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Growth Performance, Fillet Quality, and Reproductive Maturity of Rainbow Trout (*Oncorhynchus mykiss*) Cultured to 5 Kilograms within Freshwater Recirculating Systems

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Abstract

Rainbow trout are commonly cultured within aquaculture systems to one pound or less and marketed as pan-sized fillets. Production of larger rainbow trout provides a distinguishable product. Research that describes the growth performance and fillet quality of large rainbow trout is limited, particularly for trout cultured in recirculating aquaculture systems. A study was conducted evaluating the growth performance and fillet quality attributes of all-female rainbow trout reared using freshwater recirculating systems operated at a mean water temperature of 13°C, under constant lighting, and with around-the-clock feeding. Rainbow trout grew to 4.8 kg in 22 months post-hatch. Growth rates declined with the onset of reproductive maturity. Rainbow trout weighed 5.2 kg at 26 months. The mean ratio of feed provided to biomass gain was 1.36:1 from first feeding to 22 months but increased substantially from 23-25 months. As rainbow trout approached reproductive maturity, 10 fish were collected at specified intervals for assessment of fillet quality attributes. Fillet yield peaked at 20-22 months when trout were 3.8-4.8 kg. Cook yield, cooked fillet firmness, and crude fat decreased; while fillet moisture and raw fillet firmness increased from 24-26 months. Changes in fillet quality coincided with reduced growth rates, decreased feed efficiency, and increasing gonadosomatic index. Two principal components were identified that explained more than 73% of the variation in growth and fillet attribute responses: principal component 1, the growth variable (length, weight, fillet thickness, belly flap thickness, and cook yield) and principal component 2, the quality variable (fillet moisture, fillet fat, and cooked fillet firmness). This research provides rainbow trout growth performance and fillet quality results that can be referenced for the development of recirculating system production plans and for selection of harvest endpoints that balance the requirements of fish farmers and the food industry sector.

Keywords: Rainbow trout; Fillet quality; Recirculating aquaculture systems; Reproductive maturity

Introduction

Recent development and advancements in water recirculating systems for the culture of rainbow trout (*Oncorhynchus mykiss*) and other salmonids [1-3] provide opportunity to expand current production levels. Commercial production of large 2-5 kg rainbow trout provides a niche product that is distinguished from the pan-sized rainbow trout (≤ 0.5 kg whole body weight) commonly marketed in many countries, including the United States [4]. Recently, there has been growing interest in culturing rainbow trout (a.k.a. steelhead) to harvest sizes >2 kg [4]; however, research that clearly defines growth performance and quality of larger trout is limited, particularly for trout cultured in recirculating systems. Several studies have evaluated fillet quality attributes for large, sexually maturing rainbow trout [4-10]. Most of these studies were focused on physiology during reproduction, were conducted using flow through systems, and did not thoroughly describe rainbow trout growth and fillet quality with age/time. The maximum rainbow trout weights reported in previous aquaculture studies were 3.6 and 3.1 kg [5,6], respectively. Research investigating the culture of large trout (>2 kg) in recirculating aquaculture systems (RAS) is particularly limited. Successful rainbow trout culture to approximately 1.3 kg was reported in a Danish model RAS [11]; and previous on-site studies demonstrated that rainbow trout can be cultured within RAS to approximately 1.3-1.4 kg in one year from hatch [2,12,13]. However, these studies did not attempt to culture trout to larger sizes and evaluation of fillet quality was limited.

One concern when rearing trout to larger sizes is the negative effects of sexual maturation on fillet yield and quality [4,14]. During sexual maturation, female rainbow trout develop ovaries that may account for >20% of the total body weight prior to ovulation [15]. As sexual maturation occurs, female rainbow trout metabolism is reorchestrated to meet nutrient demands of egg growth that, in turn, influences mobilization of energy and other stored nutrients [16]. A primary concern in regard to fillet quality is the mobilization of lipid from energy stores [6,9,10,14,17,18]; particularly intramuscular lipid, which in addition to visceral fat, can be catabolized to support egg development. Lipid mobilization, as well as protein, from muscle profoundly affects fillet quality and reduces fillet yield, as measured by percent separable muscle [4-9,19,20]. These changes in fillet composition and yield often lead to diminished quality of the final product and reduced revenue for the fish farmer.

