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Assessing Indices of Growth for Field Studies of Juvenile Salmon: An Experiment and Synthesis

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Abstract

The hypothesis that size-selective mortality in the first marine year is a major regulator of recruitment in Pacific salmon *Oncorhynchus* spp. has led to interest in assessing the recent growth of field-caught fish. Understanding differences in relative growth across years, regions, habitats, and prey fields may provide insights into factors influencing survival. Plasma insulin-like growth factor 1 (IGF1), muscle RNA : DNA ratio (RD), and scale circulus spacing have all been used as indices of recent growth in juvenile salmonids. We concurrently assessed these growth rate indices in a laboratory study of postsmolt, young-of-the-year, ocean-type Chinook Salmon *O. tshawytscha*. We synthesized results with previous work to inform selection of appropriate growth rate indices for field studies on juvenile salmonids. Muscle samples suitable for RD analysis were obtained nonlethally and without subsequent growth impacts, even for very small juvenile salmon (75–99 mm FL). Plasma IGF1 concentration was strongly correlated with growth rate ($R^2 = 0.79$), while $\log_e(\text{RD})$ and mean spacing of the outer two circuli were moderately correlated with growth rate

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($R^2 = 0.47$ and 0.44 , respectively). Relationships between the two biochemical indices and growth rate were independent of body size at the start of the experiment. Conversely, initially larger fish formed wider circuli for a given growth rate. Insulin-like growth factor 1 and RD responded to a change in growth conditions within 4 and 6 d, respectively. Rate of circulus formation varied positively with growth rate, meaning that outer circulus spacing indexed shorter periods of growth in faster-growing fish. Our results confirm the value of plasma IGF1 as an index of recent growth in juvenile salmon. Although scales (marginal circulus spacing) and white muscle (RD) can both be sampled nonlethally, scale sampling presents a number of practical advantages.

Variation in survival of early life history stages has long been implicated in driving recruitment variability in marine fisheries (Hjort 1914; Lasker 1978). In the case of Pacific salmon *Oncorhynchus* spp., size-selective mortality of juveniles is hypothesized to occur soon after marine entry (Parker 1965; Woodson et al. 2013) or subsequently during marine residence (Beamish and Mahnken 2001; Moss et al. 2005; Duffy and Beauchamp 2011). Size-selective mortality implies growth-selective mortality. This corollary has spurred extensive research into factors influencing early marine growth rates of Pacific salmon (Fisher and Pearcy 1988; Tovey 1999; Litz et al. 2016; Wells et al. 2016). Prior growth trajectories of salmonids may be investigated by analyzing the microstructure of hard parts, including scales (Rich 1920; Ruggerone et al. 2005; Farley et al. 2007; Marco-Rius et al. 2012) and otoliths (Neilson et al. 1985; Freshwater et al. 2015; Claiborne and Campbell 2016). However, retrospective analysis of growth presents challenges, as sampled fish are unavoidably the survivors of any previous selective mortality. To study factors influencing growth, it can be advantageous to compare relative instantaneous or recent growth rates of field-collected juvenile salmon across years, seasons, and habitats (Sommer et al. 2001; Fisher and Pearcy 2005; Ferriss et al. 2014; Journey 2015). A variety of methods is available to assess recent growth of fish, with different methods representing trade-offs in terms of accuracy and logistical feasibility.

Assessing the relative instantaneous or recent growth of juvenile fish captured in the field is not a trivial endeavor. Growth may be assessed by repeated sampling of a fish population through time (e.g., Fisher and Pearcy 1988, 2005). However, the resulting “apparent growth rate” estimates may be biased by size-selective mortality, immigration, or emigration. A more robust approach is to repeatedly sample groups of marked fish of known release sizes (e.g., Healey 1980a; Fisher and Pearcy 1988, 2005; Sommer et al. 2001). This method is also subject to the aforementioned biases, with the addition of potential size-selective recapture rates. Logistical challenges of releasing and recapturing adequate numbers of marked fish may also be limiting.

Recently formed regions (outer margins) of hard parts can provide an index of recent growth rate. Fish otolith

growth is proportional to somatic growth, and increments (rings) are generally deposited daily (Neilson et al. 1985; Zhang and Beamish 2000; Middleton 2011). This allows measurements near the otolith margin that correspond to recent growth periods of known duration. However, the relationship between otolith and somatic growth is not constant (Campana 1990). Changes in increment spacing may lag changes in somatic growth and may be influenced by temperature and/or ration independently of growth (Bradford and Geen 1992; Stormer and Juanes 2016), calling into question increment spacing at the otolith margin as an index of recent growth conditions (Fey 2005). Otolith extraction and polishing are also labor intensive and require lethal sampling of fish. Conversely, scale circulus spacing is simple to measure, and scales can be obtained nonlethally. As fish grow, their scales also grow. In many species with ctenoid or cycloid scales, the circuli (concentric ridges) formed during periods of rapid growth are more widely spaced than those formed during periods of slower growth (Doyle et al. 1987; Fisher and Pearcy 1990). Circulus spacing at the outer scale margin may therefore be correlated with recent growth rate. Width of the scale’s outer few circuli has been used as an indicator of recent growth in salmonids (Ball and Jones 1960; Thomas 1964; Fisher and Pearcy 2005). A limitation of this method is that circulus formation rate has been found to vary with growth rate (Fisher and Pearcy 1990), meaning that a given number of circuli may correspond to shorter recent growth periods in faster-growing fish and vice versa. Circulus formation intervals in salmon are also relatively long (several days to weeks), thus limiting the resolution of the pre-capture period for which they can index growth.

Biochemical indices can also be employed to infer relative rates of recent somatic growth. In fish, numerous potential biochemical growth indices have been evaluated, including plasma insulin (Beckman et al. 2004a), insulin-like growth factor binding proteins (Beckman et al. 2004b; Kawaguchi et al. 2013), and RNA or DNA to protein ratios (Azuma et al. 1998; Caldarone et al. 2016). Perhaps the most well-established and widely used biochemical growth index in fish is the ratio of RNA to DNA (Bulow 1970). Tissue growth requires protein synthesis. Messenger RNA, transfer RNA, and ribosomal RNA (rRNA) are all

required for protein synthesis, with rRNA representing the great majority of total RNA in eukaryotic cells (>80% in yeast; Waldron and Lacroute 1975). The DNA content of tissue is primarily a function of the number of cells (and, hence, nuclei), whereas the total RNA content varies with protein synthesis requirements. If rapidly growing and dividing cells are synthesizing more protein than slowly growing cells, they will have a higher RNA : DNA ratio (RD). This ratio has been most widely used as a growth index for larval fish (Buckley 1979; Clemmesen 1993), where small body size allows assays to be conducted on homogenized whole individuals or groups. In larger juvenile fish, liver and white muscle RD have also been assessed as growth rate indices (Bastrop et al. 1991). Recently, RD from a nonlethally obtained muscle biopsy was correlated with individual growth rates of juvenile Atlantic Salmon *Salmo salar* (MacLean et al. 2008; Caldarone et al. 2016). Significant responses in RD can occur as soon as the second day after a change in food availability in larval and juvenile fish (Wright and Martin 1985; Malloy and Targett 1994), and response times of 4–8 d have been observed in juvenile Atlantic Salmon (Arndt et al. 1996; Caldarone et al. 2016). As with other growth indices, RD has drawbacks. Temperature may affect the relationship between RD and growth (Buckley 1982; Buckley et al. 2008), and the assumption that the DNA content of cells is constant only holds when growth is occurring by cell division (hyperplasia) rather than cell enlargement (hypertrophy; see discussion by Caldarone et al. 2016).

Another well-validated biochemical index of relative growth rate, particularly in salmonid fishes, is the plasma concentration of insulin-like growth factor 1 (IGF1). The causal relationship between IGF1 and growth in vertebrates is well established. Growth hormone produced by the pituitary stimulates the production of IGF1 in the liver and other tissues, which in turn acts directly to induce both cell growth (hypertrophy) in muscle cells (Vandenburgh et al. 1991; Adams and Haddad 1996) and cell proliferation (hyperplasia) in structural tissues in general (Lowe 1991; Lupu et al. 2001; Mommsen 2001; Castillo et al. 2004). The majority of studies on fish have found positive correlations between plasma IGF1 concentration and metrics of recent growth (Beckman 2011). Factors that may influence the relationship between IGF1 and growth rate include sexual maturation (Moriyama et al. 1997), stress (McCormick et al. 1998), and time of year (Beckman et al. 2004a). Plasma IGF1 has been found to be positively correlated with growth rate measured over periods ranging from days to months (reviewed by Beckman 2011). Levels of IGF1 have also been shown to change significantly in response to a change in growth conditions (ration) over periods as short as 4 d (Gabillard et al. 2006; Caldarone et al. 2016). Although it is possible

to draw blood from fish nonlethally for IGF1 assays (Pierce et al. 2001; Caldarone et al. 2016), this may be problematic with very small fish. Additionally, the need to centrifuge blood and freeze plasma within hours of sampling may present logistical challenges where field facilities are limited.

