of its sample reach was in the above-barrier Hamma Hamma River, and it migrated downstream from Rkm 23.3 to Rkm 12.0 between 2013 and 2014. Fish above the barrier in the Duckabush River tagged in 2013 and recaptured in 2014 displayed the strongest site fidelity. Of the 361 tagged *O. mykiss* released above the barrier in the Duckabush in 2013, 19 were recaptured in 2014. No tagged fish from above or below the anadromous barriers were recovered at the smolt traps during any of the sampling years. Three below-barrier fish were detected at the PIT tag antennas in each watershed, with several detected multiple times. A single age-2 fish tagged in lower Hamma Hamma River in August 2014 was detected at the fish ladder at the Ballard Locks, Washington during January of 2015 (a minimum distance of 105km traveled through marine waters).

Genetic analysis: In both watersheds fish below the barriers exhibited relatively high membership in the above-barrier populations, while smolts typically exhibited a much higher membership in the below barrier populations than other life stages. In the Duckabush River, the below-barrier fish exhibited an average membership of 90% in the above-barrier population, demonstrating that upstream contribution was dominant below the barrier and that some level of gene flow occurred from the above-barrier population into the below-barrier population. However, in the Hamma Hamma River, the below-barrier fish showed an average membership of 41% in the above-barrier population. The smolt trap samples had a higher percentage in the below-barrier population, 15% in the Duckabush River and 76% in the Hamma Hamma River (Table 2).

The sex ratio deviated from a 1:1 ratio increasingly with age, and the results were similar between rivers. Both the above-barrier and the smolt trap groups contained significantly more females than males. In the above-barrier groups the sex ratio was increasingly biased towards females for older age classes, while the ratio was nearly 1:1 ratio for age-1 fish (Figure 3). In the below-barrier resident populations, males represented a greater proportion of the populations, whereas smolt trap samples contained significantly higher proportions of females.

Abundance and CPUE among reaches: The reach immediately above the barrier in the Duckabush River contained an estimated 1,165 fish per km. In sufficient recaptures precluded accurate population estimates in the Hamma Hamma River above the barrier. However, catch per unit effort was significantly lower in the Hamma Hamma immediately above the barrier at 2.0 versus

5.4 fish per angler hour above the barrier in the Duckabush River (Table 1), suggesting that abundance was much lower above the barrier in the Hamma Hamma River.

The lowest abundance of fish >130 mm FL was lowest in lower reach of the Duckabush River at 150 fish per Rkm, which had a CPUE of 1.5 fish per angler hour. CPUE was similar in the lower Hamma Hamma River, at 2.1 fish per angler hour suggesting a similar abundance. Abundance in the middle reach of the Duckabush River was estimated at 907 fish per Rkm. In the sub-reaches immediately below the anadromous barriers, CPUE was similar between the Duckabush and Hamma Hamma rivers at 4.0 and 4.6 fish per angler hour respectively (Table 1). Smolt abundance was not available for the Hamma Hamma in any year. The smolt production estimate ranged from 896 to 2,938 in the Duckabush, although trap efficiencies were low; 2% in 2014 and 3% in 2015 (Table 3).

Annual Survival rates: Annual survival rates in the Duckabush River were low ranging from 5.7% to 21.4%, with 2013-2014 generally having better survival than 2014-2015 (Table 4). The highest survival rates for age 2-3 fish in 2013-2014 were above the barrier, and the lowest were in the lower reach. However during 2014-2015 when regional drought conditions occurred, this pattern reversed. In the lower reach, survival improved with age and age 3-4 survival ranged from 21.4% in 2013-2014 to 20.0% in 2014-2015. However, the opposite pattern was observed in the middle and above-barrier reaches where annual survival declined with age. The lowest age 3-4 survival occurred in middle reach and ranged from 5.7% in 2013-2014 to 7.4% in 2014-2015. Despite differences in the age specific survival, the overall 2013-2015 age 2-4 cohort survival was similar in the middle (13.7%) and lower reach (14.9%).

Annual survival was also low in the Hamma Hamma River, with lower survival in 2014-2015 than 2013-2014. Age 2-3 survival rates were similar above and below the barrier in 2013-2014 at 14.2% (above-barrier) and 15.3% (below-barrier). During 2013-2014 survival declined with age above the barrier and increased with age below the below. In 2014-2015, survival below the barrier was similar between for age 2-3 at 12.2% and 11.8% for age 3-4. Above the barrier during 2013-2014 the annual survival estimates were 12.1% (± 4.5%) for age-2 and 5.7% (± 8.0%) for age-3. The 2013-2015 age 2-4 cohort survival was higher than those observed in the Duckabush River, at 18.5% below the barrier. In both watersheds, reductions due to mortalities versus emigration by smolts could not be accounted for separately, thus survival was likely underestimated below the barriers. However as age-3 migrants comprise only a small portion of the out-migrating smolts, emigration was not expected to significantly change the survival estimates (Table 4).

Discussion

The results of our study indicate that gene flow is occurring from the above-barrier populations in both watersheds, but we were unable to determine the rate of dispersal because fish tagged above anadromous-barriers could not be detected below the barriers. The lowest abundance estimates and CPUE patterns were observed in the lower reaches of the Duckabush and Hamma Hamma Rivers and upper reach in the Hamma Hamma River. In contrast the middle (below-barrier) and upper (above-barrier) reaches on the Duckabush exhibited much higher abundance. Size-at-age was similar among the reaches, although age-1 fish were smaller in the lower reach. In the Hamma Hamma River size at age was greater below the barrier than above the barrier. Additionally, the lower reaches of both watersheds contained a higher frequency of larger individuals than the middle or upper reaches. Sex ratio above the barriers became increasingly skewed towards females, whereas below the barriers the frequency of males increased with age. The annual survival rates were low across the study, with survival declining with age above the barriers in both years. In contrast, survival below the barriers typically improved with age, with the exception of the Hamma Hamma in 2014-2015, when age-3-4 survival was slightly lower than that of age 2-3 fish.

The PIT tagging operation was ineffective for quantifying downstream migration over the anadromous barriers. The lack of detection of PIT tagged fish may have been influenced by a combination of higher than expected abundance of *O. mykiss* and lower than expected annual survival rates. Low survival rates may have been significant limitation, as of the 205 age 2-3 fish tagged in the upper Duckabush in 2013, only 27 would be available for recapture the following year, based on our survival estimates. If migration over the barrier is relatively rare in the population as a whole, the number of tags available in 2014 were not high enough to detect migration downstream over the barrier. As 10 fish were recaptured within the age group above the barrier, the overall recapture rate of the available tags was likely high. Another factor that limited detection of any above-barrier tags below the barriers, was an inability to get within 0.7 km (Hamma Hamma 2013-2015) and 0.8 (Duckabush 2013-2015) of the barrier due to restrictive terrain, and flow conditions may also have limited our ability to directly detect and dispersal over the barrier. Hayes et al. (2012) used a similar sample size but conducted their study in a smaller watershed and were able to utilize several PIT tag arrays, which was not feasible for our study.

Previous studies have shown that *O. mykiss* are able to survive passing downstream over barrier waterfalls (Pearse et al. 2009, Van Doornik et al. 2010, Hayes et al. 2012), suggesting that gene flow from above-barrier to below-barrier populations is possible. Pearse et al. (2009) indicated that above-barrier *O. mykiss* that descend over barriers remained reproductively isolated from the anadromous population in Scott Creek, California. In many watersheds, resident and anadromous populations have some degree of difference in spawn timing which limits gene flow between the life history forms. Other studies have also indicated that above-barrier populations are often genetically distinct from below-barrier populations of *O. mykiss* (Northcote et al. 1970, Currens et al. 1990). Van Doornik et al. (2013) showed that the Duckabush and Hamma Hamma River had distinct populations above and below the barriers, but that the Hamma Hamma populations were more distinct.

Our study confirmed this, suggesting that Duckabush fish were nearly genetically identical above and below the barrier. Ninety percent of the below-barrier fish identified with the above-barrier population, indicating that the two populations were not spatially isolated. The two Hamma Hamma populations appeared to be more distinct, with only 41% of the below-barrier fish assigned to the above-barrier population. It is possible that the differentiation between above-barrier and below-barrier populations in the Hamma Hamma River is a result of past stocking of hatchery rainbow trout, which may have introgressed with native population as suggested by Van Doornik et al. (2013). *O. mykiss* abundance upstream of the barrier has a significant effect on downstream dispersal and gene flow into the below-barrier population. The Hamma Hamma River immediately above the barrier had a low CPUE, suggesting that the relative abundance was low and that there is little opportunity for *O. mykiss* to disperse downstream over the barrier. Conversely, the Duckabush River contained high abundance directly above the barrier, offering increased opportunity for *O. mykiss* to disperse downstream over the barrier. Additionally, 85% of the smolt trap samples in the Duckabush River assigned back to the above-barrier population, indicating that the immigrants from the above-barrier population were not reproductively isolated from the anadromous population. Previous work in the Duckabush River indicated that above-barrier fish have the ability to produce smolts (Van Doornik et al. 2013).

Abundance of *O. mykiss* immediately above the barrier on the Hamma Hamma was the lowest of any sampled reach, whereas abundance was high in the reach above the barrier in the Duckabush River. This contrast could suggest that habitat can influence interactions between above- and below-barrier populations and that poor habitat quality above the barrier in the Hamma Hamma reduced the pool of fish available above- the and in the proximity to migrate over the anadromous barrier. Additionally, as the Duckabush River above the barrier is high gradient and does not have significant tributary or off channel habitat, juvenile *O. mykiss* may be more susceptible to being washed downstream over the barrier. Age-0 fish were not included in the study design and these fish were the most likely to be swept downstream during high flows and could represent a significant source of gene flow from the above-barrier populations. Flooding events are an assumed source of emigration and mortality in juvenile salmonids (Sandercock 1991) and may cause wash down for juvenile steelhead, which are associated with shallow stream margins during winter (Bustard and Narver 1975). Juvenile salmonids typically move from main channel habitat to off-channel areas and tributaries during floods as velocity refugees to avoid wash down and improve survival (Harvey et al. 1999, Bell et al. 2001, Bramblett et al. 2002). While off-channel habitat is available above the barrier in the Hamma Hamma River, it is lacking in the higher gradient reaches immediately above the barrier in the Duckabush River. Additionally, the highest number of inter-annual recaptures was in the Duckabush above the barrier, suggesting that resident *O. mykiss* above the barrier exhibited relatively strong site fidelity after establishing territories.

The sex ratio in the populations varied among above-barrier, below-barrier and smolt trap samples. Above-barrier populations were biased towards females, although the sex ratio shifted more towards females with age. This may have been a result of males dispersing downstream over the barrier, which was supported by the below-barrier population becoming increasingly biased towards males with age. Olsen et al. 2006 did not find any evidence of sex biased dispersal of *O. mykiss* in an Alaskan stream; however, several studies have shown male biased dispersal in other species of salmonids (Bekkevold et al. 2004, Fraser et al. 2004), which could the potentially affect gene flow between populations. Hutchings and Gerber (2002) showed that male brook trout *Salvelinus fontinalis* were more mobile than females and dispersed six times further. However, as the smolt trap samples were dominated by females, it is unclear what effect sex-biased dispersal might have had on the overall sex ratio below the barrier. As resident males generally begin to mature at age-2, while resident females typically mature at age-3 (Berejikian et al. 2014), post-spawning mortality could be significant and influence the sex ratio in the population. Little data are available on post-spawning survival rates for resident trout, but male steelhead experience higher post-spawning mortality as they typically remain on the spawning grounds longer seeking additional opportunities to spawn (Withler 1966, McGregor 1986, McMillan et al. 2007). This is supported by the low annual survival rates for age-2 and older fish in the above-barrier populations. It is likely that both post-spawning mortality and sex biased dispersal contribute to the observed changes in the sex ratio with age.

The abundance and CPUE in the watersheds showed substantial variation and were lowest in lower reaches of the watersheds and immediately above the barrier on the Hamma Hamma River. The abundance observations in the middle reach and above the barrier in Duckabush River were comparable to those observed on similar sized rivers. Porter and Hodgson (2016) showed that the average abundance of *O. mykiss* in the Crooked River, Oregon was 1485 per km between 1989 and 2016. The relatively high abundance of *O. mykiss* in the reach immediately below the barrier in the Duckabush River, the low overall smolt abundance, and the high rates of gene flow from above- to below-barrier populations suggest that these populations may be dominated by resident individuals. Additionally, low abundance and CPUE in the lowest portions of the watershed suggests that these areas may not contain sufficient rearing habitat or are being under seeded by the depressed steelhead stocks. Juvenile steelhead prefer riffle and pool habitat with relatively high velocities (Bisson et al. 1988), but because these lower reaches are typically low gradient, the amount of this preferred habitat is likely limited. Conversely, density dependent effects may impact portions of the watersheds with high abundance, which could limit the growth and overall survival of juvenile steelhead.

This study highlights the importance of considering above-barrier populations of *O. mykiss* in the conservation of downstream steelhead populations, because they could provide significant gene flow into the below-barrier populations. Abundance upstream of barriers appears to have a major role in regulating gene flow from above- to below-barrier populations. We were unable to determine the rate of downstream dispersal, or detect any direct evidence of above-barrier fish emigrating as smolts, but other studies indicate that above-barrier *O. mykiss* may retain the ability to produce smolts and have the potential to raise the effective gene pool of low abundance populations (Hayes et al. 2012, Van Doornik et al. 2013). Additionally, the results of this study imply that low annual freshwater survival may be a significant factor limiting these *O. mykiss* populations. Further research is needed to determine how above-barrier fish interact with below-barrier populations and how habitat characteristics influence abundance, growth and survival patterns in the watersheds. These results suggest that it is important to consider all life history forms of *O. mykiss* above and below anadromous barriers in the conservation of depleted steelhead populations.

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Chapter 2: Tables and Figures:

Table 1. Abundance estimates, Catch per unit effort (CPUE) and number of in-season recaptures of *O. mykiss* >130mm FL by reach in the Duckabush and Hamma Hamma Rivers. Abbreviations: C= Chapman estimator, LP = Closed Population formulation of the Lincoln-Peterson model.