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Defining optimal rainbow trout fillet quality (and for that matter seafood quality) is a complex task, because quality encompasses a wide range of traits and variables, and the manufacture and sale of product involves many levels (farmers, processors, wholesalers, distributors, retailers, caterers, and consumers) with varying perceptions of quality [21]. For example, taste, odor, nutritional value (fatty acid content), firmness, and food safety are high ranking quality attributes to consumers, chefs, and caterers; while sex, maturity, fillet size, fat content, and product consistency have greater value to wholesalers, distributors, and retailers [19,21]. The form of the final product (i.e. filleted, smoked, canned, etc.) can also influence expected quality. For example, high quality smoked products require the raw material to be higher in fat than fillets used for other, non-smoked purposes [19,22]. At the farm level, multiple variables and their interactions contribute to these quality attributes, including: genetics [23], fish age/growth rate [24], diet [19], feeding regimen [25], photoperiod-which influences sexual maturation [26], and water quality [27]. Hence, the farmer is responsible for production of fish that meet a wide range of quality demands, while also optimizing criteria that are essential to profitability such as rapid growth rates, efficient feed conversion, and maximum fillet yield.

Research that combines the evaluation of rainbow trout growth performance and coinciding fillet quality attributes would provide information for fish farmers and food industry sectors that could be useful for determination of best harvest endpoints. This information would be particularly valuable for trout farmers using or planning to use innovative fish production technologies that recirculate water and optimize environmental variables. Therefore, the primary objective of this study was to characterize rainbow trout growth, processing, and fillet quality attributes as rainbow trout approached sexual maturity, while being cultured to 5 kg in freshwater recirculating systems with relatively constant water temperature averaging 13°C, constant lighting, and around-the-clock feeding. The effects of the culture conditions on the reproductive and growth axes will be described elsewhere.

Materials and Methods

Fish and fish culture systems

All-female diploid, Kamloops strain rainbow trout were purchased as eyed eggs from Troutlodge, Inc. (Sumner, WA, USA). Eyed eggs were incubated and hatched within Heath-Tecna 8-stack incubator trays (Marisource, Tacoma, WA, USA) in a RAS maintained at a constant water temperature of 10°C. Designation of Day 1 of the growth cycle corresponded with 50% egg hatch. After hatch, water temperature in the incubation system was adjusted to approximately 13°C to acclimate the fry for transfer to culture tanks. When rainbow trout had absorbed the majority of the yolk sac, the fish were transferred into 0.5 m^3 (1.1 m diameter; 0.5 m deep) circular tanks within a single-pass system and raised at an average water temperature of 13.6°C until the fish reached a mean size of 0.13 kg at 6 mo post-hatch. Approximately 6,000 fish were then transferred into six replicated RAS; 1,000 fish were stocked in a 5.3 m^3 (2.4 m dia., 1.0 m deep) circular, dual-drain culture tank within each system. Details of the replicated RAS are described elsewhere [12]. Rainbow trout were cultured in these systems at a mean temperature of 13.2°C until approximately 12 mo post-hatch. At this time, a total of 330 rainbow trout were randomly selected and transferred to an 11 m^3 (3.7 m dia., 1.2 m deep) circular dual-drain culture tank within a partial reuse system where they were raised until 26 mo post-hatch. Approximately 60% of the water flowing through this tank was treated through a cascade aeration column and reused. As fish density approached 80 kg/m^3, fish were randomly divided and removed from the study to reduce density to 40-50 kg/m^3. Due to limited space and the need to maintain numbers, rainbow trout density exceeded 80 kg/m^3 for two brief periods during the fingerling phase. Throughout the study, random population sampling was conducted through crowding and dip-netting.