Given the multiple external and intrinsic factors that can influence indices of recent growth, they should be validated where possible in the species and life stage of interest prior to application in the field. The present study evaluates three relative growth rate indices in postsmolt Chinook Salmon *Oncorhynchus tshawytscha*. Scale and otolith microstructure have been used extensively to investigate Chinook Salmon growth trajectories over their entire life cycle and within selected stanzas (e.g., Rich 1920; Reimers 1971; Neilson et al. 1985; Bradford and Geen 1992; Tovey 1999; Sommer et al. 2001; Middleton 2011; Woodson et al. 2013). Recent work has also investigated scale circulus formation in age-0 Chinook Salmon in freshwater (Walker and Sutton 2016). However, we are not aware of any work with this species that has validated or applied circulus spacing at the scale margin as an index of relative growth rate immediately preceding sampling. We are also unaware of any published work that has utilized RD as an index of Chinook Salmon growth in the field, although Hackmann (2005) did attempt to validate RD as an index of growth rate in age-0 Chinook Salmon parr. The relationship between IGF1 concentration, growth rate, and smoltification has been described for juvenile Chinook Salmon in freshwater (Beckman et al. 1998, 1999, 2003), and the relationship between nutritional state, IGF1 regulation, and growth has been characterized in some detail (Pierce et al. 2005). Insulin-like growth factor 1 concentration has also been applied in the field to investigate spatiotemporal variation in growth conditions for Chinook Salmon during early marine residence (Ferriss et al. 2014; Journey 2015; Chamberlin et al. 2017).

There are many technical and logistical factors to consider when selecting the most appropriate indices of recent growth for ecological field studies on juvenile salmon. One obstacle to evaluating the relative merits of different approaches is that morphological and biochemical indices of growth are rarely directly compared on the same cohorts of fish. Here, we examine the relationships of marginal circulus spacing, RD, and plasma IGF1 with individual specific growth rate over a 30-d period for ocean-type Chinook Salmon postsmolts while accounting for the influences of initial size and ration level. We also investigate integration times of the biochemical indices by assessing the time frame over which each index responded to an increase or decrease in ration. Performance of these growth indices is evaluated under feeding conditions that were selected to be ecologically relevant to those experienced in the wild (low, moderate, and high rations) rather

than to elicit a significant physiological response (fasted versus fed). We discuss our results in the context of previous studies on growth indices in juvenile salmonids and summarize the relative merits and drawbacks of different approaches.

METHODS

Animals.—Progeny of ocean-type Chinook Salmon broodstock spawned at the Nitinat River Hatchery (Canadian Department of Fisheries and Oceans [DFO]) were transported as eyed eggs to the Howard English Hatchery (Goldstream Volunteer Salmonid Enhancement Association) near Victoria, British Columbia, where they were reared in surface-water-fed tanks. On May 26, 2015, pre-smolts were transported to the University of Victoria Outdoor Aquatics Unit, where they were housed in an outdoor, 390-L, fiberglass round tank supplied with 12–16°C recirculating freshwater and covered with a lid fitted with a translucent window to admit dim, natural photoperiod light (this description applies to all tanks used in this study). Fish received BioOregon 1.2-mm pelleted salmon feed, which was administered by hand twice per day at an estimated total ration of 3% body weight (BW)/d. On May 27, 10 individuals (mean mass = 4.57 g, SD = 0.68; mean FL = 75 mm, SD = 4) were euthanized with a 300-mg/L solution of tricaine methanesulfonate (TMS), preserved in formalin, and shipped to the Animal Health Center of the British Columbia Ministry of Environment for health surveillance histopathology. Aside from one acanthocephalan gut parasite in a single fish, no pathogens were detected. On June 2, approximately 25% seawater by volume was added to the tank containing the juvenile Chinook Salmon before reconnecting to recirculating freshwater. This process was repeated with approximately 50% seawater on June 3. On June 4, the tank was gradually brought up to 100% seawater and was thereafter supplied with 12–14°C recirculating, bead-filtered, ultraviolet-sterilized seawater. Chinook Salmon continued to be fed twice per day at a ration of 3% BW/d, which was recalculated daily based on an assumed growth rate of 2.5% BW/d.

Tagging and pre-experimental sampling occurred on June 22, 2015. The allocation of fish among tanks, numbers of fish sampled, and mortalities throughout the remainder of the study are illustrated in Figure 1. Anaesthesia occurred in aerated buckets containing 80-mg/L TMS in seawater, which was maintained at 12–14°C by using ice-packs. Fish were individually weighed and measured for nose-to-caudal fork length (FL). Fine forceps were used to remove a smear of scales from the preferred area (just above the lateral line and immediately posterior to the dorsal fin), and these were spread across a single numbered square of a gummed scale card. Fish were individually marked with a 12.5- × 2.1-mm PIT tag (Biomark

HPT12 FDX-B) injected intraperitoneally in a posterior to anterior direction on the midline of the ventral surface just anterior to the pelvic fins. To assess potential growth and survival impacts of muscle biopsy, 145 fish were also subject to biopsies in conjunction with tagging. Fine forceps were used to remove scales from a small area of skin (~4 mm²) on the left side of the dorsal surface 2–5 mm posterior to the dorsal fin. A Miltex 1.5-mm biopsy punch was then inserted at this site at an anterior angle of approximately 45° while rotating between thumb and forefinger to cut through the skin. In cases where the muscle plug was not initially removed by the punch, the tip of a pair of fine forceps was pressed against the site of the biopsy in an anterior to posterior direction to extrude the plug from the hole. Fish were transferred to an aerated bucket of seawater until they regained equilibrium and were then returned to a single tank. Fish were subsequently maintained at a ration of 1% BW/d, which was administered in a single morning feeding; this ration was calculated based on the remaining number of fish and the weight at the time of tagging (mean = 8.20 g, SD = 0.68) and was adjusted daily assuming growth of 1% BW/d. On July 8, 2015, all fish were again anaesthetized, weighed, and measured as described above. Sixty fish were haphazardly selected for inclusion in the growth rate experiment, while 226 fish were returned to a single tank and maintained on a continued ration of 1% BW/d in preparation for the start of the integration time experiment.

Daily percent growth rates in length and weight were calculated using the formula:

$$\text{Growth (\%/d)} = [\log_e(s_2) - \log_e(s_1)] / (t_2 - t_1),$$

where t_1 and t_2 are the experimental day numbers at the beginning and end of the period of interest; and s_1 and s_2 are measurements of fish mass (g) or FL (mm) at these time points.

Growth rate experiment.—On July 8, 2015 (day 0), 60 fish were measured and sampled for scales as described above and were haphazardly distributed among three tanks ($n = 20$ per tank). Tanks were assigned to receive a high (3% BW/d), medium (1% BW/d), or low (0.5% BW/d) ration. To maximize individual variation in growth rate (through within-tank competition for food), the ration was administered in four daily feedings between 0730 and 1530 hours. Total ration was adjusted daily based on an assumption of 3.0, 1.3, and 0.5% growth in weight per day, respectively, in the three treatments (1.3% was used for the 1% BW/d treatment, as this was the observed growth rate between tagging and day 0 of the growth rate experiment). The original study design was to maintain treatments at these rations for 3 weeks prior to terminal sampling. However, on July 13, 2015, a pump failure caused mortality of multiple species in tanks connected to

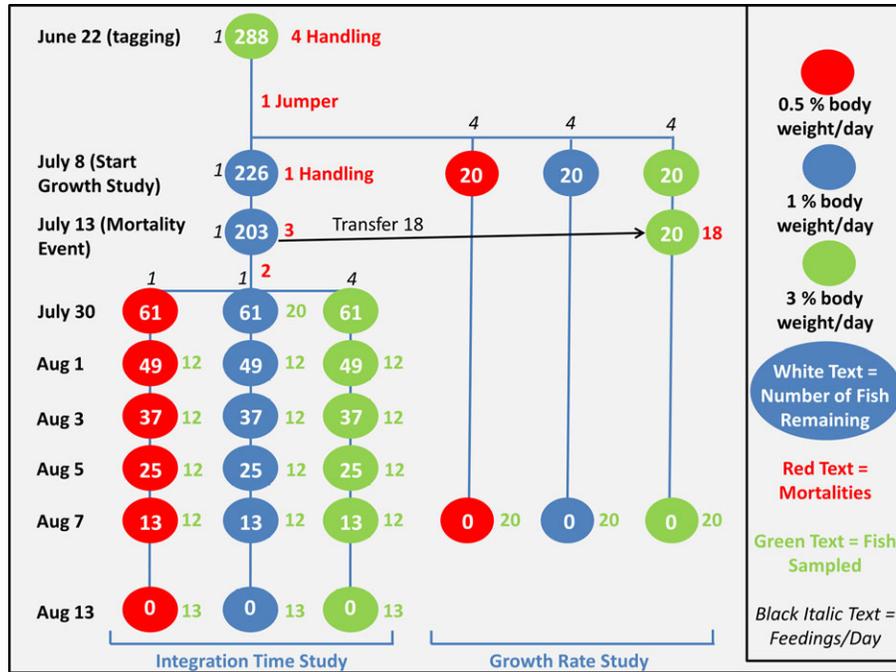


FIGURE 1. Schematic describing the disposition of Chinook Salmon postsmolts among tanks (ellipses) prior to and during experiments that were used to evaluate the relative value of plasma insulin-like growth factor 1, RNA : DNA ratio, and spacing of the outer scale circuli as indices of recent growth.

the recirculating seawater system at the University of Victoria Outdoor Aquatics Unit. This mortality event included 18 juvenile Chinook Salmon in the high-ration tank and three fish in the tank being held for the integration time experiment. Fish that survived this event showed no evidence of adverse effects, so 18 individuals were transferred from the integration time tank to the high-feed tank, and the endpoint of the growth rate experiment was extended to August 7. The experimental period for the low- and medium-ration treatments and for 2 of 20 fish in the high-ration treatment was therefore 30 d, while for 18 of 20 fish in the high-ration treatment, it was 25 d. On August 7, fish were haphazardly selected in groups of three from the three feeding levels for sampling. Each fish was euthanized in 300-mg/L TMS; was scanned for a PIT tag number; and was weighed, measured, biopsied, and sampled for scales as described above. Fish were not fed on the morning of sampling. Biopsies were taken from the right side of the dorsal surface to avoid potential scar tissue associated with the original biopsy. Muscle plugs were transferred to a glass slide, trimmed with a scalpel if necessary to remove any visible skin, transferred to a microcentrifuge tube, flash frozen in liquid nitrogen, and stored at -80°C for subsequent RD assays (see below). A razor blade was used to sever the posterior end of the fish at approximately a 45° anterior angle from the posterior tip of the anal fin to the dorsum. The fish was then held

inverted and up to 0.1 mL of blood was drawn from the caudal vein by using a heparinized Natelson capillary tube. Blood was blown from the capillary tube into a microcentrifuge tube and stored on ice for no more than 4 h. To separate blood plasma, tubes were spun-down at 5,000 revolutions/min for 5 min in a chilled (1°C) centrifuge. Up to 50 μL of plasma were drawn from each tube with a micropipette, taking care not to disturb the pellet. Plasma was transferred to clean tubes and frozen at -80°C for subsequent IGF1 assay (see below).