River	Reach	Gradient	Lower Rkm	Upper Rkm	Model	Size class (mm)	Total in-season recaptures	CPUE (angler hours)	N-Hat	Fish per km (Fpkm)	Fpkm lower 95%	Fpkm upper 95%
Duckabush Above Barrier	Lower	High	12.8	13.6	C	≥130	6	5.4	863	1,165	896	1,802
	Upper	High	14.3	14.7	C	≥130	3	4.5	218	544	371	917
Duckabush Below Barrier	Lower	Low	2.9	4	LP	≥130	5	1.5	168	150	84	329
	Middle	Moderate	8.3	9.4	LP	≥130	9	4.0	1,025	907	519	1,685
Hamma Hamma Above Barrier	Lower	Low	4	4.9	C	≥130	3	2.0	204	241	180	441
	Upper	High	17.7	18.1	C	≥130	2	4.1	259	648	481	1,365
Hamma Hamma Below Barrier	Lower	Low	1.2	2	C	130-300	2	2.1	94	122	91	259
	Lower	Low	1.2	2	C	≥300	2	NA	29	38	32	75
	Upper	High	3.2	3.6	C	≥130	2	4.6	335	864	613	1,859

Lower (Rkm 0.0 to 4.8) and Middle (Rkm 7.2 to 12.6) on the Duckabush. Lower (Rkm 0.0 to 4.4) on the Hamma Hamma River. Above the barrier was sampled from Rkm 12.6 to 15.3 on the Duckabush and Rkm 4.4 to 24.3 on the Hamma Hamma River.

Table 2. Average percent membership in two populations of *O. mykiss* in the Duckabush and Hamma Hamma Rivers based on microsatellite DNA analysis using STRUCTURE.

		Average Membership	
	N	**Above-barrier**	**Below-barrier**
	Duckabush River		
By location			
Above	89	0.996	0.004
Below	868	0.897	0.103
By type			
Above	89	0.996	0.004
Below:			
Parr	444	0.912	0.088
Resident	119	0.965	0.035
Smolt	305	0.850	0.150
	Hamma Hamma River		
By location			
Above	123	0.981	0.019
Below	792	0.406	0.594
By type			
Above			
Below:	123	0.981	0.019
Parr	345	0.498	0.502
Resident	84	0.561	0.439
Smolt	363	0.284	0.716

Table 3. Smolt trap production estimates and efficiencies using the single partial-capture trap design for the Duckabush River 2014-2016 migration year. Data were stratified by week in order to accommodate for temporal changes in trap efficiency

Year	Total Capture	Production Estimate	Efficiency	Efficiency Release Group
2014	42	2,938	1.99%	Hatchery
2015	22	896	2.99%	Hatchery
2016	135	1,251	10.14%	Natural Origin

Source: Josh Weinheimer, WDFW 2016.

Table 4. Annual cohort based survival estimates for the Duckabush and Hamma Hamma Rivers using catch curve analysis (Miranda and Bettoli 2007).

River	Age class	2013-2014	95% CI	2014-2015	95% CI	2013-2015	95% CI
Upper Duckabush	2-3	16.6%	± 0.056	5.8%	± 0.029		
	3-4	9.1%	± 0.054	NA	NA		
	2-4					NA	NA
Middle Duckabush	2-3	12.1%	± 0.045	8.2%	± 0.040		
	3-4	5.7%	± 0.078	7.4%	± 0.101		
	2-4					0.137	± 0.047
Lower Duckabush	2-3	10.3%	± 0.073	16.1%	± 0.092		
	3-4	21.4%	± 0.223	20.0%	± 0.261		
	2-4					0.149	± 0.082
Upper Hamma Hamma	2-3	14.2%	± 0.042	5.7%	± 0.027		
	3-4	9.5%	± 0.063	NA	NA		
	2-4					NA	NA
Lower Hamma Hamma	2-3	15.3%	± 0.053	12.2%	± 0.047		
	3-4	18.2%	± 0.134	11.8%	± 0.110		
	2-4					0.185	± 0.056

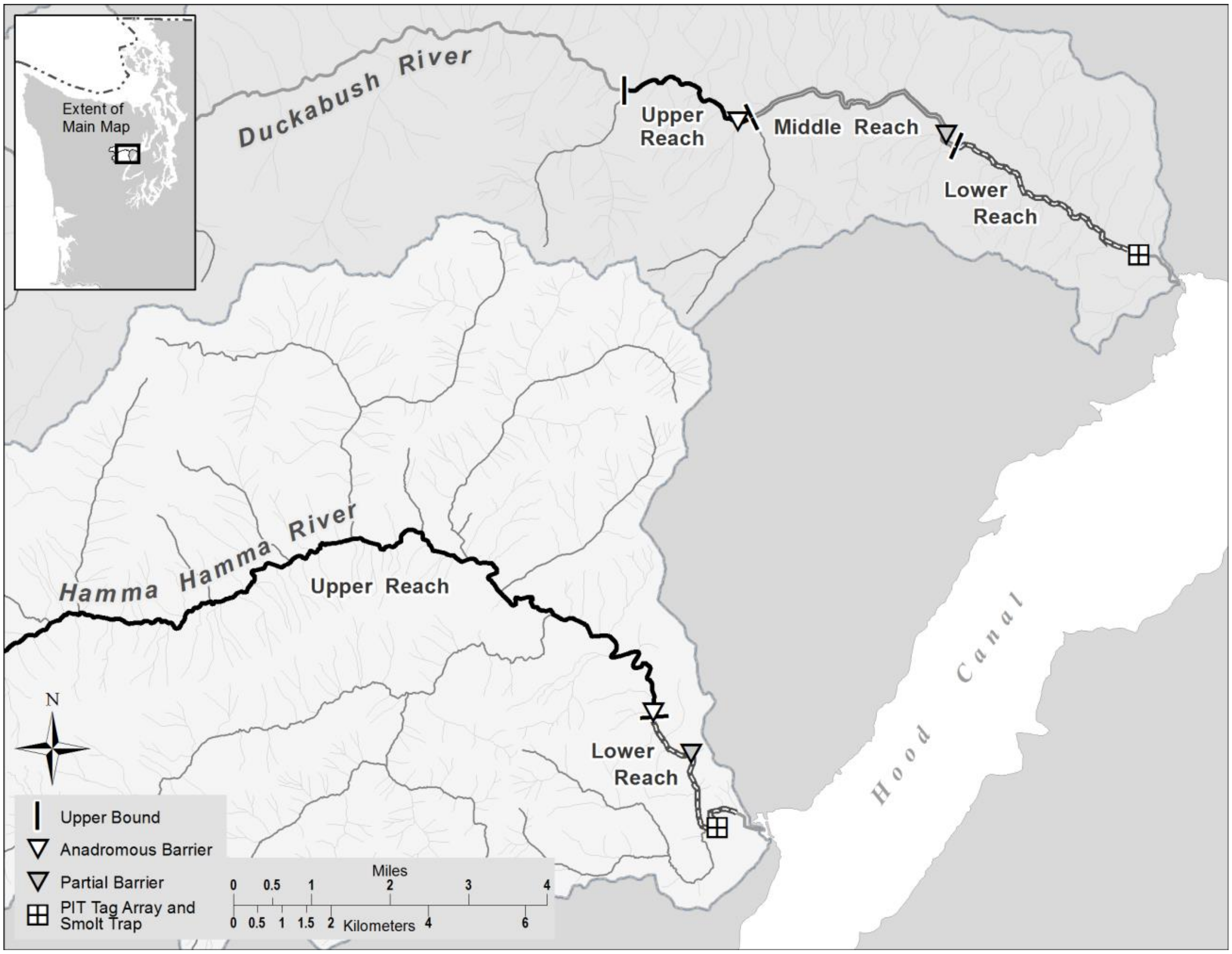

Figure 1: Map of the Duckabush and Hamma Hamma watersheds, showing the location of the sampling reaches, smolt traps, and PIT tag arrays.

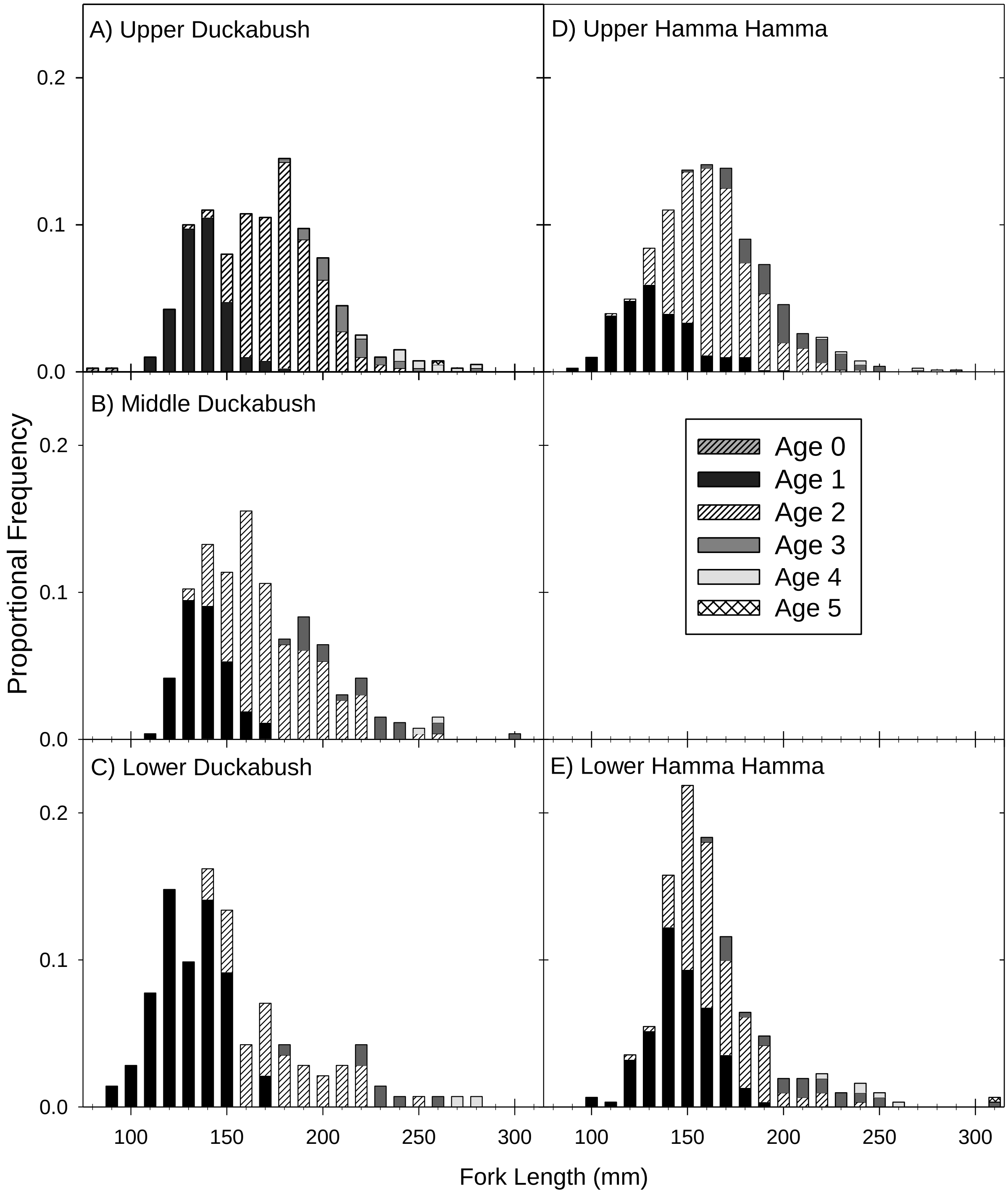

Figure 2. 2014 summer low flow period length frequency data for each age class of *O. mykiss* in the Duckabush and Hamma Hamma Rivers.

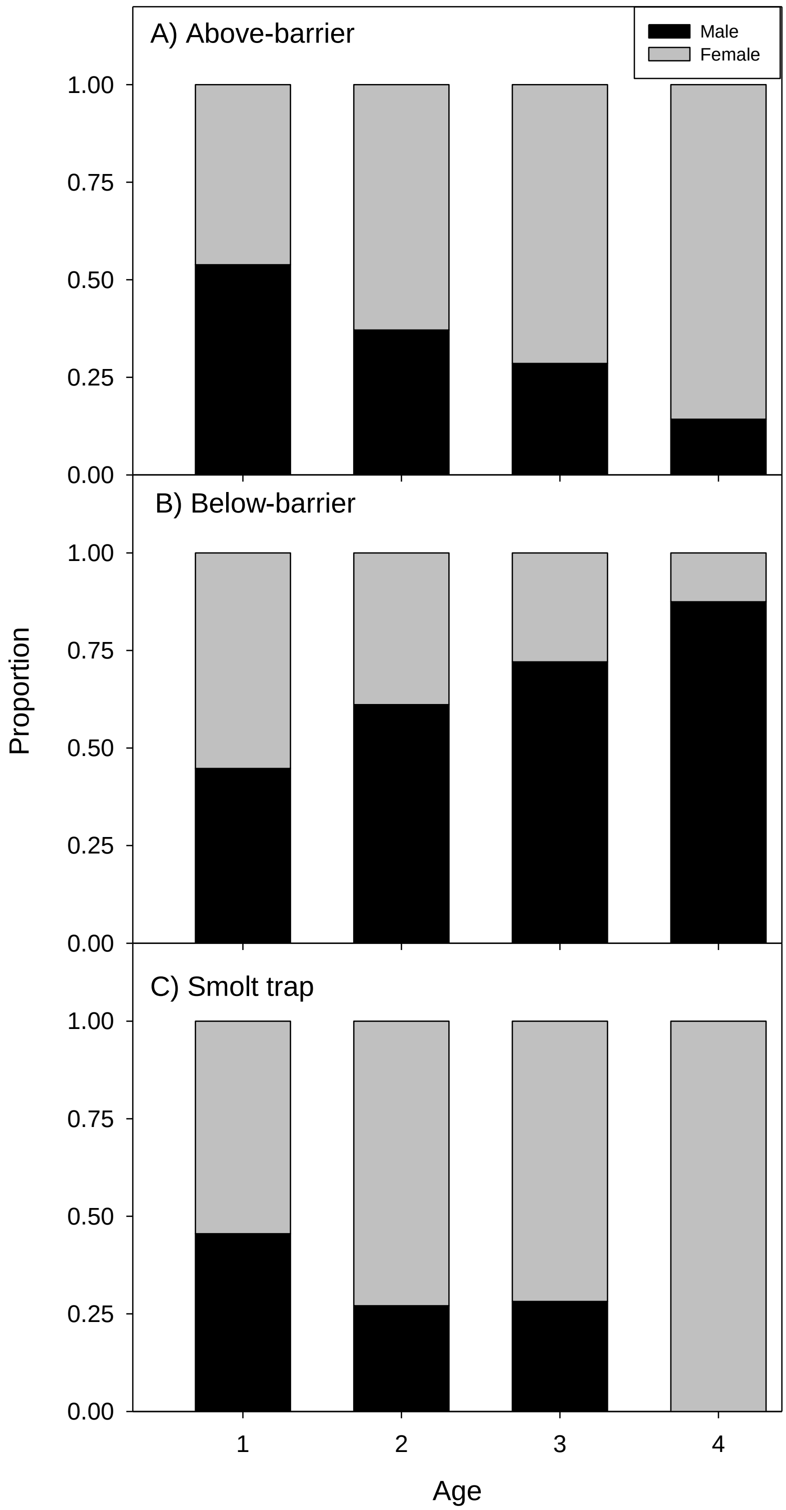

Figure 3. Sex composition at age in the Duckabush and Hamma Hamma Rivers.