Photoperiod

A constant 24 h photoperiod (overhead lights on) was provided throughout the study. From 0-12 mo external natural light was limited and the primary photoperiod was provided by overhead metal halide and fluorescent lights. When fish were relocated to the 11 m^3 partial reuse system (approximately 12 months), constant 24 h overhead fluorescent lighting was provided. However, the greenhouse-style building in which this system was housed was made of a canvas material that filtered natural light, allowing a diurnal fluctuation in light intensity at the surface of the tank: from ~ 40-200 lux at night, depending on location, to ~ 2000 lux midday.

Feeding

Timer-controlled mechanical feeders were used to provide uniformly distributed feed events that delivered equal feed portions around-the-clock through all phases of culture. From first feeding (18 days post-hatch) to 0.13 kg, Sterner Fish Tech AS, Model 907 feeders (Ski, Norway) were used to feed once every 30 min; from 0.13-1.40 kg, Arvotec, Model T-drum 2000CE feeders (Huutokoski, Finland) were used to feed once every 2 h; and from 1.4-5 kg, an Aquatic Eco-Systems, Auger Feeder Model FS4022 (Apopka, FL, USA) was used to feed once every 6 h. Daily feeding rates and feed size were determined using standard trout feeding charts as well as observations of feeding activity and wasted feed. Fish were fed to satiation as much as possible. Although we attempted to avoid overfeeding, it did occur on occasion, especially as the fish approached 5 kg. From first feeding to 0.13 kg, trout were fed Silver Cup Feed (Nelson & Sons Inc., Murray, UT, USA) and from 0.13 kg to the end of the study, trout were fed standard trout diets produced by Zeigler Brothers Inc. (Gardners, PA, USA). A slow sinking, extruded commercial trout growers diet, (42% protein: 16% fat) was fed for the majority of the study, with the exception of a 45:20 diet fed from 6 to 8 mo post-hatch. Daily feed amounts were recorded.

Water quality analysis

Dissolved oxygen and temperature were measured 5 times weekly using a handheld dissolved oxygen meter while trout were in the fry system and 11 m^3 growout system (Table 1). When trout were

Parameter	Method	Units
Dissolved oxygen	Hach Model HQ10 LDO probe; Hach SC100 Universal Controller with LDO probe	mg/L
Temperature	Hach Model HQ10 LDO probe; Hach SC100 Universal Controller with LDO probe	°C
Dissolved carbon dioxide	Standard Methods 2320	mg/L
Alkalinity	Standard Methods 2302	mg/L (as $CaCO_3$)
Total suspended solids	Standard Methods 2560	mg/L
Total ammonia nitrogen	Standard Methods 4500-NH_3	mg/L (as NH_3-N)
Nitrite-nitrogen	Standard Methods 4500-NO_2	mg/L (as NO_2-N)
Nitrate-nitrogen	Standard Methods 4500-NO_3	mg/L (as NO_3-N)
$cBOD_5$	Standard Methods 5210 5-day BOD	mg/L

Table 1: Standard methods [28,29] or a handheld probe were used to measure water quality.

cultured in the six replicated RAS, SC100 Universal Controllers with Luminescent Dissolved Oxygen (LDO) probes (Hach Company, Loveland, CO, USA) displayed oxygen and temperature continuously and these values were recorded daily each morning. Water samples were collected once weekly and analyzed for dissolved carbon dioxide (CO_2), alkalinity, total ammonia nitrogen (TAN), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), total suspended solids (TSS), and 5-day carbonaceous biochemical oxygen demand ($cBOD_5$) according to methods described in Table 1 [28,29].

Fish sampling and performance assessment

During the first five months of culture, after first feeding, representative bulk weight samples were collected monthly. Thereafter, 60-100 fish were sampled monthly from the population to collect individual length and weight data. Fish were returned to culture tanks after each sampling event. Fulton's condition factor (CF), thermal growth coefficient (TGC), and feed efficiency ratio (FER) were calculated using the following equations. Mean weight was expressed in grams, length in millimeters, and temperature as ºC.