Integration time experiment.—On July 30, 2015 (day 0), 183 individually PIT-tagged Chinook Salmon postsmolts that had been held together in a single tank were haphazardly divided among three tanks ($n = 61$ per tank). To minimize stress, fish were not anaesthetized and measured at this time. In order to provide baseline values for the integration time experiment, the remaining 20 individuals were euthanized and sampled as described for the growth rate study. The three tanks were designated as low, medium, and high (0.5, 1.0, and 3.0% BW/d, respectively) feeding treatments, with the ration adjusted daily based on an assumption of 0.5, 1.3, and 3.0% growth in weight per day. Ration was administered in four feedings per day to the high-feeding treatment and was administered in one morning feeding to the other two treatments. Twelve fish were removed from each tank for terminal sampling (as described for the growth rate experiment) on days 2, 4, 6,

and 8; the remaining 13 fish in each tank were sampled on day 14. On each sampling day, the fish to be sampled were caught in the morning prior to feeding and were moved to a separate tank to minimize stress on the fish remaining in each treatment. Due to a mistake on the final day of the experiment (day 14), fish were fed in the morning 1–3 h prior to sampling.

RNA : DNA assay.—An RNase-free work environment was maintained by treating surfaces, glassware, and other equipment with RNaseZap (Ambion). Nucleic acid quantification from muscle biopsies followed the assay outlined by Caldarone et al. (2001), with slight modifications. One-hundred microliters of 0.5% STE buffer (0.5% weight/volume N-lauroylsarcosine; 0.05-M Tris; 0.1-M NaCl; 0.01-M EDTA, pH 8.0; and 0.1-mg/mL proteinase K) were added to 1.5-mL microcentrifuge tubes containing muscle biopsies. Samples were homogenized by vortex (Vortex Genie 2; Scientific Industries, Inc., Bohemia, New York) for 30 min at room temperature. Samples were then diluted with 400 μ L of Tris-EDTA buffer (final concentration of N-lauroylsarcosine = 0.1%) and centrifuged for 15 min (14,000 \times g, 20°C) to sediment out any undissolved particles, and a 300- μ L aliquot was removed for fluorometry. Duplicate 75- μ L aliquots of the centrifuged sample were added to an OptiPlate-96 black-walled, opaque, 96-well microtiter plate. Standard solutions of RNA (*Escherichia coli* Total RNA; Ambion Catalog Number AM7940) and DNA (Calf Thymus DNA; Invitrogen Catalog Number 15633019) were prepared at 10 μ g/mL, and twofold serial dilutions (75 μ L per well; minimum concentration = 0.63 μ g/mL) of the standards were added to the plate. Every well then received 75 μ L of Tris-EDTA buffer (containing 2- μ g/mL ethidium bromide), and the plate was incubated for 5 min. Fluorescence was recorded on a Perkin Elmer Victor³ V 1420 Microplate Reader (excitation = 531 nm; emission = 600 nm), with the signal representing the combined fluorescence of RNA and DNA in each sample. Ribonuclease A (7.5 μ L; Thermo Scientific Catalog Number EN0531) was added to each well and incubated at room temperature for 30 min. Fluorometry was recorded again, and the remaining signal was empirically determined to be from DNA only. The RD was calculated for each well, and samples where the coefficient of variation (CV) for either the RNA or DNA concentration in duplicate wells exceeded 15% were excluded from further analysis.

Insulin-like growth factor 1 assay.—Frozen plasma was shipped on dry ice to the National Marine Fisheries Service, Northwest Fisheries Science Center, where the concentration of plasma IGF1 for individual fish was measured using the time-resolved fluorescence immunoassay developed by Small and Peterson (2005), as modified by Ferriss et al. (2014). Uniformity and speed in processing samples were enhanced by using an automated

pipetting workstation (Perkin Elmer). Across individual assays, all samples were standardized using interassay pools of juvenile Coho Salmon *Oncorhynchus kisutch* plasma at three known IGF1 concentrations (low, medium, and high) corresponding to approximately 75, 50, and 25% binding in the immunoassay. Reported individual IGF1 concentrations are the standardized means of duplicate measurements. In the event that duplication between wells was poor, as classified by a CV greater than 7%, the sample was dropped from the final data set. Additionally, samples falling outside of the linear range of the immunoassay standard curve (>80% or <20% binding) were dropped from the final data set. Data standardization and complete laboratory techniques are detailed by Ferriss et al. (2014).

Scale circulus deposition and spacing analysis.—Impressions of Chinook Salmon scales were made at the DFO Pacific Biological Station by pressing scale cards onto acetate sheets using a DK20SP Automatic Digital Swinger swing-away heat-transfer press (40.64 \times 50.80 cm [16 \times 20 in]) following the methods described by Hudson and Crosby (2010). Scale impressions were photographed at 8 \times magnification using bright field illumination with an Olympus DP26 digital camera mounted on an Olympus S2X16 stereomicroscope. Scale impressions were only analyzed when a clearly defined origin indicated that the scale was not regenerated. CellSense software was used to draw a line from the center of the origin to the scale margin in an anterior direction along the longest axis of each scale. The point tool was then used to place points sequentially beside this line at the center of the origin, the margin of the origin, and the outer margin of each circulus out to the edge of the scale (Figure 2). The line length measurements and Cartesian coordinates of points for each scale were exported as comma-separated value (csv) files, and custom code in the R statistical language was used to count the number of circuli and calculate the linear distance between sequential points. When the sum of these linear distances (the radius of the origin and spacing of each circulus) differed by more than 2% from the scale radius measured using CellSense software, the measurement process for the scale was repeated.

Up to three good scales (when available) were imaged and analyzed for fish sampled at the end of the growth study, while one scale was imaged and analyzed for fish sampled at the time of tagging and at the beginning of the growth rate study. In addition to the single scale that was analyzed for each fish at the time of tagging, visual circulus counts were made for up to two additional scales (when available) for those fish that were used in the growth rate experiment. Where more than one scale from a single fish was measured or counted for circuli, average values for each fish were used in analyses. For the growth rate experiment, circulus formation intervals for each fish were calculated as

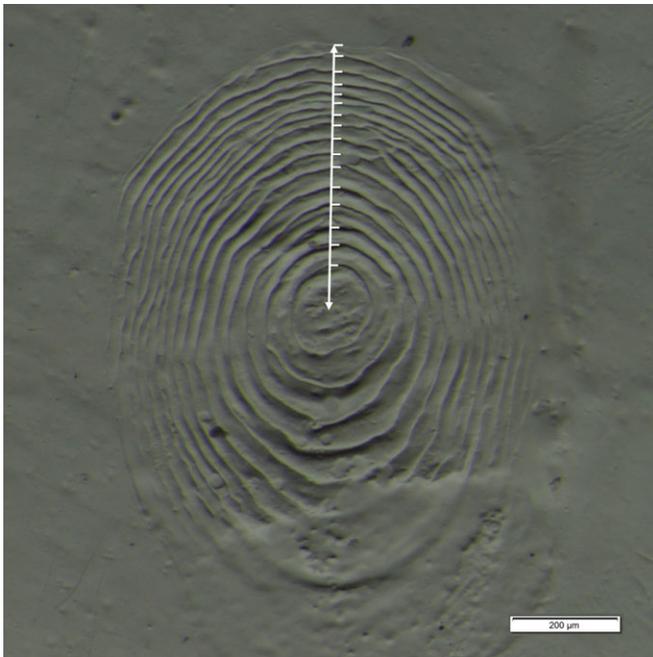


FIGURE 2. Acetate impression of a scale from a postsmolt Chinook Salmon (120 mm FL; 20.68 g) sampled at the end of the growth rate experiment from the high-growth-rate treatment (3% body weight/d). The measurement radius (double-ended arrow) and locations of points used to calculate circulus spacing (hatch marks) are indicated.

the elapsed days (46 d) divided by the difference between the mean circulus count at the time of terminal sampling and the time of tagging. Two fish for which the increase in circulus number over this period was calculated as being less than 1 were not included in the analysis.

Analysis.—To assess relationships between individual growth rates and growth rate indices (growth rate experiment), we employed a small-sample corrected Akaike's information criterion (AIC_c) model selection approach to evaluate alternative multiple regression models. Identical candidate model sets (Table 1) were developed for each response variable (RD, plasma IGF1, spacing of the outermost scale circulus or outer two circuli, and circulus formation interval). The hypothesis of no relationship between response variables and individual growth was described by a "Null" model. A "Growth" model represented a monotonic relationship between each response variable and individual growth rate in percent length per day (henceforward, "growth rate"). To account for nonindependence of fish within tanks or ration levels, models including ration as a fixed factor either with ("Growth \times Ration" model) or without ("Growth + Ration" model) an interaction with growth rate were also considered. Finally, to assess whether response variables were related to size at the beginning of the study, three additional models were specified that included a term for FL on day

0 ("Growth + Day-0 FL," "Growth + Ration + Day-0 FL," and "Growth \times Ration + Day-0 FL"). Candidate models were fitted using the "lm" function of the base stats package in R. Heteroscedasticity and normality of residuals were examined graphically, and response variables were \log_e transformed when necessary. Best models for each response variable were selected based on minimum AIC_c values using the "aictab" function in the "AICcmodavg" library. Goodness of fit of relationships between response variables and growth rate ("Growth Rate" model) was compared using the coefficient of determination (R^2).