Chapter 3: Factors Limiting Growth of Juvenile Anadromous and Resident *Oncorhynchus mykiss* in the Duckabush and Hamma Hamma Rivers, WA.

Abstract

Growth is a significant factor regulating the survival and life history expression of salmonids, and as such is an important conservation consideration for species like *Oncorhynchus mykiss*. This study evaluated the annual and summer growth potential of *O. mykiss* at various life stages (age 1-2, age 2-3 and age 3-4) above and below anadromous barriers in the Duckabush River and Hamma Hamma River, Washington. The objectives of the study were to determine whether growth was limited by environmental conditions within the watersheds, and if so, whether the greatest influence over growth was the availability of food or the thermal regime. Bioenergetic modeling was used with empirical data on growth, diet, and thermal experience to estimate the feeding rate, growth trajectory and the amount of food required to attain the growth observed between life stages in the, middle and lower reaches below the barrier and the upper reach above the barrier on the barrier Duckabush River and below the barrier and above the barrier on the Hamma Hamma River. Model outputs indicated that *O. mykiss* fed at 25% (age-2) to 32% (age-4) of their maximum consumption rate (%Cmax) annually in the middle reach and above the barriers in the Duckabush and Hamma Hamma Rivers and between 20% (age-2) and 26% (age-4) of Cmax in the lower reaches of the Duckabush and Hamma Hamma Rivers. Summer (June-September 15) was the primary growing season and accounted for 67% to 85% of the annual growth in the middle and above-barrier reaches. However, food was insufficient to support adequate growth for age-2 and older *O. mykiss* in these reaches on both rivers. There was little scope for growth beyond age-2 in the middle reach and above the barriers, and prey quality and consumption rates exerted the strongest influence over growth rates. Feeding rates were also relatively low in the lower reaches of both rivers at 20% (age 2 and 3) to 25% (age-4) of Cmax where salmon-derived resources accounted for the majority of the annual growth. Overall, food availability and quality appeared to limit growth of juvenile *O. mykiss* in the middle reach and above the barriers in the Duckabush and Hamma Hamma Rivers, making these

populations vulnerable to density-dependent effects on growth and production. Prey supply and quality were less limiting in the lower reaches below the barriers, largely due to marine subsidies from spawning salmon; however, other factors might limit the populations in these reaches. With steelhead facing declines across their range, this study highlights the importance of identifying factors limiting growth during critical life stages in freshwater.

Introduction

Steelhead are the anadromous form of rainbow trout (*O. mykiss*), and have experienced population declines across the Pacific Rim over the last century with nine of the 15 Distinct Population Segments (DPS's) in the United States currently listed under the Endangered Species Act (ESA) (Ford 2011, Gayeski et al. 2011). Among these listed populations is the Puget Sound DPS, which has declined substantially in the past two decades, and was listed as threatened under the Endangered Species Act (ESA) in 2007 (NMFS 2007). These declines have been linked to a number of factors, including habitat degradation, overfishing, interactions with hatchery fish and poor marine survival (Hard et al. 2007, Ford 2011). Across the Puget Sound DPS smolt to adult survival rates have decreased since the 1990's (Kendall et al. in press), but the mechanisms leading to this reduced survival remain largely unexplored. During recent years, survival rates of smolts migrating through Puget Sound have been especially low, ranging from 5% to 30% (Moore et al. 2013), over mean travel times of only nine days through the Puget Sound to the ocean (Moore et al. 2010, Goetz et al. 2008). Size selective mortality in both freshwater and marine life stages has been associated with juvenile growth during freshwater rearing (Thompson and Beauchamp 2014). Ward et al. (1989) showed a strong correlation with the size of juveniles entering marine waters to the adult return rate in the Keogh River, British Columbia; however, this relationship has since disappeared and marine survival has persisted at a relatively constant lower rate (Ward 2000). Very little information is available on how environmental or ecological factors in the freshwater influence the growth and subsequent survival of juvenile steelhead in the Puget Sound region.

As juvenile steelhead generally spend two years in freshwater before migrating to marine waters (Behnke 2003, Quinn 2005), growth during this period is crucial to long-term survival. This extended period of freshwater rearing makes steelhead particularly sensitive to seasonal changes in temperature, food availability and quality (McCarthy et al. 2009, Hardiman and Mesa 2014, Thompson and Beauchamp 2016). Stream environments vary seasonally with the highest production of aquatic invertebrates typically occurring during spring, while inputs of terrestrial invertebrates often dominate during the summer months (Nakano and Murakami 2001, Wipfli and Baxter 2010, Richardson and Sato 2015). Terrestrial invertebrates and adult forms of aquatic insects are high-energy prey sources relative to the immature forms of aquatic invertebrates due to lower water or higher lipid content, and can influence the overall growth for juvenile *O. mykiss* (McCarthy et al. 2009). For populations that co-exist with Pacific salmon, salmon eggs and flesh can provide a high energy food source and may contribute substantially to annual growth. Although they are only available for a short fraction of the year, Lowery and Beauchamp (2015) showed that salmon eggs and flesh accounted for approximately 50% of the annual energy budget of adult bull trout (*Salvelinus confluentus*) in the Skagit River, Washington. Similar results have been reported for juvenile Coho Salmon (*O. kisutch*) and Dolly Varden (*Salvelinus malma*) in Alaska (Armstrong et al. 2010, Jaecks and Quinn 2014). Increased energetic quality of prey can improve growth and compensate for reduced food availability or buffer against extreme temperatures by broadening the range of temperatures at which optimum, or at least positive growth, is achieved (Beauchamp 2009, McCarthy et al. 2009). As such, summer and early fall may be a particularly important period for juvenile *O. mykiss*, accounting for the majority of the annual growth.

When food supply is limiting, density dependent effects further reduce growth and survival of juveniles. *O. mykiss* form dominance hierarchies in streams, with larger individuals holding the best territories and dominating smaller fish when resources are limited (Abbott et al. 1985, Keeley and McPhail 1998, Keeley 2001, Imre et al. 2004). Dominant individuals grow faster than sub-dominants (Abbott and Dill 1989), increasing variability in the size and amount of prey consumed by individuals. However, when prey is abundant, dominance hierarchies break down and size becomes less variable (Slaney and Northcote 1974, McCarthy et al. 1992). In instances of low steelhead abundance and continued immigration of rainbow trout from above anadromous barriers, juvenile steelhead could be placed at a competitive disadvantage. This would be especially true if the rainbow trout are large, as they would be able to competitively dominate the smaller steelhead and would require larger territories (Keeley 2003). Reduced growth associated with prey limitations could result in size-selective mortality and lower survival in juvenile steelhead. In such cases competition could potentially pose a significant impact to already imperiled anadromous populations.

Food supply and quality also influence the life history pathway that juvenile *O. mykiss* adopt. Rates of anadromy can increase with decreasing food supplies in salmonids (Olsson et al. 2006, O'Neal and Standford 2011). McMillan et al. (2012) showed that resident males contained higher lipid levels than non-maturing anadromous males. Residency tends to be more common in watersheds with abundant prey and particularly dominant where high levels of salmon derived nutrients are available (Pavlov et al. 2001, Pavlov et al. 2008; Savvaitova 1975). Salmon eggs are two to three times as energy dense as terrestrial and aquatic invertebrates (Armstrong et al. 2010, Lowery and Beauchamp 2015), often allowing individuals to mature at sizes and ages similar to anadromous individuals (Kuzishchin et al. 2007). This suggests there is a balance between food supply and the life history strategy adopted, although a number of other factors such as genetics and other environmental variables play a role in life history expression.

As freshwater rearing is a critical stage for juvenile steelhead and influences their life history pathway and survival to later life stages, it is crucial to understand whether environmental factors are limiting growth during this period and to identify where bottlenecks might occur. Bioenergetic modeling has been used increasingly in recent years to simulate the growth potential for various life stages of fish associated with different environments (Hartman and Kitchell 2008, McCarthy et al. 2009, Lawrence et al. 2015) and provides a useful tool for identifying factors that limit growth. These models provide estimates of how changes in feeding rates, thermal experience, diet or metabolism affect the overall growth of individuals (Hanson et al. 1997). The model accounts for mass and temperature-dependent effects on consumption and metabolism, and allows for the use of species-specific parameters for maximum consumption (Cmax), metabolism, waste, and activity costs. Additionally, the models allow population and age-specific inputs for growth and time varying temperature, diet, and energy density of prey and consumer, thus allowing for comparisons across seasons, populations and watersheds.

This study used a bioenergetics approach to determine the factors limiting growth during the freshwater rearing stage for *O. mykiss* in reaches above and below anadromous barriers on the Duckabush and Hamma Hamma Rivers, Washington. We examined growth both annually and across the summer-lower-flow period. The objectives were to: 1) identify periods of slow and fast growth and to identify which age classes were most vulnerable to growth limitations between rivers and among reaches; and 2) determine the relative influence of body size, temperature, and the availability and energetic quality of food on growth to determine the primary limiting factors for these anadromous and non-anadromous reaches.

Methods

Study Area: The Duckabush and Hamma Hamma Rivers, WA are snow-rain dominated watersheds that drain from the eastern slopes of the Olympic Mountains into the Hood Canal (Figure 1). The Duckabush watershed has a drainage area of 194 km^2, with the headwaters in Olympic National Park and National Forest and the Brothers Wilderness Area. The Hamma Hamma River has a drainage area of 220 km^2, with headwaters in Olympic National Park, Olympic National Forest, and the Brothers and Mount Skokomish Wilderness Areas. Both watersheds support populations of Chinook *O. tshawytscha*, Coho *O. kitsuch*, Chum *O. keta* and Pink salmon *O. gorbuscha*, as well as Coastal Cutthroat O. *clarki clarki* and Rainbow/Steelhead trout *O. mykiss*. Several species of small-bodied sculpin *Cottus sp.* are also present and represent a significant fraction of the fish biomass in the watersheds. Waterfalls at river km (Rkm) 12.1 on the Duckabush and Rkm 4.0 on the Hamma Hamma are full barriers to anadromous access, and only resident *O. mykiss* are found above the barrier. Additional partial anadromous barriers at Rkm 7.2 on the Duckabush and Rkm 3.2 on the Hamma Hamma block access to all anadromous species except *O. mykiss*. The watersheds were separated into reaches for this study, with three segments on the Duckabush: the lower reach with full access by all anadromous species (Rkm 0.0 to 7.2); the middle reach with resident fish and steelhead (Rkm 7.2 to 12.6); and the upper above-barrier reach containing only resident species (Rkm 12.6 to 15.3). The Hamma Hamma was divided into two reaches: the lower below-barrier reach (Rkm 0.0 to 4.0) was accessible to all anadromous species except the upper 0.8 km which was only accessible by steelhead and resident fishes, and the upper above-barrier resident reach (Rkm 4.0 to 24.3). The smolt traps were located in the lower watersheds at Rkm 0.5 on the Duckabush and Rkm 0.8 on the Hamma Hamma.

Juvenile Sampling: *O. mykiss* were sampled by angling from June through October of 2013 (N = 1,393), 2014 (N = 1,560) and 2015 (N = 632) to determine their size, diet and growth rates. Captured *O. mykiss* were anesthetized in buffered Tricaine Methanesulfonate (MS-222) at a concentration of 40 mg/L. All fish were measured for fork lengths (FL to the nearest mm), weighed (to the nearest 0.1 g) and a scale sample taken to determine age and growth (Table 1). Diet samples were collected from a subsample of fish via non-lethal gastric lavage (Giles 1980), from a subsample 133 fish in 2015. Diet samples were collected in 150 micron mesh sieve and preserved in 70% ethanol for laboratory examination. During 2013 and 2014 all *O. mykiss* ≥ 70 mm captured by hook and line angling were implanted with a PIT Tag (12.5 mm long, 2.1 mm in diameter: Prentice et al. 1990) by a concurrent mark-recapture study. Hood Canal steelhead populations exhibited median emergence dates of July 8-13 (~80 days in the gravel) (NMFS unpublished data), thus age-0 juveniles were not vulnerable to hook and line angling during the growing season. Consequently, backpack electrofishing was used in the upper Hamma Hamma River in 2015 to collect FL and W data for young of the year fish. All sampled *O. mykiss* were allowed to recover in a holding area until they regained equilibrium prior to release. All activities involving the capture and handling of fish for this study conformed to the University of Washington's IACUC protocol 3286-21and all necessary research permit (NMFS 4d Research Permit #'s 17604, 18327, 19222) and ESA consultation requirements were met.

Scale Analysis: Scales were taken from the preferred region of the fish above the lateral line between the dorsal and adipose fins (DeVries and Frie 1996). Scales were mounted on a gummed card in the field and were pressed into heated acetate impressions for analysis. All scale samples were aged by the Washington Department of Fish and Wildlife (WDFW) aging laboratory and annuli were identified as a region of the scale where circuli spacing was constricted, indicating a period of slow growth. Only fish with scales that could be used to determine age were considered for bioenergetics modeling.