$CF=100{,}000 * Weight/(Length)^3$

$TGC=(End\ Weight^{1/3}–Start\ Weight^{1/3})/((Days\ Between\ *Mean\ Temp.)\times 1000)$

FER=Cumulative Feed Delivered to Fish/Fish Biomass Gain

Fish processing

Fish were sampled bimonthly to evaluate processing and fillet attributes, from 14-24 mo, and then monthly from 24-26 mo. During each sampling event, at least 10 fish were randomly netted from the tank and humanely euthanized immediately after removal according to approved procedures. The following data were collected on individual fish: total body length; as well as total body, head-on-gutted (HOG), skin-on fillet, skinless fillet, total visceral, and total gonadal weights. These weights were used to calculate HOG, skin-on fillet, and skinless fillet yields (product mass/total body mass *100), as well as gonadosomatic index (GSI; gonad mass/total body mass *100) and viscerosomatic index (VSI; visceral mass/total body mass *100). Fillet thickness and belly-flap thickness for each fish were measured using calipers at three standardized locations along the length of the ventral midline; these locations were just cranial to the pectoral fin, just caudal to the pelvic fin, and at the vent. Data were used to determine the effect of age (from 14-26 mo) on mean fish size, TGC, FER, GSI, VSI, fillet yield and quality attributes.

In preparation for proximate composition analysis, boneless-skinless fillets were frozen in liquid nitrogen, powdered using a Waring commercial grade blender (Model 51BL31; Waring Commercial; Torrington, CT, USA), and stored at -20°C until analysis. Moisture, crude lipid, crude protein, and ash analyses were completed according to AOAC approved methods [30].

Cook yield and fillet texture analysis

A 3-by-8 cm section of the dorsal musculature was removed 2 cm caudal to the pectoral girdle from each boneless, skinless fillet. The section was thermally processed in a microprocessor-controlled smoke oven (model CVU-490, Enviro-Pak, Clackamas, OR, USA) to an internal temperature of 65.5°C. Following cooking, fillet portions were removed from the oven and cooled to room temperature. Cooked portions were weighed to calculate cook yield (cooked weight as a percent of raw weight). The precut, cooked portion was placed, with the skin-side up, in a Kramer Shear Cell. Shear force was measured using a Texture Analyzer (model TA-Hdi, Texture Technologies Corp., Scarsdale, NY, USA) equipped with a 5-blade Kramer shear attachment at a cross speed of 127 mm/min. Values were expressed as peak force generated per gram of sample.

Statistical analysis

This experiment was conducted as a completely randomized design with *age* as an independent variable. One-way ANOVA was used to evaluate the effect of *age* on growth and fillet attributes using the GLM procedure of SAS® system for Windows, version 9.1 [31]. Significance was defined at $P<0.05$. Principal component analysis (PCA) was used to create a few key variables that characterized as fully as possible the variation in the data set while reducing its dimensionality. The varimax rotation on the first two principal components was computed by FACTOR procedure of SAS® system for Windows, version 9.1 [31]. Significance of principal component loadings was defined at 0.50. Linear and nonlinear models were fitted to the rotated standardized principal components (PCs) to characterize their relationships with the variable *age*. The nonlinear least squares method was used to fit a four-parameter logistic (4PL) model to the first principal component (PC1) scores as a function of age [32]. A segmented regression model was fitted to the second principal component (PC2) scores [33,34]. Regression analysis by PROC REG procedure and Pearson's correlation analysis by PROC CORR procedure were used to assess the relationships of select fillet attributes, as well as GSI, with fish age [31].