To visualize the response of biochemical growth rate indices to a change in growth conditions (integration time experiment), we plotted 95% confidence interval-bounded, locally weighted scatterplot smooths (loess; span = 0.8) of individual RD and plasma IGF1 against experimental day for each ration level (day-0 values were reused for each ration level). We also generated loess smooths in the same manner for final length and growth rate (% length/d) since day -22 (the most recent time point at which fish were measured prior to the experiment).

RESULTS

Survival and Growth Effects of Muscle Biopsy and PIT Tagging

Only six mortalities occurred over the 16 d between PIT tagging and remeasuring fish prior to the growth rate experiment. Of these, one resulted from a handling accident, and another involved a fish jumping from the tank. The other mortalities occurred when fish failed to recover from anaesthesia at the time of tagging ($n = 3$) or remeasuring ($n = 1$). Two of these latter four mortalities were of fish that had been subjected to a biopsy in conjunction with tagging. Of the 21 fish that died at the time of the pump failure, 10 had been subjected to a muscle biopsy at the time of tagging. Two fish lost their PIT tags between tagging on June 22 and remeasuring on July 8; they were included in the 20 fish that were sampled at the beginning of the integration time experiment. Percent daily growth in length of Chinook Salmon postsmolts between tagging and remeasuring was almost identical for biopsied fish (mean = 0.43%, SD = 0.10; $n = 142$) and nonbiopsied fish (mean = 0.43%, SD = 0.11; $n = 143$).

Analytical Results

All fish exhibited positive growth in length and weight between tagging and final sampling. During the growth rate experiment, individual daily growth rates were 0.46% (SD = 0.10), 1.27% (SD = 0.13), and 2.05% (SD = 0.14) in weight and 0.18% (SD = 0.05), 0.39% (SD = 0.07), and 0.61% (SD = 0.07) in length over 30 d for Chinook

TABLE 1. Log likelihood, regression statistics, and small-sample corrected Akaike's information criterion (AIC_c) for candidate multiple regression models relating muscle RNA : DNA ratio (RD), plasma insulin-like growth factor 1 (IGF1) concentration, circulus formation interval, and mean spacing of the outermost circulus or outer two circuli to daily percent growth in length for Chinook Salmon postsmolts ($n = 60$) reared for 25–30 d at rations of 0.5, 1.0, and 3.0% body weight (BW)/d. Models including FL on day 0 were included to account for possible size-based differences in growth indices that were independent of growth rate. Models including ration level and the growth rate \times ration level interaction were included to account for possible nonindependence of individuals with each tank or ration level. Growth rates were measured over 30 d; circulus formation interval was measured over 46 d (25–30 d of differential rations following 16–21 d of 1.0% BW/d). Best models (based on AIC_c) are indicated in bold italics.

Response and model	Log likelihood	N	K	AIC_c	ΔAIC_c	R^2	P
Log_e(RD)							
Null	-19.9	53	2	44.0	31.6	NA	NA
<i>Growth</i>	-2.9	53	3	12.4	0.0	0.47	<0.0001
Growth + Ration	-1.7	53	5	14.7	2.3	0.50	<0.0001
Growth \times Ration	-1.5	53	7	19.5	7.1	0.50	<0.0001
Growth + Day-0 FL	-2.8	53	4	14.5	2.1	0.47	<0.0001
Growth + Ration + Day-0 FL	-1.6	53	6	16.9	4.6	0.50	<0.0001
Growth \times Ration + Day-0 FL	-1.4	53	8	22.0	9.7	0.50	<0.0001
Plasma IGF1 (ng/mL)							
Null	-186.8	47	2	377.9	79.6	NA	NA
Growth	-146.4	47	3	299.3	0.9	0.79	<0.0001
<i>Growth + Ration</i>	-143.5	47	5	298.4	0.0	0.81	<0.0001
Growth \times Ration	-141.8	47	7	300.4	2.1	0.83	<0.0001
Growth + Day-0 FL	-146.0	47	4	301.0	2.6	0.79	<0.0001
Growth + Ration + Day-0 FL	-143.2	47	6	300.4	2.0	0.82	<0.0001
Growth \times Ration + Day-0 FL	-141.2	47	8	302.1	3.7	0.83	<0.0001
Log_e(circulus formation interval, d)							
Null	-28.9	53	2	61.9	30.0	NA	NA
<i>Growth</i>	-12.7	53	3	31.9	0.0	0.45	<0.0001
Growth + Ration	-11.9	53	5	35.1	3.2	0.47	<0.0001
Growth \times Ration	-11.6	53	7	39.7	7.9	0.48	<0.0001
Growth + Day-0 FL	-12.0	53	4	32.9	1.0	0.47	<0.0001
Growth + Ration + Day-0 FL	-11.3	53	6	36.5	4.6	0.48	<0.0001
Growth \times Ration + Day-0 FL	-10.8	53	8	40.9	9.0	0.49	<0.0001
Outer circulus width (μm)							
Null	-187.1	59	2	378.4	25.9	NA	NA
Growth	-173.8	59	3	354.0	1.5	0.29	<0.0001
Growth + Ration	-172.8	59	5	356.7	4.3	0.32	<0.0001
Growth \times Ration	-171.2	59	7	358.6	6.1	0.35	0.0003
<i>Growth + Day-0 FL</i>	-171.9	59	4	352.5	0.0	0.34	<0.0001
Growth + Ration + Day-0 FL	-170.4	59	6	354.5	2.0	0.37	<0.0001
Growth \times Ration + Day-0 FL	-169.3	59	8	357.6	5.1	0.39	0.0002
Mean width of outer two circuli (μm)							
Null	-181.3	59	2	366.8	48.1	NA	NA
Growth	-161.3	59	3	329.1	10.4	0.44	<0.0001
Growth + Ration	-160.2	59	5	331.6	12.9	0.46	<0.0001
Growth \times Ration	-157.3	59	7	330.8	12.1	0.52	<0.0001
<i>Growth + Day-0 FL</i>	-155.0	59	4	318.7	0.0	0.55	<0.0001
Growth + Ration + Day-0 FL	-153.0	59	6	319.7	1.0	0.58	<0.0001
Growth \times Ration + Day-0 FL	-150.9	59	8	320.7	2.0	0.61	<0.0001

Salmon postsmolts reared for 25–30 d on estimated rations of 0.5, 1.0, and 3.0% BW/d, respectively. As ration calculations assumed growth rates of 0.5, 1.3, and 3.0%

per day, the amount of food provided to the high-ration group was slightly greater than 3% per day by the end of the study period. Estimated ration levels for the other two

tanks were relatively accurate. Growth rate in length was highly correlated with growth rate in weight ($R^2 = 0.97$; Figure 3a); growth rate in length was used in all subsequent analyses. For the integration time experiment, overall mean growth rates from 22 d prior to the start of the experiment to final sampling (a period of 22–36 d) was 0.43% per day (SD = 0.07) in length and 1.27% per day (SD = 0.27) in weight.

In total, 263 muscle biopsies were assayed for RD on eight 96-well microplates. The DNA and RNA standard slope ratio ($m_{\text{DNA}}/m_{\text{RNA}}$) was 3.15 (SD = 0.11). Twenty-five samples (9.5%) were rejected due to high variation between duplicates. Values of RD averaged 3.47 (SD = 1.11; range = 1.39–6.55) across all treatments in both experiments. Blood plasma was successfully obtained for 260 fish and assayed for IGF1 concentration; 228 (88%) of these assays met quality control standards for inclusion in analyses. Plasma IGF1 concentrations averaged 29.62 ng/mL (SD = 9.65; range = 10.53–49.75 ng/mL) across all treatments in both experiments. For fish that were included in the growth rate experiment ($n = 60$), scales that were suitable for circulus counts were obtained for 56 individuals, and the average number of countable scales (target = 3) was 2.6. Scales were sampled again at the start of the growth rate experiment; however, due to the pump failure that occurred on day 5 and the need to transfer fish into the experiment, circulus formation interval was calculated over 46 d from the time of tagging to the end of the growth rate study. Nevertheless, circulus counts were obtained for 38 fish on both the day of tagging and at the start of the growth rate experiment. Mean circulus count over this 16-d period increased from 11.8 (SD = 1.0) to 12.8 (SD = 1.3). Scales that were suitable for analysis were obtained from all fish at the end of the growth rate experiment, and the average number of countable scales (target = 3) was 2.85. Considerable scale loss was observed for some fish at the time of sampling.

Growth Rate Experiment

Residuals of linear models relating RD and circulus formation interval to growth rate were somewhat nonnormal and heteroscedastic; therefore, both variables were log-transformed for the final candidate model set. Models including a growth rate term were strongly favored over null models for all response variables ($\Delta\text{AIC}_c > 25$; Table 1). Model selection suggested that the relationship with growth was independent of ration level for all response variables except IGF1 concentration. The best model for IGF1 (Growth + Ration; Table 1) included independent intercepts for each ration level. Fish size (FL) at the start of the experiment was only retained as a term in the best models relating spacing of the outer circulus or outer two circuli to growth rate (Table 1). Initial FL was marginally significant for the spacing of the outermost

circulus ($P = 0.0575$; Figure 3f) but was highly significant for the mean spacing of the outer two circuli ($P = 0.0005$).