Thermal Environment: The mean daily temperature observed from temperature data loggers (HOBO Water Temperature Pro v2 Data Logger –U22-001) was used as the thermal environment experienced by *O. mykiss* in the Duckabush and Hamma Hamma Rivers (Table 2). The data loggers were tethered to the bank with parachute cord and weighted to rest on the streambed at Rkm 9.7 in the middle reach of the Duckabush and Rkm 15.3 above the barrier in the Duckabush, at Rkm 2.0 in the lower reach on the Hamma Hamma and Rkm 11.7 above the barrier on the Hamma Hamma. A temperature logger was installed in the lower reach of the Duckabush River at Rkm 2.0 during 2013-2014 but was lost during a high flow event. Water temperatures were recorded at 20 minute intervals continuously between September 15, 2013 and September 15, 2014. Data anomalies indicating potential dewatered periods during extreme flow events were removed from the data set. Where temperature data were not available, the temperature was estimated from the mean percent difference between the temperature data from the nearest logger using historical records from 2008-2011 (NMFS unpublished data).

Diet Analysis: Invertebrates in the stomach contents were identified to order, by type (insect, non-insect and terrestrial or aquatic origin) and life stage (unknown, larvae, pupae or adult) and blotted wet weights of each prey group were measured for each stomach. Diet items were combined into functional groups based on energy content of the prey items (Table 3; McCarthy et al. 2009, Lowery and Beauchamp 2015). The percent of the total biomass in each stomach was calculated for each functional group in the diet, with highly digested items distributed proportionally among the identified items. To estimate seasonal mean energy densities (J g^{-1}) in the diet items for each site, blotted wet mass (g) of each energy density group was converted to total energy (J), and the summed amount from all energy groups was divided by the total wet mass (g) of the composite diet sample. Fish were only considered to be available as prey for age-2 and older *O. mykiss* due to gape limitations for younger fish. Late fall and winter diet and drift inputs assumed that aquatic winged adult and terrestrial invertebrates were not available, and these prey categories were distributed proportionally to the low-energy aquatic larvae, high-energy aquatic larvae and aquatic non-insect prey categories. Late-fall diets used September diets (simulation day 1) and winter diets used June diets (simulation day 271-273) (Table 3).

Drift Samples: Drift samples were collected to determine the composition and availability of prey throughout the watersheds. Drift samples were collected over three periods (June, August and September) at two sites in the Hamma Hamma (below-barrier Rkm 1.6 and above-barrier Rkm 11.3) and three sites in the Duckabush River (in lower Rkm 2.0 and middle Rkm 8.4 below-barrier reaches and within the upper above-barrier reach at Rkm 14.1). Two drift nets (0.3 m x 0.3 m, 500 μm mesh) were deployed in areas downstream of riffles associated with fish holding areas at depths of <0.5 m and flow of >0.3 m/s. The frame opening of the nets was 0.3 m wide by 0.3 m high, and the net was

1.1 m long with a 6.0 cm wide cod end. Deployment times were recorded to the nearest minute and varied (range 60–147 minutes, mean: 91 minutes; SD: 22 minutes; Culp et al. 1994) depending on the amount of time needed for concurrent fish sampling. The top 5 cm of the cross-sectional area of the net opening was kept above the surface, to capture surface drifting prey, while the remainder was submerged to capture subsurface prey items. Water volume through the net was determined by measuring water velocity (m/s) and depth in front of each net with a Swoffer Current Velocity Meter (Swoffer Instruments, Inc., Seattle, Washington). Large organic matter was removed and attached invertebrates were washed into the cod end upon net retrieval. Captured macroinvertebrates were transferred into vials and preserved in 70% ethanol diluted with water. Benthic macroinvertebrates were not sampled.

Invertebrates were sorted by order, life stage (larvae, pupae or adult), size (>1mm or <1mm), and grouped by energy density (Table 3). Invertebrates <1mm were omitted from the sample as salmonids feed on larger drift items (Filbert and Hawkins 1995, Keeley and Grant 2001). Monthly drift composition was calculated as the proportional biomass of each energy density group by blotted wet weight (g). To estimate seasonal mean energy densities (J g^{-1}) in the drift sample for each site, blotted wet mass (g) of each energy density group was converted to total energy (Joules), and the summed amount of joules in each energy group was divided by the total wet mass (g) of the composite drift sample. Drift invertebrate biomass (g) and abundance values were expressed as delivery rates per hour through the net. Daily prey supply was calculated by multiplying drift (g)/hour by the hours of daylight between civil twilight at dawn and dusk to approximate the period over which trout could feed on drifting prey. Prey supply (g) was converted to prey energy available (J). This was compared with the energetic needs to attain the observed growth for the average drift feeding trout based on bioenergetic modelling to determine whether sufficient food was available. It was assumed that 100% of the biomass of prey > 1mm delivered to the trap location was available for drift feeding trout to consume. Salmon eggs were not present in drift samples and as such were considered separately when determining temporal food supply.

Prey Electivity: The energetic quality of prey varies by type, and predators may select for higher energy food sources over those of lower energetic value. Prey electivity compares the proportional biomass of edible-sized prey (longest solid linear dimension >1 mm) in the diet to the corresponding proportional biomass of these prey in the drift. Electivity indices were calculated for grams of each functional group found in diet samples using Manly's α (Equation 1, Manly et al. 1972, Chesson 1983).

Equation 1:

$$\alpha_i = \left(\frac{r_i/n_i}{\sum_{i=1}^{m} r_i/n_i}\right)$$

where:
i = prey type
r = proportion of prey type (g) in diet
n = proportion of prey type (g) in drift
m = number of prey types possible (7-8)

A threshold value of $\alpha_i > 1/m$ represented positive electivity for prey group i.

Bioenergetics Modeling: Bioenergetics model simulations were used to estimate consumption and feeding rates for each age class of juvenile *O. mykiss* in non-anadromous and anadromous reaches of the Duckabush and Hamma Hamma Rivers, given observed annual growth rates, and temporal change in diet composition and thermal regimes. We used the Wisconsin Fish Bioenergetics Model (Hanson et al. 1997), parameterized for Rainbow trout and Steelhead (Rand et al. 1993).

The model is an energy balance equation (C = M + W + G) that estimates consumption C (g/d of each prey type) from the net energy required for metabolism M and waste W losses by fitting C to observed growth G (g) of the consumer. The model operates on a daily time step and accounts for the mass- and temperature-dependent effects on maximum consumption and metabolism, and temporal changes in the thermal experience, diet composition, and energy density of the consumer (J/g wet mass). The model also provides consumption as a percentage of theoretical maximum consumption (% Cmax), which can be used as an indicator of food availability. Annual growth simulations were run from September 15, 2013 (day 1) to September 14, 2014 day 365) for ages 1-2, 2-3, and 3-4 (Table 4). The mean initial and final weights used for the annual bioenergetics model simulations were obtained from field observations collected during September of 2013 and 2014.

In addition to the age-specific growth (Table 4), the simulations also used distinct empirically-based inputs from each river and reach for the temporal changes in thermal experience (Table 2), diet composition and the associated energy density of prey and consumer (Table 3). The energy density of O. *mykiss* included seasonal variation, but the same values were used for all age classes. Lowery and Beauchamp (2015) indicated that the initial energy density of *O. mykiss* in the Skagit River was 3,906 J/g in January, 3,900 J/g in May and 3,806 J/g in July, which were used as energy density values to fill in seasonal gaps in model simulations. Bomb calorimetry results from *O. mykiss* captured in the upper Duckabush (n=10) and upper Hamma Hamma (n=10) in early September provided starting and ending values for the energy density (5,815 J/g) of the consumers (Table 4).

Growth Sensitivity: Based on the majority of feeding rates estimated from the annual simulations fitted to observed growth (20-30% Cmax; Table 4), we examined the sensitivity of temperature-dependent growth potential in response to plausible ranges in variability of composite energy densities of seasonal diets and increased or decreased feeding rates. Temperature-dependent growth curves assuming consumption values of 20%, 25%, 30% and 50% Cmax were generated for each age class (1-4) of *O. mykiss*, assuming a mean energy density of 5,815 J/g for the consumers. Curves were produced for each age class at the observed summer (June-September) composite energy diet. Reach-specific curves were combined, with the average composite energy density used where values were similar. A single set of growth curves was produced for the middle and upper reaches of the Duckabush and Hamma Hamma Rivers as composite energy densities of diets in these reaches were similar (Table 3; 3858 J/g, 3854 J/g, 3853 J/g) and the lower reaches of the Duckabush and Hamma Hamma Rivers for age-2 to age-4 (6,394 J/g and 6,406 J/g). Additional curves were produced for high energy drift invertebrate diets (5000 J/g) and diets with higher energy salmon eggs available (12,000 J/g) for each age class. Scope for growth was compared at temperatures corresponding to the mean summer low flow period and different consumption rates and diet compositions to determine the relative effect of increased food quality and quantity for juvenile *O. mykiss*.

Results

Thermal Environment: The thermal environments exhibited considerable seasonal variation and varied among reaches and between the two rivers (Figure 2). In the Duckabush River, temperatures in the upper reach ranged from 1.5 °C to 13.4 °C with an annual mean temperature of 7.1 °C; the middle reach was approximately 2.4% warmer on average, with an annual range from 0.8 °C to 13.8 °C and a mean of 7.3 °C; the lower reach averaged 5.0% warmer than the middle reach, and the annual temperature ranged from 0.9°C to 14.5°C with a mean of 7.6°C. The thermal environment in the Hamma Hamma River is modulated by numerous headwater tributaries and lakes, and exhibits less seasonal variation than in the Duckabush River. Temperature in the upper reach ranged from 3.9 °C to 11.4 °C with an annual mean temperature of 7.6 °C. Temperature in the lower reach of the Hamma Hamma River averaged 15.4 % warmer than the upper reach and ranged from 4.5 °C to 14.0 °C with an annual mean temperature of 8.9 °C.

Seasonal Diet and Drift Comparisons: The seasonal (summer, fall and winter) composite energy densities of diet samples were higher than concurrent drift samples for all sample reaches except during June in the upper reaches of the Duckabush and Hamma Hamma rivers. Seasonal energy densities of diets varied relatively little among reaches and months except for the large increases in the lower reaches of both rivers in during September (Figure 3). The mean composite energy density for the summer was higher than the annual level due to the availability of terrestrial invertebrates (i.e. August in the lower Duckabush) and salmon derived resources in the lower reaches of both rivers during September. Salmon derived resources resulted in significantly higher summer composite energy density for diets in the lower reaches at 6,400 J/g compared to 3855 J/g in the upper below-barrier reaches-barrier reach and above the barriers. The energy budget of summer diet samples middle reach of the Duckabush and above the barriers in both rivers was similar and was dominated by 67% aquatic larvae, while high energy adult terrestrial insects contributed between 8% and 14%. Once salmon eggs became available, they dominated the diets in the lower reaches at 82% in the Duckabush and 57% in the Hamma Hamma River during September (Figure 3). In the Hamma Hamma during the peak of the pink salmon and summer chum runs in early September, sea lice accounted for 19% of the diets by weight, but were absent in subsequent diet samples.

Prey electivity analysis indicated that *O. mykiss* in the middle reach and upper reaches selected against low energy aquatic larvae but showed strong selection for high energy aquatic larvae and a slight to strong selection for terrestrial invertebrates (Figure 4). In the lower reaches, fish selected high-energy prey such as terrestrial invertebrates and salmon eggs, while avoiding low-energy aquatic invertebrates. The supply of higher energy resources such as terrestrial invertebrates in the middle and upper reaches, as estimated by the energy delivered to a drift sampler from dawn to dusk, were insufficient to meet the energetic demands of *O. mykiss* needed to satisfy their observed growth rates, and the greatest limitations occurred during August (Figure 5). The overall indicator of per capita prey supply was limiting during August and September in the middle reach of the Duckabush and during September in the upper reach of the Duckabush (Figure 5). The presence of large winged caddis adults resulted in significantly higher prey availability in the lower reach of the

Duckabush River than in other reaches during September. Age-4 fish were most strongly affected by prey supply limitations across all reaches.

Bioenergetics Modeling: The model simulations indicated that consumption rates associated with observed growth were moderate to low across all age classes. In the middle reach of the Duckabush and upper reaches of both rivers, fish fed at between 25% (age-2) and 32% (age-4) of Cmax. In the lower reaches of the rivers, consumption rates were lower than the upper reaches, ranging between 19% (age-2) and 28% (age-4) of Cmax and increased with age (Table 4). The highest feeding rates were in the upper Duckabush River and the lowest were in the lower Hamma Hamma River.

The bioenergetics simulations indicated that the highest growth occurred between June and September 15, corresponding with the period of warmest water temperatures (Figure 6). In the middle reach of the Duckabush and upper reaches of both rivers, this period accounted for 67%-78% of the total annual growth for age 1-2 *O. mykiss*, 65%-85% for age 2-3, and 67%-81% for age 3-4. In the lower reaches of both rivers, the highest simulated growth occurred during late summer and fall when salmon were spawning. The period between June and September 15 accounted for between 27% and 38% of the annual growth for age 1-2 *O. mykiss*, 28% and 34% for age 2-3, and 25% and 37% for age 3-4. Annual growth efficiency was similar among reaches, ranging from 9.6% to 12.2% for age-2 fish (Table 4). Annual growth efficiency was generally higher for age-2 fish compared to older age classes in the middle and upper reaches of the Duckabush, but showed no clear pattern in the lower Duckabush or either reach in the Hamma Hamma River. Across all age classes, *O. mykiss* experienced weight loss or no growth between mid-November and April during the coldest water temperatures of the year (Figure 6). This was corroborated by empirical data from recaptures. Of three age-2 recaptures tagged above the barrier in the Duckabush September 2014 and caught in June 2015, the average weight gain was 7.2g, with one showing weight loss. During the same period, the model predicted 8.5g of weight gain. Similarly in the above-barrier reach of the Duckabush, weight frequency data for mid-August was within 4g of the weight predicted by the model, at 72g (observed) versus 68g (model) for age-2 to age-3 fish.