Results and Discussion

Fish growth

Rainbow trout grew to a mean size of 1.4 kg by 12 mo, to 4.8 kg by 22 mo, and to 5.2 kg and 62.9 ± 0.6 cm in 26 mo (Figure 1 and Table 2). Cumulative growth from 0-26 mo followed a sigmoidal curve typical for trout as they advanced from the larval and juvenile stages to maturing adult [35]. Condition factor increased steadily from 0-12 mo and thereafter remained relatively constant at approximately 2.1g/cm³ (Table 2). Thermal growth coefficient averaged 2.13 from first feeding to 12 mo (1.4 kg) as fish grew to 1.4 kg, 1.49 as fish grew from 1.4-4.8 kg (13-22 mo), and 0.30 as fish grew to 5.2 kg (23-26 mo) (Table 2). Growth rates declined with onset of sexual maturation, as nutrient

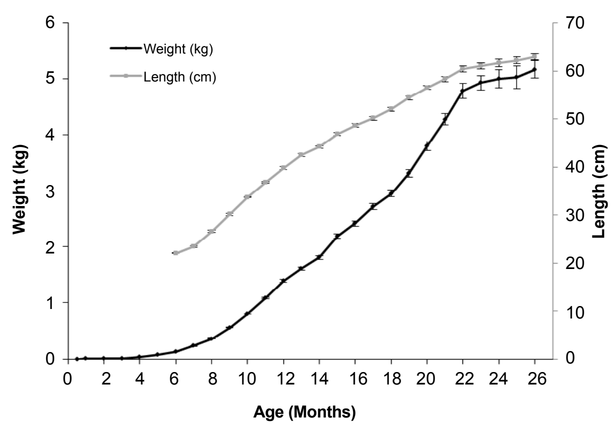


Figure 1: Mean length (cm) and weight (kg) of rainbow trout during the 26 month culture period. Note that bulk weights were conducted over the first 5 months of the life cycle, thus length measurements were not collected.

Age (Months Post-hatch)	Fish Weight (kg)	Condition Factor	Water Temperature (°C)	Thermal Growth Coefficient	Feeding Rate (% Body Weight Daily Feed)	Feed Efficiency Ratio (Kg Feed/Kg Biomass Gain)
1	0.001	-	12.0	0.84	7.00	0.40
2	0.002	-	13.9	0.92	6.32	1.91
3	0.012	-	14.0	1.59	4.80	1.59
4	0.037	-	13.5	2.66	3.51	0.73
5	0.068	-	13.4	1.93	2.14	1.07
6	0.130	1.59	13.3	2.06	2.33	1.29
7	0.182	1.82	12.5	2.84	1.91	1.05
8	0.297	1.89	12.7	2.85	1.78	1.17
9	0.566	2.02	13.5	2.97	1.69	1.38
10	0.685	2.06	13.6	2.68	2.10	1.72
11	0.943	2.13	14.8	2.33	1.62	1.54
12	1.230	2.16	15.2	2.09	1.42	1.64
13	1.501	2.09	13.0	1.28	0.85	1.93
14	1.713	2.11	13.0	1.29	0.83	1.90
15	2.002	2.14	13.1	2.17	0.82	1.19
16	2.307	2.11	13.0	1.10	0.67	1.94
17	2.570	2.16	12.7	1.32	0.69	1.84
18	2.836	2.09	12.6	1.13	0.40	1.28
19	3.136	2.05	12.5	1.44	0.41	1.11
20	3.556	2.10	12.5	1.90	0.49	0.88
21	4.038	2.16	12.5	1.64	0.45	1.15
22	4.533	2.16	12.7	1.62	0.47	1.20
23	4.858	2.17	12.9	0.56	0.30	2.23
24	4.970	2.13	13.4	0.19	0.29	6.18
25	5.012	2.07	13.6	0.06	0.34	21.6
26	5.097	2.14	13.4	0.37	0.15	0.71

Table 2: Rainbow trout performance summary including growth, survival, and feeding rates throughout the life cycle from fry to maturing adult.