Of the growth rate indices, IGF1 concentration correlated most strongly with growth rate ($R^2 = 0.79$), followed by $\log_e(\text{RD})$ ($R^2 = 0.47$), mean spacing of the outer two circuli ($R^2 = 0.44$), and spacing of the outermost circulus ($R^2 = 0.29$; Table 2). There was also a relatively strong correlation between growth rate and the \log_e of the circulus formation interval ($R^2 = 0.45$), indicating that the effect of fish growth rate on scale growth was not limited to circulus spacing. Circulus spacing declined over the initial 10 circuli and remained low after the beginning of the experiment (mean circulus number = 12.8) in the low- and medium-ration treatments; in the high-ration treatment, circulus spacing increased by the end of the experiment (Figure 4).

Integration Time Experiment

The sizes (FL) of low-, medium-, and high-ration groups of Chinook Salmon postsmolts were not clearly different by the end (day 14) of the integration time study (Figure 5a). However, growth rates measured over 22–36 d (depending on sampling day) had separated by day 8 of the experiment (Figure 5b). Mean IGF1 of fish sampled on day 0 of the integration time experiment (26.14 ng/mL, SD = 7.05) was lower than that of fish fed at 1% BW/d in the growth rate experiment (34.95 ng/mL, SD = 8.94). In the high-ration treatment, IGF1 increased rapidly, and by day 4, it was higher than values in both the medium- and low-ration treatments, remaining relatively constant through day 14. Concentrations of IGF1 for the low- and medium-ration fish remained low initially, but on day 14, the concentrations had increased to levels similar to that of the high-ration treatment. Unlike IGF1 concentration, mean RD of fish sampled on day 0 (3.82, SD = 1.13; $N = 17$) was higher than that of fish fed at 1% BW/d in the growth rate experiment (2.55, SD = 0.66; $N = 19$). The RD values of fish sampled from low- and high-ration treatments had separated by day 6 and remained so until the end of the experiment. The RD graded with ration level from day 6 to day 14; however, 95% confidence intervals of the loess smooth for the medium-ration treatment overlapped with those of high- and low-ration treatments throughout this period.

DISCUSSION

Factors to consider when selecting indices of relative growth rate for fish ecology studies include the strength of the relationship with growth, integration time, logistical feasibility, and potential for nonlethal sampling. Couture et al. (1998) and Beckman et al. (2004a) further suggested that an ideal growth rate index should be required for growth (causal) rather than a result of growth

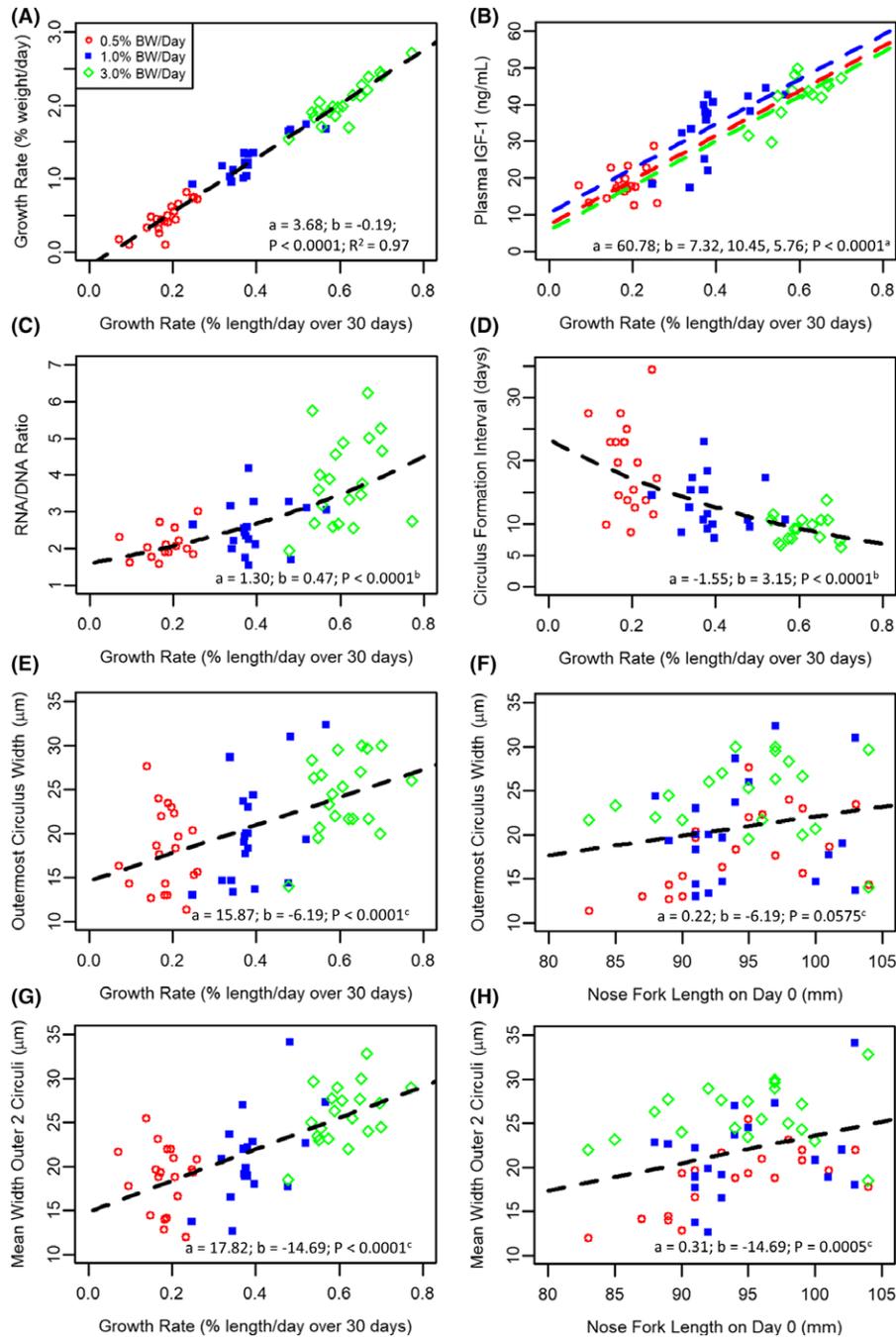


FIGURE 3. Plots of the best regression models (see Table 1) of (A) instantaneous growth rate (weight; %/d) over 30 d, (B) plasma insulin-like growth factor 1 (IGF1), (C) RNA : DNA ratio, and (D) rate of circulus formation over 46 d (since tagging) against instantaneous growth rate (length; %/d) over 30 d for postsmolt Chinook Salmon maintained on rations of 0.5, 1.0, and 3.0% body weight (BW)/d for 25–30 d. Rate of circulus formation and RNA : DNA ratio were \log_e transformed to improve normality and reduce heteroscedasticity of residuals; however, model fits to untransformed data are plotted here to facilitate comparison to other indices. Best models for the (E), (F) mean spacing of the outermost circulus and (G), (H) mean spacing of the outer two circuli against instantaneous growth rate (length) included a term for FL at the time of tagging; therefore, these relationships are plotted separately with initial FL (E, G) and individual growth rate (F, H) held at their respective means (94.5 mm; 0.39% length/d). Regression parameters are slope (a) and intercept (b). Superscripts are defined as follows. ^aBecause the best model for IGF1 included intercepts for each ration level, b -values listed are for rations of 0.5, 1.0, and 3.0% BW/d, respectively. ^bRegression parameters listed are for the linearized form of the illustrated relationships with the response variable \log_e transformed. ^cRegression parameters provided are for the complete model, including both variables; therefore, the b -values do not correspond to those for the illustrated partial regression lines.

TABLE 2. Theoretical, technical, and logistical comparison of indices of relative recent growth rate in juvenile salmon; italic text indicates interpretation of results from the present study of Chinook Salmon postsmolts (IGF1 = insulin-like growth factor 1; RD = RNA : DNA ratio).

Characteristic	Plasma IGF1	RD	Mean spacing of the outer scale circuli
Theoretical relationship to tissue growth rate	Proximal cause	Corollary of mechanistic requirement	Conditional consequence
Correlation with recent growth rate	<i>Strong</i>	<i>Moderate</i>	<i>Moderate</i>
Linear relationship with growth rate	<i>Yes</i>	<i>No</i>	<i>Yes</i>
Independent of initial size	<i>Yes</i>	<i>Yes</i>	<i>No</i>
Able to detect very slow or zero growth	<i>Yes</i>	<i>Yes</i>	<i>No</i>
Response time for a change of growth conditions	4 d ^a	6 d ^a	>14 d
Provides information on prior growth history	No	No	Yes
Nonlethal sampling possible	Not on small fish	<i>Yes</i>	Yes
Usable on dead fish	No ^b	No ^b	Yes ^c
Feasibility for remote field work	Moderate	Moderate	High

^aOur results were consistent with some response by these indices over the time frames indicated; however, biologically meaningful results will depend on the scale of differences in growth rate.

^bIn fish that have been dead for an extended period (>30 min), blood will clot and become impossible to draw, and nucleic acids will be subject to degradation. Sampling of freshly killed fish should be possible.

^cAlthough sampling of scales from dead fish is possible, a caveat is that fish killed by trawl gear or other mechanical injury may have lost their scales.

(correlational). The growth rate indices evaluated in the present study fall on a causal to correlational spectrum with respect to their theoretical relationship to somatic growth. Insulin-like growth factor 1 is a proximal cause of growth; elevated RNA content (and, hence, RD) is required for growth; and scale circulus spacing is correlated with the growth rate at the time of circulus formation.