Growth Sensitivity: The temperature-dependent growth curves indicated that the annual mean temperature was well below the optimum for all age classes, and growth potential in the rivers was highest during the summer when water temperatures were warmer (Figure 7). The temperature range for mid-November to April was unlikely to allow growth for juvenile *O. mykiss*. The mean summer temperatures of 11.0 (upper reach) and 11.2 (middle) and 11.7 °C (lower) in the Duckabush River and 12.1°C in the lower Hamma Hamma were near the optimum temperature for growth for *O. mykiss* feeding at the observed 20-30% Cmax with composite prey energy density of 3,855 J/g (for the middle and upper reaches) and 6,400 J/g (lower below-barrier). The mean temperature of 10.1 °C in the upper Hamma Hamma was slightly below the optimum growth rate, but still provided relatively high potential growth under ambient feeding conditions. Given a higher rate of consumption, the water temperatures would have allowed much faster growth than was observed. At 25% Cmax, the highest growth rates were 0.006 $g^{\bullet}g^{-1}d^{-1}$ for age-1, 0.004 $g^{\bullet}g^{-1}d^{-1}$ for age-2 and 0.002 $g^{\bullet}g^{-1}d^{-1}$ for age-3 fish (Figure 7).

With higher energy prey available, such as pulses of terrestrial invertebrates in late summer and fall, the growth potential of *O. mykiss* could increase even if consumption rates remain low. Despite lower consumption rates in the lower anadromous reaches (20% Cmax age 2-3 and 25% age-4), the increased prey quality (higher energy density) in late summer and fall resulted in higher growth rates than observed in the middle reach of the Duckabush or upper reaches in both rivers (Table 4). Age-2 fish grew at 0.008 $g^{\bullet}g^{-1}d^{-1}$, while age-3 fish grew at 0.006 $g^{\bullet}g^{-1}d^{-1}$. If prey quality in the middle and upper reaches of the rivers was increased to 5,000 J/g (e.g., from a higher proportional contribution of adult or terrestrial insects), *O. mykiss* would experience an 81% increase in growth rate to 0.012 $g^{\bullet}g^{-1}d^{-1}$ for age-1 fish, a 108% increase to 0.007 $g^{\bullet}g^{-1}d^{-1}$ for age-2 and 142% to

0.006 $g^{\bullet}g^{-1}d^{-1}$ at age-3 (Figure 7). Increasing feeding rates in the summer to 50% Cmax in the middle and upper could increase growth rates by 288% (0.023$g^{\bullet}g^{-1}d^{-1}$) for age-1 and 352% (0.016 $g^{\bullet}g^{-1}d^{-1}$) for age-2 fish, 460% (0.013 $g^{\bullet}g^{-1}d^{-1}$) age-3. In the lower reaches, the same change in consumption rate would increase growth by 348% (0.021$g^{\bullet}g^{-1}d^{-1}$) for age-1 and 333% (0.035 $g^{\bullet}g^{-1}d^{-1}$) for age-2 fish, and 377% (0.029 $g^{\bullet}g^{-1}d^{-1}$) age-3 (Figure 8). At the observed prey quality (3,855 J/g), there was little to no growth potential for age-3 and age-4 individuals at 20% Cmax in the upper reaches. These results show that older age classes have the most potential gain from both increases in consumption rate and prey quality. Given that larger, older individuals cannot achieve positive growth under ambient food supply and thermal conditions in the upper watersheds they may be shifting to more profitable habitats or adopting an alternative life history strategy to maintain growth.

Discussion

The results of this study indicated that food supply limits the growth potential of *O. mykiss* in the watershed in the Duckabush River and Hamma Hamma Rivers despite near-optimal temperatures during the peak summer growing season. Differences in prey resources among the reaches significantly affected growth, with the lower anadromous reaches less limited where salmon derived resources were seasonally available. Older age classes of fish were typically more limited as growth rates declined with age, resulting in higher overall consumption rates to attain sufficient growth. Armstrong and Schindler (2011) reported that 66 different species of fish fed at the median of 43% Cmax. In comparison our observed consumption rates were low across the study, but were higher in the middle and upper reaches (25%-32%) than in the lower reaches (20%-26%). The lowest consumption rates were observed in the lower reaches of the Duckabush and Hamma Hamma Rivers, but the seasonal availability of higher energy salmon eggs compensated for the lower overall food supply and actually supported higher growth rates for age-2 and older *O. mykiss* than in the middle and upper reaches. It is also possible that these consumption rates were underestimated as we were unable to collect diets during spring when juvenile salmon are available as a potential prey source. Pflug et al. (2013) showed that fish contributed 57% to 91% of the diet of *O. mykiss* smolts in the Skagit River during the spring of 2009 and 2010, with pink salmon fry being the primary prey source in 2010. Consumption rates observed in this study were low compared to similar studies in other regions (Railsback and Rose 1999, Godby et al. 2007, McCarthy et al. 2009, Weber et al. 2014), but were similar to feeding rates reported in the Skagit River within the Puget Sound region (Thompson and Beauchamp 2016). Rainbow trout exposed to a thermal environment in a Sierra Mountain, CA stream similar to that during the low flow period in the Duckabush displayed consumption rates of 35% to 42% of Cmax (Railsback and Rose 1999). A study by McCarthy et al. (2009) showed similarly low consumption rates during the summer low flow period in the Russian River of coastal northern California, but indicated that elevated stream temperatures were also a primary factor limiting growth. In the Duckabush and Hamma Hamma Rivers, the observed temperatures were near the optimum range for growth during the summer low flow period. Railsback and Rose (1999) showed that when temperature regimes are not extreme, factors controlling consumption affect growth more than the direct effects of temperature. A similar effect was observed in tributaries to the Skagit River, WA, where prey density and quality appeared to be limiting growth in the watershed (Thompson and Beauchamp 2016).

Food quality and quantity exerted the greatest influence over growth in the Duckabush and Hamma Hamma Rivers, and seasonal inputs of terrestrial invertebrates and salmon derived resources were likely crucial to the growth and survival of *O. mykiss* in the watershed. The exploitation of seasonally available high energy food sources by salmonids is well documented (Needham 1930, Kawaguchi and Nakano 2001, Sweka and Hartman 2008). Nakano et al. (1999) showed that over a 24 hour period, *O. mykiss* in forest streams relied on terrestrial invertebrates for up to 70% of their daily food intake during the summer low flow period. The observed proportion of terrestrial invertebrates varied among reaches in our study but ranged from 10% to 30% of the diet of *O. mykiss*. While this represents a significant portion of the diet, it is considerably lower than that reported in other studies (Nakano et al. 1999, McCarthy et al. 2009, Thompson and Beauchamp 2016). This may be due to differences in the climate or habitat available in the Duckabush and Hamma Hamma Rivers. Both watersheds have intact riparian habitat. Other studies have indicated that streams with high quality riparian habitat typically have higher densities of terrestrial insects in the drift (Allan et al. 2003). As terrestrial insects in the drift typically peak near dusk (Nakano et al. 1999), they may be underestimated in the diets included in this study, which were collected during the day. Additionally inputs of terrestrial insects are often episodic in nature and may have been missed by the limited diet samples.

During 2015 the lower reaches of the Duckabush and Hamma Hamma Rivers received sizeable returns of pink (~200,000 to 225,000) and summer chum (~6,000 to 1,500) salmon (SCoRE WDFW 2017); salmon-related resources provided a high energy food supply and accounted for the majority of the annual growth. While invertebrate prey quality was higher in the middle and upper reaches of the Duckabush and Hamma Hamma Rivers than in the lower reaches, the availability of salmon-derived resources in the fall resulted in more energy-rich diets on average. Seasonally available inputs from anadromous salmon (eggs, flesh, etc.) represent an important resource. Jaecks and Quinn (2014) indicated that eggs and flesh contributed up to 80% of the annual energy budget of Dolly Varden (*Salvelinus malma*) in Southeast Alaska. Similarly, our results suggest that the relatively brief period when salmon eggs were available resulted in majority of the annual growth for *O. mykiss* in the lower reaches of the Duckabush and Hamma Hamma Rivers. Similar to bull trout in the Skagit River (Lowery and Beauchamp 2015), we observed that *O. mykiss* showed an aversion to pink salmon eggs. However, sea lice falling off of pink salmon as they entered freshwater, accounted for 19% of the diet in early September.

ESA-listed summer chum return during a period of near optimum water temperatures and provided the majority of the eggs preferred by *O. mykiss*, and as such summer chum salmon may be particularly important to sustaining *O. mykiss* populations in the lower reaches below the barriers. Our results showed that *O. mykiss* lost weight during the winter months when growth opportunities were limited, suggesting that access to these seasonal high energy prey resources may be crucial in allowing fish to build up sufficient lipid reserves going into winter, when higher mortality rates are common (Biro et al. 2004).

Food limitation occurred to varying degrees in all of the sample reaches. Typically, the supply of high energy aquatic invertebrates and terrestrial invertebrates were insufficient to fully satisfy the energetic demands of *O. mykiss* in the watersheds, thus necessitating inclusion of varying proportions of lower-energy prey in the diet. Our results also suggest that these higher energy prey were preferred by *O. mykiss* and had substantial effects on growth. In the middle and upper reaches of the watersheds, where salmon were absent, growth potential declined with age and size of consumer, and even became negative for ages 3 and older *O. mykiss*. These reaches also contained the highest densities of *O. mykiss* (Chapter 2), which suggests that density-dependent effects influenced the population. The degree of these limitations was typically much less severe for age-2 *O. mykiss* compared to older age classes due to the higher absolute metabolic demands of larger fish. The presence of salmon in the lower reaches likely resulted in additional prey resources due to the addition of marine derived resources. Wipfli et al. (1998) reported that salmon-bearing streams in Southeast Alaska supported 8-25 times higher aquatic invertebrate densities compared to those without salmon populations. In the lower reaches the lowest observed abundance of invertebrates in the drift, when limitations did occur, was in September during salmon spawning. Minakawa (1997) showed that salmon spawning can temporarily reduce aquatic invertebrate abundance. While drifting invertebrates were less available in September, salmon eggs during this period were readily available and were selected for by *O. mykiss*. While prey items were not as limiting in the lower reaches below the barriers in the watersheds, the observed abundances of *O. mykiss* (Chapter 2) were typically low suggesting that rearing habitat may be a primary limiting factor.

While reduced growth opportunities in freshwater have the potential to reduce survival at later life stages, they may also influence the life history trajectory of *O. mykiss*. Model simulations indicated that the growth of older age classes of fish was most limited by prey quality and quantity. Our results suggest that in the upper/mid Duckabush River and Hamma Hamma River, there is little scope for growth for fish older than age-2, except for the fastest growing individuals that fed at higher than average rates. Benjamin et al. (2013) suggested that when food is limiting *O. mykiss* are more likely to adopt an anadromous life history strategy. This may be the case in the middle reach of the Duckabush, where food resources were most limited. However, in reaches above the anadromous barriers in the Duckabush and Hamma Hamma Rivers fish are less likely to smolt as a result of food supply limitations. It is possible that above-barrier fish migrate downstream over the barriers (Chapter 2), and declining growth potential could potentially trigger these migrations. In the lower anadromous reaches, where food was less limiting, there was substantial scope for growth past age-2 suggesting that fish may be more likely to adopt a resident life history strategy. A number of studies have indicated that residency is common in watersheds with high levels of salmon derived resources, which allow *O. mykiss* to mature at sizes similar to anadromous individuals (Russell 1974, Russell 1977, Pavlov et al. 2001, Kuzishchin et al. 2007). Our observations support this as the largest *O. mykiss* of the study were encountered in the lower rivers (Chapter 2). However, as anadromy is also influenced by genetics (Neave 1944, Thrower et al. 2004, Hayes et al. 2012) and other environmental factors (McMillan et al. 2007, Narum et al. 2008), it is unclear to what extent limitations on growth influence the life history trajectories of *O. mykiss* in these populations.

The low consumption rates and relative scarcity of food resources often result in increased intraspecific competition (Keeley and McPhail 1998, Keeley 2000, Keeley 2001), and density-dependent effects. *O. mykiss* is a territorial species and agonistic behavior is common when resources are limited (Li and Brocksen 1977). Abbott et al. (1985) showed that juvenile steelhead were able to assert dominance over their conspecifics when their body mass was 5% greater. As older and larger fish have a much greater scope for growth (in terms of absolute weight gain) at higher consumption rates, adopting this competitive behavior may be particularly beneficial to these individuals. When similarly-sized dominant and sub-dominant juvenile steelhead where fed equal rations, the dominant fish grew 12.3% faster than sub-dominant individuals (Abbott and Dill 1989). This behavior generally leads to higher variability in size and the amount of food individuals can consume. However, when food is relatively abundant, feeding hierarchies tend to break down, and overall variation in size of individuals in the populations decreases (McCarthy et al. 1992). Slaney and Northcote (1974) showed that at higher prey levels, juvenile rainbow trout defended smaller territories and exhibited lower frequency of aggressive behaviors. With food limitations in the watershed, it is possible that reduced flows during summer associated with future climate change could exacerbate this problem, resulting in a deficit of available habitat and increased competitive interactions. As size selective mortality has been identified as a factor (Thompson and Beauchamp 2014) influencing poor early-marine survival (Moore et al. 2010, Moore et al. 2013), reduced growth opportunities and density-dependent effects in freshwater may be important factors restricting the growth of these populations. However, high mortality rates in freshwater caused by delayed smoltification due to low growth rates may be another significant factor limiting these steelhead populations.

Inter-annual survival rates for age-2 and older *O. mykiss* in the Duckabush and Hamma Hamma Rivers were low across all years and reaches (Chapter 2), and were likely influenced by the observed growth limitations. In the middle and upper reaches where growth opportunities are limited past age-2, survival declined with age. However in the lower reaches where salmon-derived resources are available and growth is less limited, survival improved with age. Reduced growth opportunities can restrict survival in salmonids, with coho salmon exhibiting higher survival rates in years when individuals were larger (Hartman et al. 1987). Similarly with *O. mykiss* larger individuals had higher survival rates than smaller individuals, even when they were protected from predation (Smith and Griffith 1994). The growth limitations and low survival may be a significant impact on juveniles that are unable to reach a threshold smolt size, and then delay an additional year during which they are exposed to the high mortality rate. In the Duckabush River where genetic analysis indicated that 90% of the below-barrier population assigned to the above-barrier population (Chapter 2), there may be significant competition between steelhead and rainbow trout life history forms for the limited prey resources.