Insulin-Like Growth Factor 1 Relation to Growth

Given a direct causal relationship to growth, it is perhaps unsurprising that of the indices evaluated, plasma IGF1 concentration showed the strongest correlation with recent growth rate in Chinook Salmon postsmolts. The R² for this relationship (0.79; Table 1) compared favorably with literature values, falling above the values reported in 21 of 25 studies of IGF1 and fish growth reviewed by Beckman (2011). Although our model selection approach suggested that this relationship was not independent of ration level (Table 1), the slope coefficients were very similar whether or not ration was included in the model (60.8 and 58.1, respectively). The intercepts for treatment levels also did not grade with ration level (Figure 3), indicating no consistent relationship between ration and IGF1 independent of growth rate. The fact that FL on day 0 was not included in the best model suggested that IGF1 was independent of initial size in our experiment. This contrasts with the results of Shimizu et al. (2009), who found that for fasted juvenile Coho Salmon, basal IGF1 levels were dependent on fish size. On the other hand, Beckman et al. (1998, 2003) sorted juvenile Chinook Salmon at the

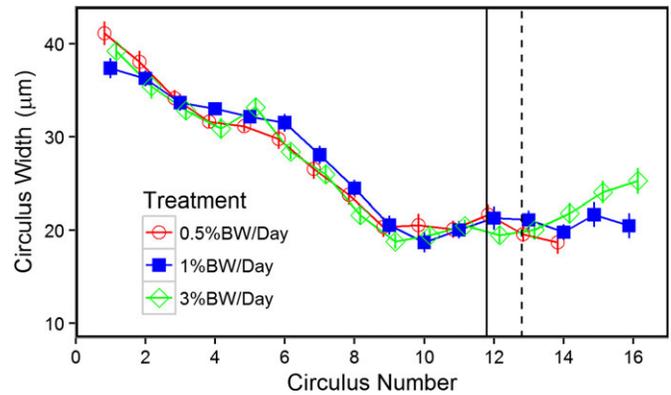


FIGURE 4. Circulus spacing by circulus number for postsmolt Chinook Salmon reared for 25–30 d on rations of 0.5, 1.0, and 3.0% body weight (BW)/d. Points are not shown where circulus measurements were available for fewer than 8 fish in any treatment; error bars represent SE. The vertical solid line indicates the mean number of circuli (11.8) for these fish at the time of PIT tagging (day -15), and the dashed line indicates the mean number of circuli (12.8) at the start of the experiment (day 0).

same developmental stage into small and large individuals and then induced different growth rates by using differences in temperature and ration, respectively. In both cases, faster-growing small fish achieved higher IGF1 values than slower-growing large fish, indicating that IGF1 concentration was more strongly related to growth than to size. Concern that a relationship between IGF1 and size could confound the ability of IGF1 to resolve differences in growth has led some authors to standardize IGF1

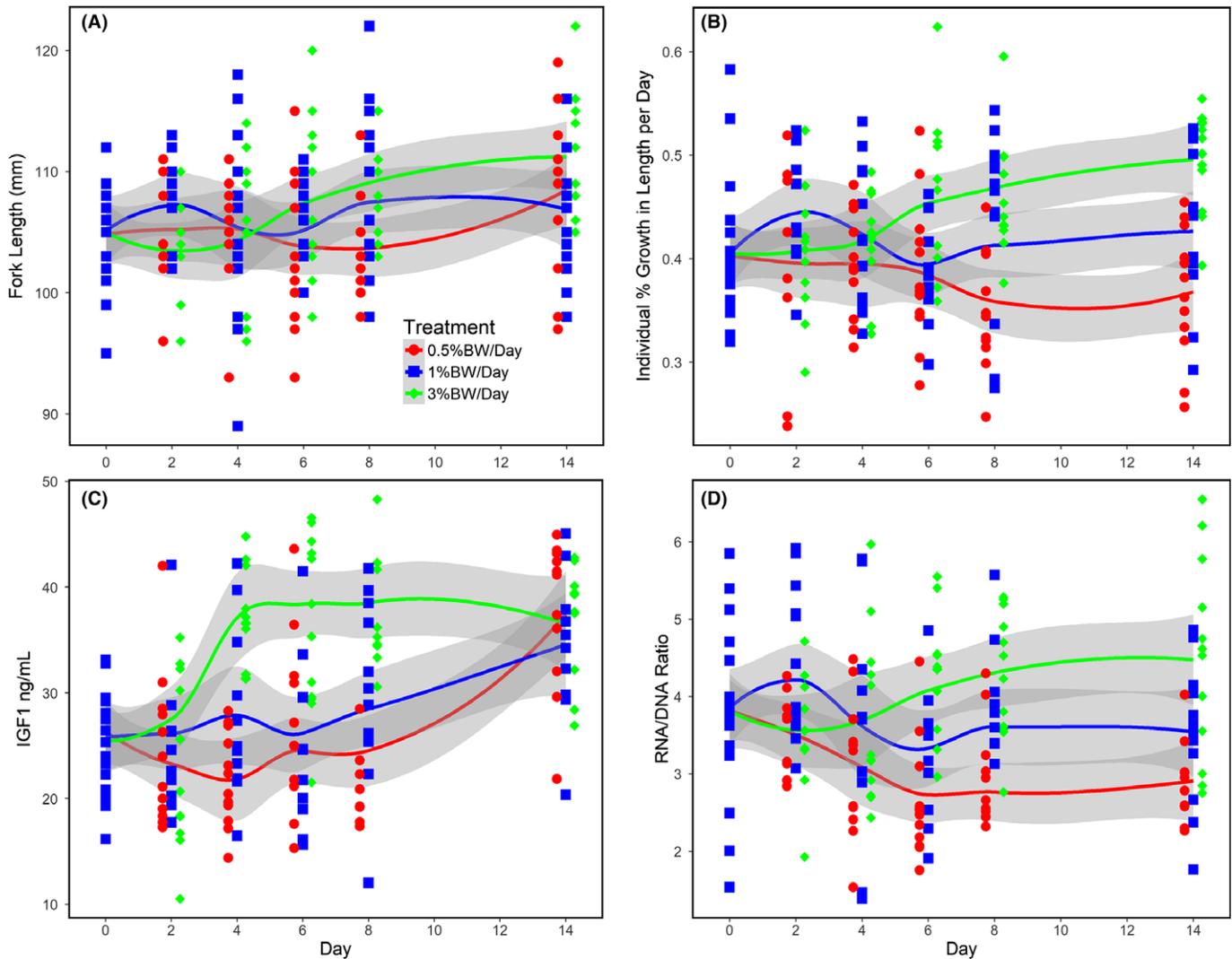


FIGURE 5. Locally weighted scatterplot smooths (loess; span = 0.8) of (A) FL, (B) growth rate (length; %/d) since day -22 (i.e., integrates a period of 22–36 d), (C) plasma insulin-like growth factor 1 (IGF1) concentration, and (D) RNA : DNA ratio for postsmolt Chinook Salmon maintained on a ration of 1.0% body weight (BW)/d up to the beginning of the experiment (day 0) and subsequently split into treatments of 0.5, 1.0, and 3.0% BW/d and subsampled ($n = 12$ –13 per treatment) on days 2, 4, 6, 8, and 14. Shaded regions indicate 95% confidence intervals of loess smooths. Smooths were fitted by replicating day-0 values for each ration level. Note that fish were last fed no later than 24 h prior to sampling in the low- and medium-ration treatments and 17 h prior to sampling in the high-ration treatment on all days except day 14, when all fish were fed 1–3 h prior to sampling.

values to fish length for field studies (Ferriss et al. 2014). One drawback of this approach is that it may reduce the significance of results if differences in size are in part a consequence of differences in recent growth rates. While challenging, more research to tease apart the relations among growth rate, size, and IGF1 concentration is warranted.

The absolute values of plasma IGF1 concentration we observed (10–50 ng/mL; Figure 3) cannot be directly compared with most literature values for juvenile Chinook Salmon (e.g., Beckman et al. 1998, 1999; Pierce et al. 2005), as IGF1 standards and methodologies have varied.

However, samples from the present study were run in the same laboratory using the same standards, interassay pools, and protocols as two recent field studies (Ferriss et al. 2014; Journey 2015). For age-0.0 and age-1.0 Chinook Salmon sampled in early summer across eight regions of the British Columbia coast over 3 years, Ferriss et al. (2014) observed year \times region mean IGF1 concentrations ranging from 38 to 64 ng/mL. Similarly, Journey (2015) reported year \times region \times life history type mean concentrations ranging from 43 to 92 ng/mL for age-0.0 and age-1.0 Chinook Salmon sampled over 3 years in three coastal regions of British Columbia. The higher

IGF1 concentrations observed for these postsmolt Chinook Salmon in the marine environment than in our laboratory study could reflect more rapid growth rates in the wild. Based on a change in size frequency distributions of Chinook Salmon caught from June to October in the Gulf Islands, British Columbia, in 1976, Healey (1980b) reported a consistent increase in size of 0.75–0.85 mm/d for three distinct modes corresponding to June FLs of 80, 140–160, and 320–360 mm (likely corresponding to age-0.0, age-1.0, and age-0.1 fish, respectively). A very similar estimate of growth rates for age-0.0 Chinook Salmon (0.7–1.0 mm/d) was derived by Trudel et al. (2007) based on sizes of fish caught in summer trawl surveys from California to the Bering Sea. Applying a growth rate of 0.8 mm/d to the range of FLs observed at the end of our growth rate study (89–106 mm) yields growth rates of 0.6–0.9% of length per day, which are at or above the growth rates observed for the high-ration treatment.

Insulin-Like Growth Factor 1 Integration Time

Plasma IGF1 concentrations responded to an increase in ration (improved growth conditions) within 4 d. However, IGF1 concentration did not decrease for fish that were on a reduced ration (Figure 5). This may have been because the mean IGF1 concentration for fish on day 0 was already quite low (lower than for fish fed at the same ration level [1% BW/d] in the growth rate study). This lower IGF1 level could have been a consequence of providing food in a single daily feeding, while fish in the growth rate study were fed four times per day. Shimizu et al. (2009) detected higher plasma IGF1 in juvenile Coho Salmon 2–10 h after receiving a meal relative to unfed controls (although this pattern was not significant in fish that had previously been fasted for 1 week or longer). Peddu et al. (2009) found that IGF1 in juvenile Mozambique Tilapia *Oreochromis mossambicus* was elevated 1 h after a scheduled feeding time whether or not feeding occurred, suggesting that IGF1 regulation could become conditioned to diel food availability. Fish in the medium-ration treatment of the growth rate study had been fed over a longer diel period and more recently prior to sampling than those at the beginning of the integration time study, possibly resulting in elevated levels of IGF1. Short-term effects of feeding may also explain the increase in plasma IGF1 concentration observed on day 14 for both the low- and intermediate-ration treatments (Figure 5). Unlike on other sampling days, these fish were fed (in error) 1–3 h prior to sampling. Shimizu et al. (2009) measured IGF1 values that were approximately 50% higher in fed fish than in controls 2 h after a meal. A similar response could explain the elevated IGF1 for low- and medium-ration fish on day 14, although it is not clear why a similar elevation was not observed for high-ration fish. If elapsed time since a previous meal or conditioned diel feeding chronology has a

large effect on plasma IGF1, this could have implications for field study design in cases where fish exhibit diel periodicity in feeding behavior (e.g., Schabetsberger et al. 2003).

Notwithstanding the results on day 14, the observed 4-d response time to a change in ration for IGF1 concentration is consistent with the results of other studies. Pierce et al. (2005) reported that fasted Chinook Salmon parr had significantly lower IGF1 than control or increased ration individuals after 4 d, and Gabillard et al. (2006) and Caldarone et al. (2016) reported increases in IGF1 4 d after refeeding of fasted juvenile Rainbow Trout *Oncorhynchus mykiss* and Atlantic Salmon, respectively. Slower responses have also been reported. Shimizu et al. (2009) measured IGF1 concentrations in juvenile Coho Salmon 1, 7, and 21 d after withdrawing food; although a slight decline occurred by day 7, a much larger and statistically significant decline was evident by day 21. A more extended period for IGF1 values to stabilize (2 weeks) was also observed by Pierce et al. (2005) and Gabillard et al. (2006). This contrasts with our results, where IGF1 values in the high-ration treatment remained constant after day 4. Overall, the period over which IGF1 level is able to resolve differences in growth conditions probably ranges from about 4 d to 2 weeks.

Insulin-Like Growth Factor 1 Logistical Considerations

Sampling blood plasma to assay IGF1 concentration is a somewhat delicate operation that could be challenging in certain field conditions. Blood may be drawn from the caudal vein by using a syringe in larger fish, but this is difficult in small individuals (<120 mm; Ferriss et al. 2014). In the present study, we removed the caudal fin and drew blood by using heparinized Natelson capillary tubes. Obtaining an adequate blood sample (~50 µL) using this method requires a steady hand on smaller fish. Plasma must be separated by centrifuging blood within 4 h of collection, requiring a centrifuge that is suitable for use in the field. Plasma must then be frozen as soon as possible at –80°C. Nevertheless, plasma samples for IGF1 have been obtained successfully under remote field conditions (e.g., Bond et al. 2014). Blood may be collected from recently killed fish—for example, those brought on deck during trawling (Ferriss et al. 2014; Journey 2015)—but it is necessary to process fish quickly before clotting begins. It is possible to draw blood nonlethally using a syringe in larger juvenile salmonids (Pierce et al. 2001; Caldarone et al. 2016); however, this would not be possible in smaller fish. Furthermore, in any field study design where nonlethal sampling is important (e.g., tagging studies), drawing a significant blood volume could impair feeding and predator avoidance behavior and could result in delayed mortality or abnormal behavior. Of the three evaluated growth indices, IGF1 analysis was the most complex (and therefore

expensive), requiring a well-equipped laboratory as well as specialized reagents (antibodies and IGF1 standards) and training.

RNA : DNA Ratio Relation to Growth

The significant positive relationship we observed between RD and growth rate was consistent with a large body of previous work on larval and juvenile fish (Buckley 1979, 1982; Rooker and Holt 1996; Caldarone 2005; and many others). The use of RD as an index of relative growth rate in fish is well established, whereas results for juvenile salmonids have been more equivocal. Moderate to strong positive correlations between muscle RD and growth have been reported for Arctic Char *Salvelinus alpinus* (Miglav and Jobling 1989), Rainbow Trout (Suresh and Sheehan 1998a), Brook Trout *Salvelinus fontinalis*, and Atlantic Salmon (Wilder and Stanley 1983). MacLean et al. (2008) found a positive and highly significant relationship between RD and individual growth rate for Atlantic Salmon smolts, a result that was confirmed by Caldarone et al. (2016). On the other hand, Ferguson and Danzmann (1990) investigated relationships between RD and growth (measured over periods of 7–21 d) for individual yearling Rainbow Trout held at temperatures of 5–11°C and found significant relationships in less than 50% of cases (8 of 18 regressions). Even where relationships were significant, the R^2 values reported by these authors were relatively low (0.21–0.39). Kawaguchi et al. (2013) did not find a significant relationship between growth rate and RD in yearling Cherry Salmon *Oncorhynchus masou* and observed no decrease in RD even after 6 weeks of fasting. To our knowledge, the only previous work investigating RD in juvenile Chinook Salmon was conducted by Hackmann (2005), who found only a very weak correlation between RD and individual growth rate ($R^2 = 0.06$).

Size at the beginning of the experiment was not included in our top model relating RD to growth rate, suggesting that RD was independent of size over the range of sizes in our study. As fish grow, the importance of hypertrophy relative to hyperplasia may increase, resulting in a decrease in the DNA concentration of tissues (Koumans et al. 1994; Suresh and Sheehan 1998b). This can result in a violation of the basic assumption of constant DNA content of tissues that underpins the use of RD as a growth rate index (discussed by Caldarone et al. 2016). Although our results appear to confirm that RD is related to growth rate and not size for fish at the same stage of development, the question of size and developmental stage should be considered carefully before applying RD as an index of relative growth rates in the field.

While previous studies have generally described a linear relationship between RD and growth rate (e.g., Suresh and Sheehan 1998a; MacLean et al. 2008; Caldarone et al. 2016), we fitted a regression to log-transformed RD,

which improved the normality of the residuals. It is unclear whether the somewhat nonlinear and heteroscedastic relationship that we observed between RD and growth rate reflected the actual distribution of RD values for experimental fish or whether it was an artifact of sampling technique. Our study was intended as a trial of a technique that would have minimal growth and mortality impacts on age-0.0 Chinook Salmon prior to their tagging and release. We therefore used a smaller biopsy punch (1.5-mm diameter) than the 2-mm-diameter punch used by MacLean et al. (2008) on relatively large (160–220-mm) Atlantic Salmon smolts. This approach was successful in that it apparently resulted in no increased mortality or decreased growth for the small (75–99-mm) fish in our study. However, the resulting plug of tissue was only 56% of the cross-sectional area that would be produced by a 2-mm biopsy. Fish muscle is not homogeneous; rather, it is organized into myotomes separated by myosepta. These myosepta consist primarily of connective tissue and have a higher lipid content than the muscle fibers themselves (Zhou et al. 1995). MacLean et al. (2008) found that for juvenile Atlantic Salmon, tissues other than muscle (i.e., scale, fin, and gill) had low and consistent RD ratios that were not related to growth rate. It seems plausible that our very small biopsy punch may have risked obtaining nonrepresentative samples of muscle tissue (relatively more or less muscle fibers relative to connective tissue). This could have resulted in greater variance and artificially low RD values for some high-growth-rate fish (but not vice versa if myosepta had consistently low RDs). Alternatively, it is possible that there is a minimum RNA concentration (ribosome density) for fish exhibiting nonnegative growth and that RD values converge as the growth rate decreases.

RNA : DNA Ratio Integration Time

The RD responded to a change in ration within 6 d and continued to track ration level until the end of the experiment (day 14). However, given the high variance and small sample size, RD values were only able to differentiate high- and low-ration treatments from each other and not from the medium-ration treatment. Interestingly, RD values in the integration time experiment were elevated in comparison with the corresponding ration levels in the growth rate experiment. The time required for RD to respond to a change in growth conditions was relatively consistent with results for previous work on Atlantic Salmon. Working with postsmolts, Caldarone et al. (2016) documented a consistent decrease in individual RD after 7 d of fasting and a consistent increase 8 d after refeeding the fasted fish (previous measurements at 3 and 4 d, respectively). Treatment means were significantly different after 12 d in both cases. Similarly, Arndt et al. (1996) reported that the RD of Atlantic Salmon fry responded to

changes in ration within 4–8 d. Faster RD response times of 2 d or less have been reported for larval fish (e.g., Buckley 1980; Wright and Martin 1985; Rooker and Holt 1996). It is likely that RD response time is partially a function of energetic reserves (Caldarone et al. 2016), with greater reserves corresponding to a longer response time. It seems safe to conclude that RD responds to changes in growth conditions within 1–2 weeks in juvenile salmonids.

RNA : DNA Ratio Logistical Considerations

Muscle biopsies for RD analysis are simpler to obtain from small fish in the field than blood plasma for IGF1. The only special requirement is the capacity to flash freeze samples and maintain them at -80°C . Our results and those of MacLean et al. (2008) and Caldarone et al. (2016) also confirm that biopsies can be obtained non-lethally and with little, if any, growth impairment. Nevertheless, as discussed above, we are uncertain whether the heteroscedasticity that we observed for the relationship between RD and growth was a consequence of difficulty obtaining a representative muscle sample with a very small biopsy punch.