The results of this study highlight the importance of identifying factors limiting *O. mykiss* populations in freshwater. Early growth is a critical period that affects both survival among juvenile life stages and smolt to adult returns (Thompson and Beauchamp 2014). Identifying where growth limitations are influencing survival or life history patterns of *O. mykiss* in freshwater can help guide recovery and restoration efforts. Summer chum, which return during near optimum water temperatures in the lower rivers provide an important seasonal food resource, and maintaining healthy runs may be particularly important to the growth of *O. mykiss* in these watersheds. However, the role that food supply and food quality play in life history expression remains unclear. Further research should investigate the role that additional food resources from salmon in the lower reaches of watersheds have on life history expression and whether this results in a shift towards a resident life history strategy. Additionally, as food resources were limiting in the middle and upper reaches of the watersheds, future work could determine the extent to which density-dependent effects are occurring and whether habitat quality is limiting *O. mykiss* abundance throughout the watershed. Finally, although size selective mortality has been documented in other Puget Sound watersheds it is unknown whether these populations are affected. Understanding the degree to which reduced growth opportunities at early life stages affects survival to adulthood is essential to understanding the factors limiting steelhead populations in the area.

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Chapter 3: Figures and Tables:

Table 1. Summary statistics for *O. mykiss* captured and measured above and below the barriers in the Duckabush and Hamma Hamma Rivers in 2013 and 2014.

Sample Site	Age	2013			2014		
		n	FL (mm)	SD	n	FL (mm)	SD
Upper Duckabush	0	NA	NA	NA	2	83	5
	1	121	137	16	129	132	12
	2	102	181	18	224	175	19
	3	68	211	19	26	206	15
	4	15	237	22	9	249	17
	5	NA	NA	NA	1	260	NA
Middle Duckabush	1	180	140	20	81	133	15
	2	132	178	25	140	170	26
	3	24	220	34	16	211	25
	4	2	280	33	NA	NA	NA
	5	NA	NA	NA	1	321	NA
Lower Duckabush	1	74	128	15	85	124	17.91
	2	45	169	26	31	171	27.31
	3	5	232	56	4	212	27.46
	4	NA	NA	NA	2	318	70.71
	5	NA	NA	NA	1	340	NA
Upper Hamma Hamma	1	88	131	24	103	126	18
	2	209	157	20	225	162	22
	3	61	196	24	31	197	23
	4	5	218	11	6	230	40
Lower Hamma Hamma	1	84	134	17	126	141	16
	2	105	162	20	125	159	19
	3	15	206	28	20	206	37
	4	2	245	NA	4	267	67
	5	1	383	NA	1	301	NA

Table 2. Mean monthly water temperature °C in the Duckabush and Hamma Hamma Rivers during 2013-2014. September 15, 2013 represents day-1 of the simulations.

Month	**Temperature °C**					
	Sim. Day	**Upper Duckabush**	**Middle Duckabush**	**Lower Duckabush**	**Upper Hamma**	**Lower Hamma**
September	1	10.2	10.5	11.0	9.9	11.5
October	31	7.2	7.4	7.8	8.8	10.2
November	62	5.6	5.8	6.1	7.3	8.4
December	92	3.6	3.3	3.4	6.0	7.0
January	123	4.2	4.3	4.5	5.6	6.4
February	154	3.3	3.6	3.8	4.7	5.5
March	182	4.7	5.0	5.6	5.2	6.1
April	213	5.7	6.0	6.3	6.1	7.1
May	243	6.8	7.1	7.4	7.3	8.5
June	274	8.6	8.8	8.9	8.7	10.3
July	304	11.9	12.2	12.8	10.3	12.6
August	335	12.4	12.7	13.3	10.8	13.2
September	365	10.2	10.5	11.0	9.9	11.5

Table 3. Prey diet proportions and energy densities by taxa and by energy groups used in bioenergetics model simulations. September 15, 2013 represents day-1 of the simulations.

Watershed	Sim. Day	Low Energy Aq. Larvae (2,991 J/g)	Aq. Non-Insect (3,571 J/g)	High Energy Aq. Larvae (4,272 J/g)	Winged Aq. Adult (4,258 J/g)	Terr. Larvae (4,272 J/g)	Terr. Non-Insect (3,842 J/g)	Terr. Adult (5,798 J/g)	Sea Lice (3,571 J/g)	Salmon Eggs (12,000 J/g)	Salmon Flesh (3,300 J/g)	Fish (5,552 J/g)
Upper Duckabush	1	0.377	0.000	0.258	0.330	0.000	0.002	0.033	0.000	0.000	0.000	0.000
	48	0.594	0.000	0.406	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	198	0.909	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	271	0.671	0.000	0.067	0.223	0.000	0.027	0.012	0.000	0.000	0.000	0.000
	342	0.258	0.000	0.364	0.087	0.000	0.087	0.204	0.000	0.000	0.000	0.000
	365	0.377	0.000	0.258	0.330	0.000	0.002	0.033	0.000	0.000	0.000	0.000
Middle Duckabush	1	0.411	0.001	0.179	0.038	0.056	0.028	0.287	0.000	0.000	0.000	0.000
	48	0.695	0.002	0.303	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	198	0.746	0.000	0.254	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	273	0.356	0.000	0.122	0.398	0.012	0.000	0.112	0.000	0.000	0.000	0.000
	343	0.811	0.001	0.089	0.082	0.007	0.000	0.010	0.000	0.000	0.000	0.000
	362	0.411	0.001	0.179	0.038	0.056	0.028	0.287	0.000	0.000	0.000	0.000
	365	0.411	0.001	0.179	0.038	0.056	0.028	0.287	0.000	0.000	0.000	0.000
Lower Duckabush	1	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.991	0.000	0.000
	30	0.190	0.000	0.810	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	271	0.186	0.000	0.791	0.002	0.000	0.000	0.021	0.000	0.000	0.000	0.000
	344	0.576	0.127	0.089	0.055	0.000	0.000	0.147	0.006	0.000	0.000	0.000
	365	0.000	0.000	0.002	0.065	0.001	0.000	0.005	0.000	0.923	0.004	0.000
Upper Hamma Hamma	1	0.393	0.017	0.193	0.041	0.174	0.008	0.175	0.000	0.000	0.000	0.000
	48	0.652	0.028	0.320	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	198	0.911	0.082	0.007	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	272	0.609	0.055	0.005	0.181	0.019	0.126	0.005	0.000	0.000	0.000	0.000
	340	0.270	0.000	0.536	0.033	0.080	0.000	0.081	0.000	0.000	0.000	0.000
	361	0.394	0.017	0.193	0.041	0.173	0.007	0.175	0.000	0.000	0.000	0.000
	365	0.394	0.017	0.193	0.041	0.173	0.007	0.175	0.000	0.000	0.000	0.000
Lower Hamma Hamma	1	0.000	0.000	0.000	0.094	0.086	0.002	0.010	0.000	0.807	0.001	0.000
	30	0.370	0.614	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	272	0.177	0.294	0.007	0.145	0.088	0.284	0.005	0.000	0.000	0.000	0.000
	327	0.030	0.000	0.273	0.126	0.065	0.078	0.388	0.000	0.000	0.000	0.040
	356	0.017	0.000	0.341	0.065	0.000	0.001	0.000	0.186	0.324	0.066	0.000
	365	0.017	0.000	0.341	0.065	0.000	0.001	0.000	0.186	0.324	0.066	0.000

Table 4. Bioenergetics simulation inputs (growth [G]: Wf – Wi) and outputs (feeding rate [%Cmax], and consumption [C]) for simulations conducted on fish captured during September 2013 and September 2014 at different life stages for growth estimated between ages1-2, 2-3, 3-4 and during summer for age-2, age-3 and age-4. Energy densities for all age classes were assigned values of 5,815 J/g on day 1, 3,906 J/g on day 109 (Jan 1) 3,900 J/g on day 289 (May 1), 3,806 J/g on day 290 (July 1), and 5,815 J/g on day 365 (Sep 14).

Model	Age	n_i	n_f	W_i	W_f	ED_i (J/g)	ED_f (J/g)	% Cmax	G	C	%GE
Upper Duckabush River											
Annual	1-2	121	224	30.8	80.2	5,815	5,815	28.2	49.4	455.1	10.8
Annual	2-3	102	26	67.2	125.9	5,815	5,815	29.1	58.7	710.9	8.3
Annual	3-4	68	9	107.7	210.5	5,815	5,815	32.3	102.8	1126.4	9.1
Middle Duckabush River											
Annual	1-2	180	140	36.8	66.8	5,815	5,815	25.4	30.0	429.9	9.8
Annual	2-3	132	16	73.0	107.8	5,815	5,815	26.7	34.8	672.8	5.2
Annual	3-4	24	2	122.9	221.1	5,815	5,815	31.7	98.2	1255	7.8
Lower Duckabush River											
Annual	1-2	74	31	23.2	61.0	5,815	5,815	20.1	37.8	310.4	12.2
Annual	2-3	45	4	54.5	111.8	5,815	5,815	21.7	57.3	549.5	10.4
Annual	3-4	5	2	109.8	316.7	5,815	5,815	28.1	206.9	1355.2	15.3
Upper Hamma Hamma											
Annual	1-2	88	225	29.0	72.9	5,815	5,815	26.6	43.9	456.5	9.6
Annual	2-3	209	31	49.0	124.7	5,815	5,815	29.4	75.7	737.9	10.3
Annual	3-4	61	6	104.1	207.7	5,815	5,815	31.2	103.6	1200.3	8.6
Lower Hamma Hamma											
Annual	1-2	84	125	26.1	60.1	5,815	5,815	19.6	34.0	340.8	10.0
Annual	2-3	105	20	46.0	118.4	5,815	5,815	22.5	72.4	615.9	11.8
Annual	3-4	15	4	97.9	220.8	5,815	5,815	25.0	122.9	1104.7	11.1

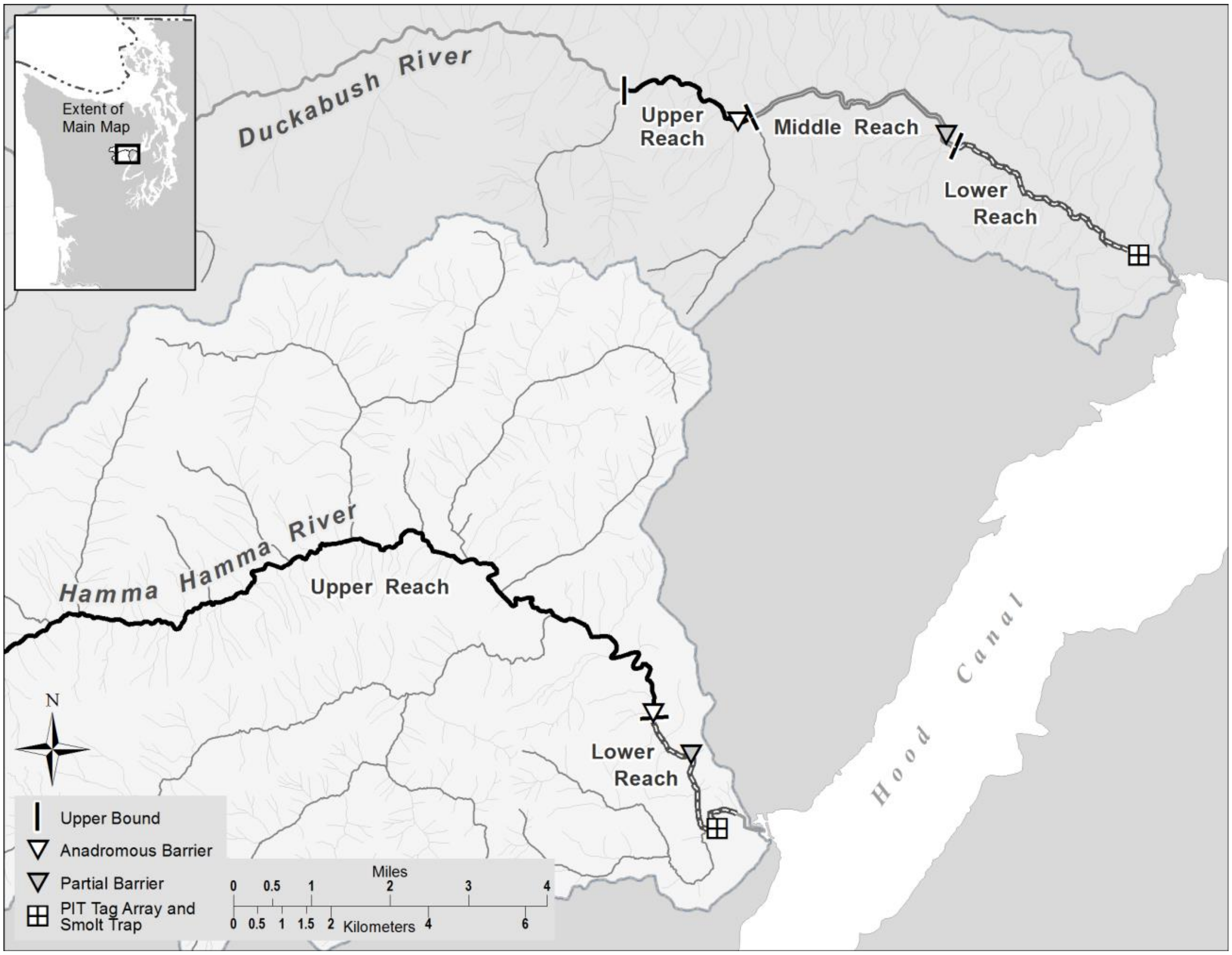

Figure 1. Map of the Duckabush and Hamma Hamma watersheds, showing the location of the sampling reaches and the smolt trap locations (Source: WDFW GIS Lab).

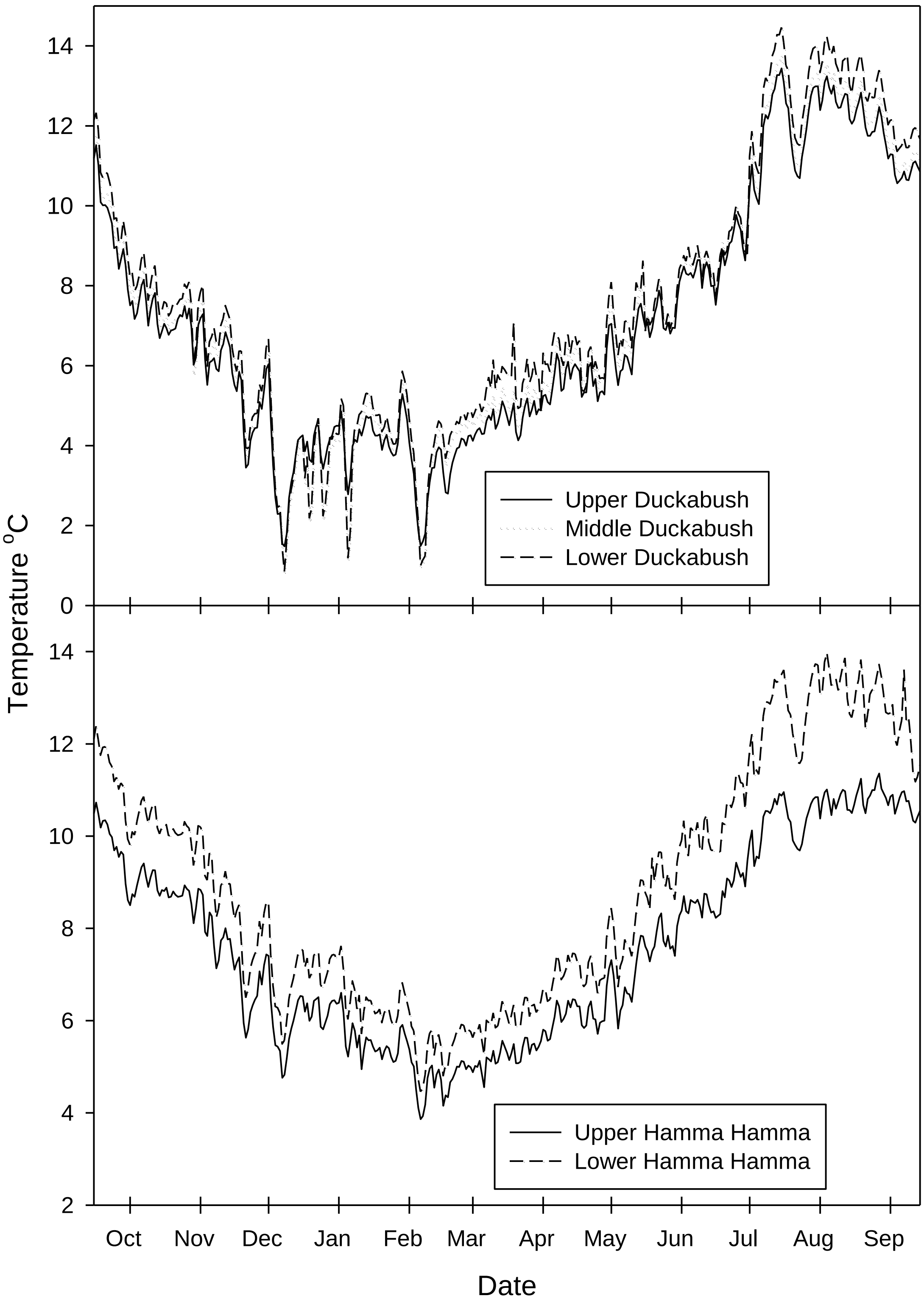

Figure 2. Annual September 2013 to September 2014 stream temperatures °C in the Duckabush and Hamma Hamma Rivers.

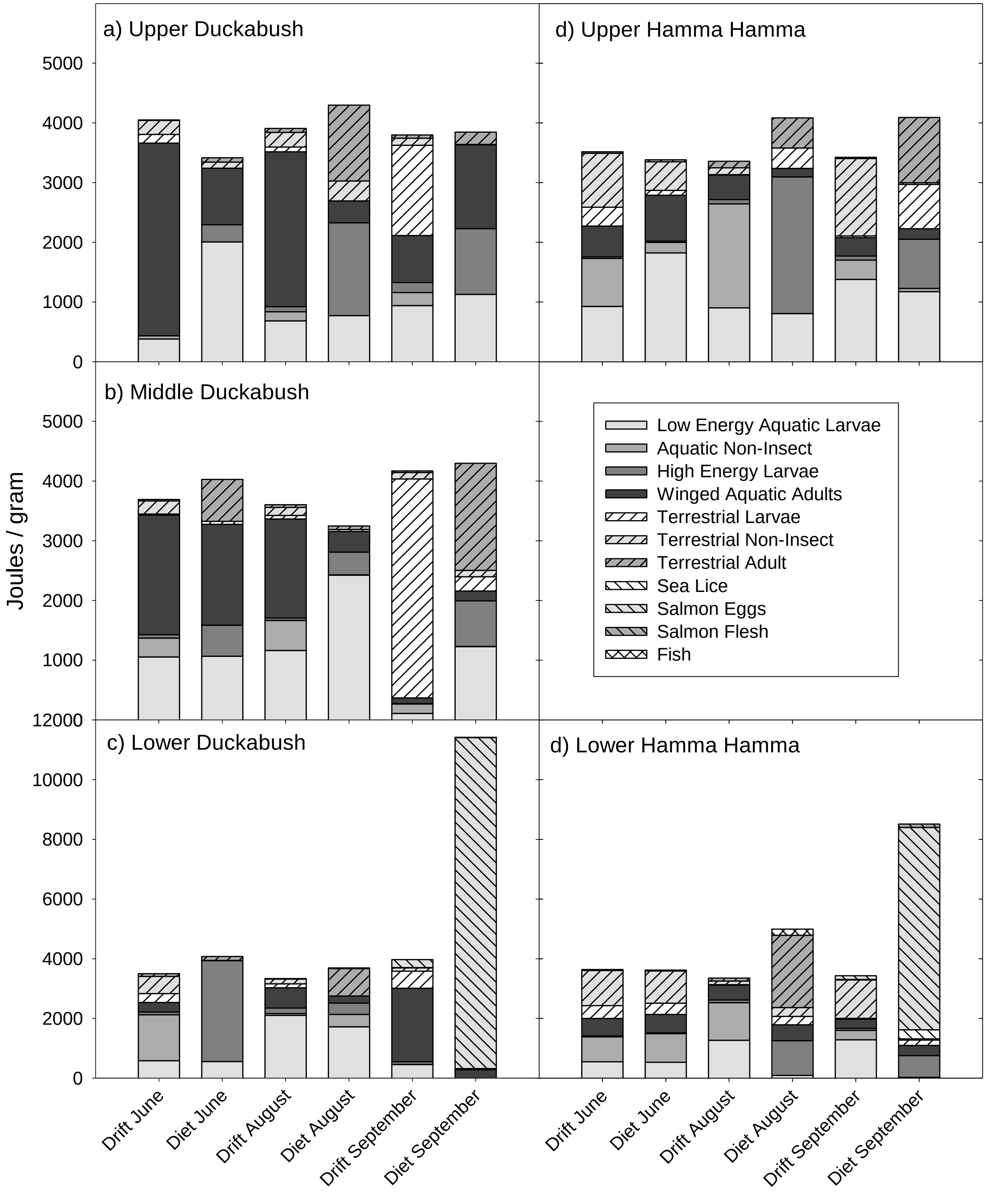

Figure 3. Composite energy density (J/g) of seasonal drift samples and *O. mykiss* diets in the Duckabush and Hamma Hamma Rivers.

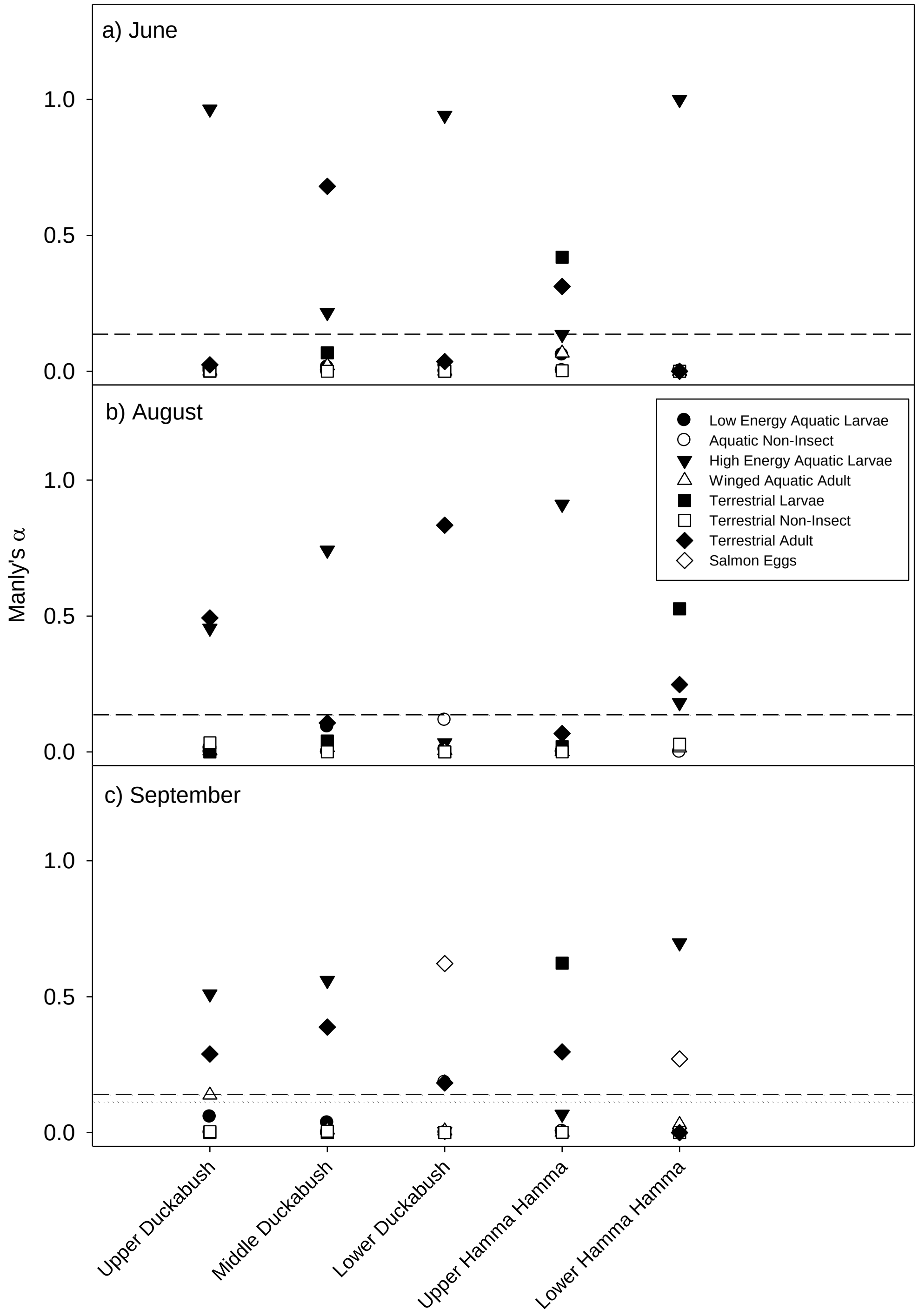

Figure 4. Manly's alpha, as calculated for *O. mykiss* during June, August, and September 2015 in the Duckabush and Hamma Hamma Rivers. Calculations were based on average diet from field collections and on prey availability from the results of drift net sample analysis. Alpha values that indicate no preference fall on the dashed or dotted (L. Duck and L. Hamma August) reference line, which is placed according to the number of prey types (1/m). Points that fall above the line indicate prey preference and points below the line indicate prey avoidance.

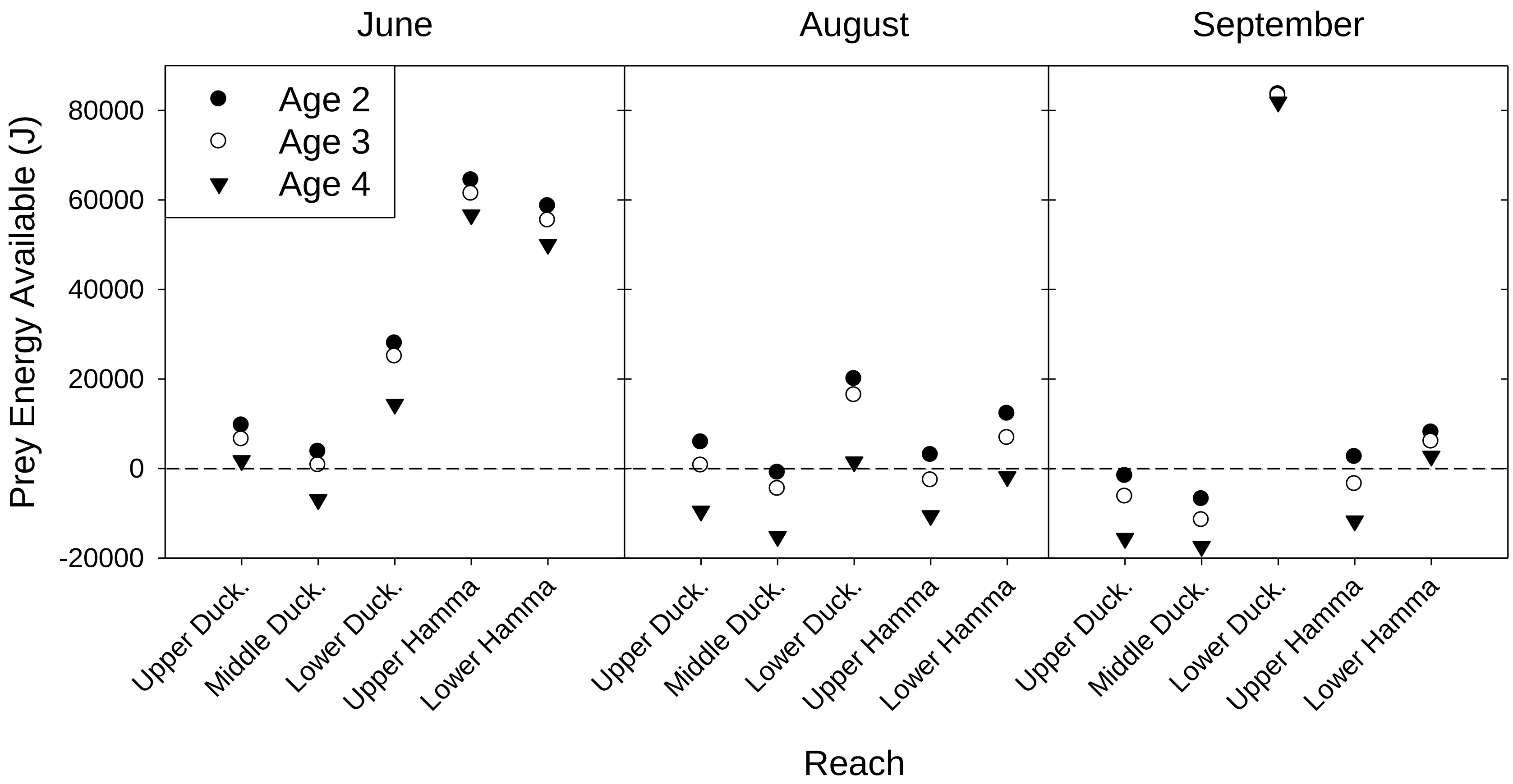

Figure 5. Prey energy availability (J) versus consumer demand in June, August and September for age-2 to age-4 *O. mykiss* in the Duckabush and Hamma Hamma Rivers. Values below the dashed line represent prey energy levels (J) below the energetic demand required for typical drift feeding *O. mykiss* to satisfy their observed growth rate.