Another major consideration impacting the validity of RD as an index of recent growth in the field is temperature. At lower temperatures, RD values may be higher for a given growth rate (Buckley et al. 1999, 2008), possibly due to the need to compensate for reduced catalytic activity of RNA at low temperature (Goolish et al. 1984). Models have been developed that attempt to derive a common RD, growth, and temperature relationship across multiple species of larval fish (Buckley 1984; Buckley et al. 2008). This challenge may be more acute for juvenile fish, where greater mobility may facilitate movement between habitats with very different temperatures over a relatively short time scale (e.g., above or below a thermocline in lakes or the ocean or into tributaries with different thermal regimes in a riverine system). Laboratory experimental work should therefore seek to determine whether the influence of temperature and growth rate on RD integrate over common or differing time frames in juvenile fish. Even if such details can be worked out, researchers will still be challenged by situations where it is not possible to be confident of the thermal regime experienced by a juvenile fish immediately prior to capture.

Circulus Spacing Relation to Growth

We observed a significant positive correlation between mean spacing of the two outermost circuli and growth and a negative correlation between circulus formation interval and growth. The R^2 values for these relationships (0.44 and 0.46, respectively) were only slightly lower than the R^2 for $\log_e(\text{RD})$ versus growth (0.47). An assumption of variation in circulus spacing with growth rate in salmonids underpins widely used aging techniques that

differentiate summer growth zones with widely spaced circuli from winter zones with closely spaced circuli (e.g., Gilbert 1912; Ricker 1962; Bilton and Ludwig 1966). A number of studies have also explicitly demonstrated positive correlations between growth rate and scale circulus spacing (Fisher and Pearcy 1990, 2005; Fukuwaka and Kaeriyama 1997).

The relationship between the spacing of the outermost circulus and growth ($R^2 = 0.29$) was weaker than that for the outer two circuli, likely due in part to measurement uncertainty. When measuring circuli on acetate scale impressions, it is not always clear whether the visible scale margin corresponds to the edge of a fully formed circulus or only a partially formed circulus. Our outermost circulus spacing measurement might better be considered a “penultimate circulus-to-scale margin” measurement. In studies of wild fish, averaging the spacing of a subset of outer circuli not including the scale margin could reduce error due to incompletely formed circuli (as long as the time period reflected is relevant for the study design; see below). Additionally, scale circuli are irregular in spacing and, in some cases, are split (Figure 2). Averaging the spacing of multiple circuli reduces the effect of this variability. The relatively strong relationship between growth rate and circulus spacing observed by Fisher and Pearcy (1990) for laboratory-reared age-0 Coho Salmon ($R^2 = 0.64$) likely reflected averaging across a greater number of circuli than in the present study (up to 16 based on a formation rate of 0–0.25 circuli/d over 63–66 d). Given the challenges discussed above, the strength of correlation between circulus spacing and growth that we observed when averaging only two circuli (including the outermost) was encouraging.

Our best models relating spacing of the outermost circulus and outer two circuli to growth both included terms for FL on day 0. This relationship was positive in both cases (initially larger fish formed wider circuli relative to growth rate). Chinook Salmon scales exhibit a pattern of initially wide circuli close to the origin, which then become narrower (Koo 1967: Figure 6; Campbell et al. 2015: Figure 2; present study: Figures 2, 4). Often, a minimum is reached with a series of closely spaced circuli (generally between circulus 10 and 20), which may be interpreted as an annulus or ocean entry check depending on life history type. A subsequent increase in circulus spacing has been interpreted as a consequence of rapid growth rate in marine waters (Koo 1967; Reimers 1971; Tovey 1999). Recent work employing scale and otolith microchemistry has called into question the ability of scale morphology to accurately identify habitat transitions in juvenile Chinook Salmon (Campbell et al. 2015). Our results suggest that early changes in circulus spacing are to some degree independent of growth rate and the phenological events that influence it. Further experiments on fish

over a wider range of sizes are necessary to determine whether the relationship between circulus spacing and growth rate becomes independent of size in larger Chinook Salmon.

Circulus Spacing Integration Time

The duration of the integration time experiment (14 d) was less than the estimated time required to form a single circulus at the growth rates exhibited by many of the fish in the study (see below). We therefore inferred potential integration times for this index from the relationship between circulus formation interval and growth rate observed in the growth rate experiment. The positive relationship between growth rate and circulus formation rate for Chinook Salmon postsmolts was consistent with previous results for Sockeye Salmon *Oncorhynchus nerka* (Bilton and Robins 1971), steelhead (anadromous Rainbow Trout; Beakes et al. 2014), Coho Salmon (Fisher and Percy 1990, 2005), and Atlantic Salmon (Haraldstad et al. 2016). This relationship has the important implication that circulus spacing, averaged over the same number of circuli for fish growing at different rates, will reflect different periods of growth. The nonlinear relationship between circulus formation interval and growth rate (Figure 3) was consistent with the observation that scale growth ceases (circulus formation rate approaches 0) in fish that are not growing (Suzuki and Kaeriyama 1990; as cited by Fukuwaka 1998). Heteroscedasticity in the relationship between circulus formation interval and growth rate was likely a consequence of error structure. Circuli were counted as integers, and formation interval was calculated by dividing a constant number of days (46 d) by the number of circuli deposited (mean of up to three scales). Variance in the number of circuli deposited therefore generated far greater variance in estimated circulus formation interval where the increase in circulus number was small.

The observed relationship between circulus formation interval and growth equated to formation of an individual circulus over 9–17 d at growth rates of 0.2–0.6% length per day. As previously discussed (in the section Insulin-like Growth Factor 1 Relation to Growth), typical growth rates for juvenile Chinook Salmon are likely greater in the field than we observed in the laboratory. Extrapolating the relationship in Figure 3, growth rates of 0.6–0.9% length per day would equate to deposition of one circulus every 6–9 d. This agrees well with formation interval estimates of 6.8 d during early marine residence of Robertson Creek ocean-type Chinook Salmon (Tovey 1999) and 4.6–8.3 d across different stocks of Puget Sound Chinook Salmon (Gamble et al., 2018). Based on these literature values for growth and circulus formation rate, circulus spacing averaged over the outermost two to four circuli could be expected to index growth over the preceding 10–

20 d and 18–36 d, respectively. Likely growth rates of field-sampled fish must therefore be considered when using circulus spacing as an index of relative recent growth rate, and the potential for very slow-growing fish to confound analyses should be taken into account.

Circulus Spacing Logistical Considerations

A great advantage of using scale pattern analysis as an index of relative recent growth rates is that scales can be collected easily and nonlethally in the field without the need for specialized equipment. Scales can also be obtained from dead or frozen fish and can be stored and archived dry for many years. Processing and measuring scales for growth rate analysis require less specialized labor than aging, as fixed protocols can be established for obtaining digital measurements that do not require a subjective assessment of check and annulus locations. Nevertheless, scale processing is time consuming and subject to error. An added benefit of scales relative to biochemical indices of growth is that they can also provide information on life history or growth trajectories prior to the period immediately preceding capture (e.g., Ball and Jones 1960; Beamish et al. 2004; Fisher and Percy 2005; Gamble et al. 2018).

One challenge is that when fish are caught using net-based gear, particularly trawling, most scales are often lost during capture. Recent work by Beakes et al. (2014) also suggested that temperature may have a significant effect on the relationship between circulus spacing and growth rate. Specifically, at lower temperatures, steelhead formed wider circuli despite a lower growth rate. If future work confirms this relationship between temperature and circulus spacing in other salmonids, similar considerations will apply as discussed above for RD and growth.

CONCLUSIONS

The relative growth rate indices evaluated here differ in terms of their theoretical basis, strength of relationship to growth rate, integration time, and logistical constraints (Table 2). Plasma IGF1 concentration has a causal theoretical relationship to growth, and our results confirm that it is strong indicator of relative recent growth rates in postsmolt Chinook Salmon. This index has been well validated in salmonids and other fish (reviewed by Beckman 2011) and has been successfully applied to study the recent growth of salmon in the field (Ferriss et al. 2014; Journey 2015; Chamberlin et al. 2017). We found that IGF1 responded to a change in growth conditions (ration) within 4 d—earlier than differences in growth rate could be measured directly. This is consistent with the causal role of IGF1 in somatic growth. Where fish may be sampled lethally (or are large enough to permit non-lethal sampling of blood plasma), IGF1 provides a robust

index of recent relative growth rates. The RD in muscle has been less comprehensively validated in juvenile salmonids than IGF1; nevertheless, our results confirm that RD values are positively related to recent growth rate in juvenile Chinook Salmon and respond quickly (within 6 d) to changes in growth conditions. We confirmed and extended the results of MacLean et al. (2008) and Caldarone et al. (2016) by demonstrating that nonlethal sampling of RD is possible even in very small fish (<10 cm); however, the use of a very small (1.5-mm-diameter) biopsy may have been responsible for high variability around the relationship between growth rate and RD. The well-documented effect of temperature on the relationship between growth rate and RD (Buckley et al. 1999, 2008) could be problematic if fish have experienced different thermal regimes prior to sampling. Nevertheless, RD represents an analytically simple, nonlethal approach to assessing recent relative growth rates. Mean spacing of the outer two scale circuli was almost as strongly related to recent growth of Chinook Salmon postsmolts as RD. Morphometric analysis of scales is nonlethal, simple, and inexpensive, but the dependence of circulus formation rate on growth rate means that this metric will index different periods of growth for fish growing at different rates. This is a particular concern for cases in which some fish may be growing very slowly. Scales can also provide additional information on prior growth trajectories and life history.

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