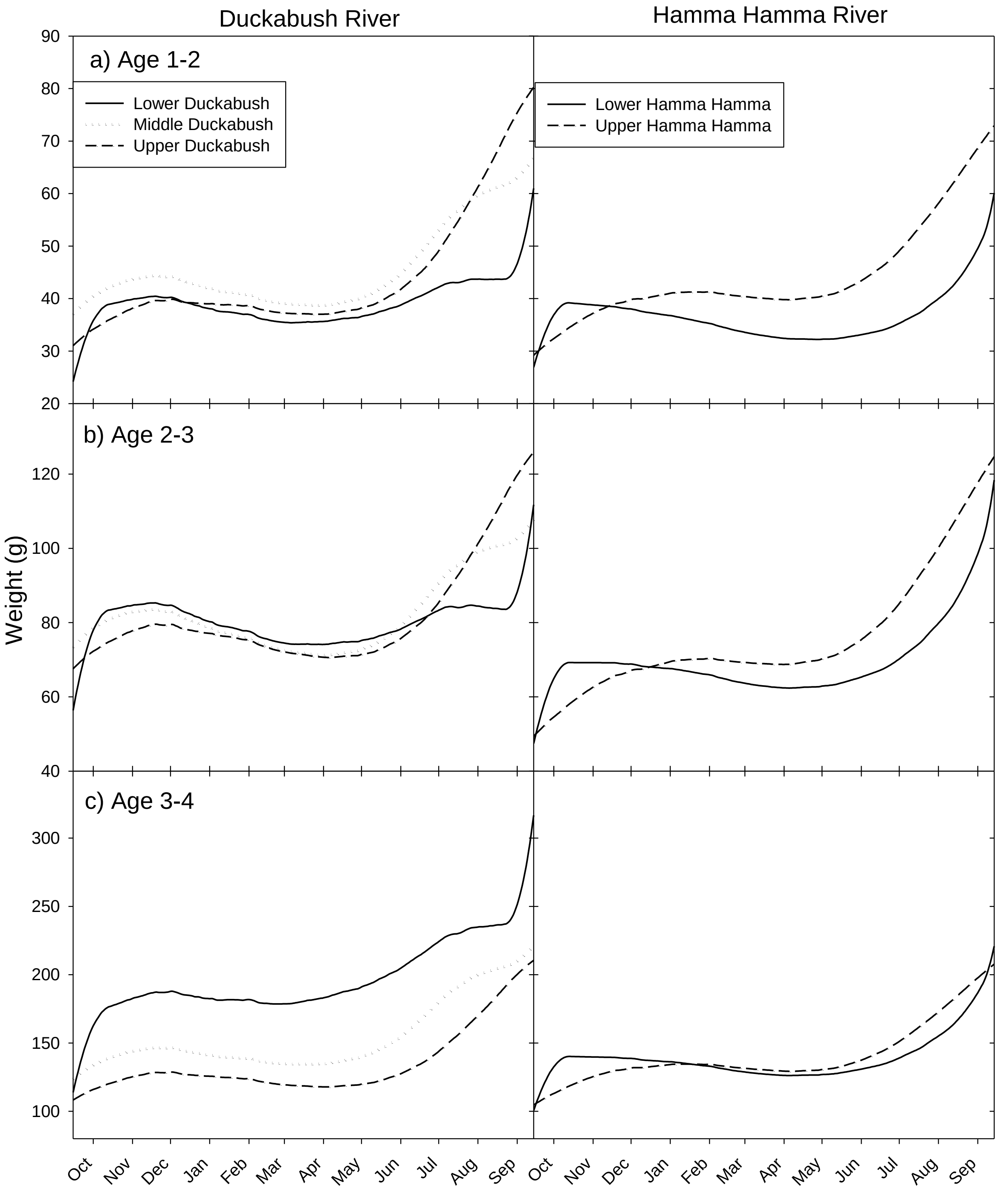

Figure 6. Bioenergetics simulation results of annual growth trajectories for age 1–2, 2–3 and 3-4 *O. mykiss* in Duckabush and Hamma Hamma Rivers between September 15 and September 14.

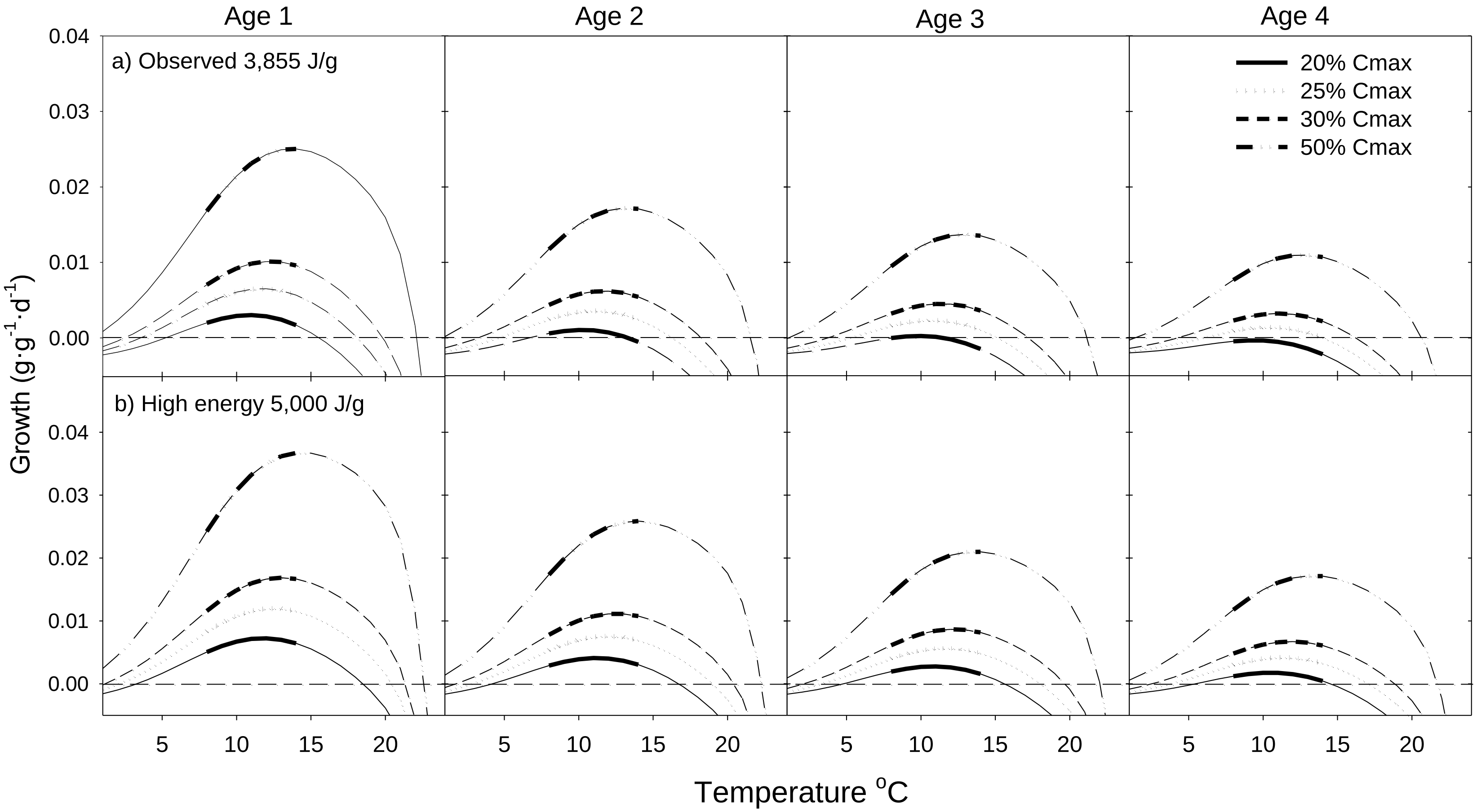

Figure 7. Temperature-dependent growth curves for age-1 (30g), age-2 (75g), age-3 (125g) and age-4 (200g) *O. mykiss* in the upper and middle Duckabush River and upper Hamma Hamma River with observed prey quality from diets (June-September) (3,855 J/g; upper panels) and a high prey quality (5000 J/g; lower panels) varying consumption rates 20%, 25%, 30% and 50% Cmax. The dashed horizontal line represents no growth. Bold portions of the curves represent the observed temperatures between June 1 and September 14.

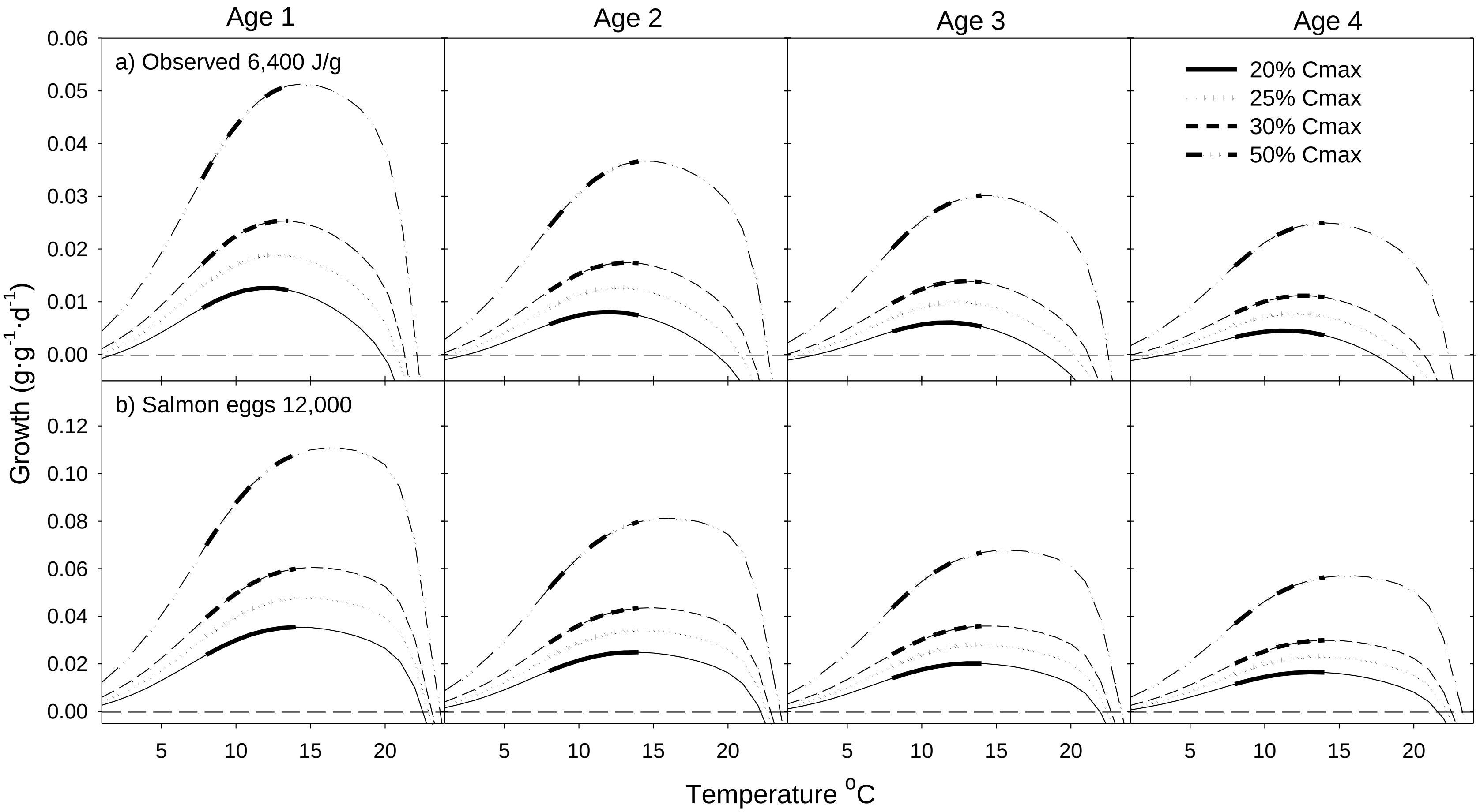

Figure 8. Temperature-dependent growth curves for age-1 (30g), age-2 (75g), age-3 (125g) and age-4 (200g) in the lower Duckabush and Hamma Hamma rivers with observed prey quality from diets (June-September) (6,400 age; upper panels) and salmon eggs (fall) (12,000 J/g; lower panels) at varying consumption rates 20%, 25%, 30% and 50% Cmax. The dashed horizontal line represents no growth. Bold portions of the curves represent the observed temperatures between June 1 and September 14.