System (fish size)	Temperature °C	Dissolved O_2 mg/L	Dissolved CO_2 mg/L	Alkalinity mg/L as $CaCO_3$	TAN mg/L	NO_3-N mg/L	NO_2-N mg/L	TSS mg/L	$cBOD_5$ mg/L
Single-pass (0.5-130 g)	12.9 ± 0.1	9.5 ± 0.2	18.4 ± 0.3	266 ± 3	0.31 ± 0.01	3 ± 0	0.01 ± 0.00	4.5 ± 0.3	1 ± 0
Fully-recycle H Fully-recycle L[a] (130-1400 g)	13.2 ± 0.0 13.2 ± 0.1	10.0 ± 0.0 10.0 ± 0.1	9.3 ± 0.1 9.0 ± 0.4	226 ± 1 214 ± 1	0.29 ± .00 0.48 ± 0.05	12 ± 0 70 ± 4	0.04 ± 0.01 0.10 ± 0.00	2.7 ± 0.1 6.4 ± 1.2	2 ± 0 6 ± 1
Partial-reuse (1400-5000 g)	12.9 ± 0.0	11.0 ± 0.1	13.6 ± 0.3	263 ± 1	0.24 ± 0.01	2 ± 0	0.01 ± 0.00	1.1 ± 0.1	0 ± 0

[a]Rainbow trout were raised in both the high (H) and low (L) water flushing treatments.

Table 3: Water quality (mean ± standard error) in the culture tanks during each phase of the 26-month growth period.

utilization was refocused from somatic growth toward gonad and gamete development [9,35,36].

To the authors' knowledge, rainbow trout growth rates (i.e., cumulative weight with age/production time) of this magnitude have not been previously reported for trout cultured to mean sizes > 2 kg [4-6,37]. The study conditions were unique in that trout of this size were cultured in freshwater recirculating systems that provided maximum control of environmental conditions (Table 3). The following culture parameters likely contributed to the rapid growth rates: constant 24 h overhead lighting, around-the-clock feeding to satiation, and near-optimal water temperatures averaging 13°C.

Survival

Cumulative rainbow trout survival was 92.9% following hatch and yolk sac absorption. After 6 months of culture in the single pass fry culture system, cumulative survival was 87.1%. From 6 to 12 months of the life cycle, while the rainbow trout were reared in six replicated RAS, mortality was minimal. Cumulative survival after 12 months of culture was 86.3%. Rainbow trout mortality from fry stage to 1-1.5 kg was slightly greater than the average mortality rate (4%) for other trout cohorts cultured on-site within the same systems [2]. Slightly increased mortality occurred from 12-22 months while a small portion of the population (330 fish) was held in the partial reuse system for growout to 5 kg. From months 12 to 20, 8.4% of the population was lost to mortality. Much of this mortality was related to repeated monthly handling of the same fish for length and weight assessment, as one or two mortalities typically were removed from the tank on days subsequent to each sampling event. Based on these results, frequent handling of large rainbow trout (1-4 kg) is not recommended in a commercial setting and production plans should be designed to minimize handling and movement of large trout. From months 20 to 26, an additional 15% of the population was lost to mortality. Some of these fish succumbed to handling stress, while others became egg laden causing them to lose equilibrium in the water column.

Feed efficiency

Feed efficiency ratio (cumulative feed delivered to the fish/ biomass gain) ranged from 1.0-2.0 for the majority of the growth cycle and averaged 1.36 from first feeding to 22 mo; however, FER increased from

2.23 to >20.0 from 23-25 mo (Figure 2 and Table 2). Feed efficiency has been reported to decrease with rainbow trout age [24,38]; but has not been thoroughly evaluated for maturing rainbow trout. During the present study, increased FER corresponded with a rapid decline in TGC and an increase in egg development, as evidenced by increasing GSI. The dramatic increase in FER can be explained in part by the reallocation of energy resources from growth to reproduction; however, it was likely that the primary driver for the increase in FER at 25 mo to >20.0 was a substantial decline in feed consumption and subsequently significant amounts of wasted feed. Between months 25 and 26, FER declined from >20.0 to 0.71 (Figure 2). The substantial drop in FER was due to recognition by the fish culturist that maturing trout were not accepting feed. Feed ration was reduced by more than 50% from months 25-26, in order to rebalance FER (Figure 2). Although, the FER from months 23-25 was far from optimal, these results provided critical information for fish farmers with prospects of raising large rainbow trout. Increased FER or simply a reduction in feeding would negatively impact a producer's profit margin because feed represents a large fraction of the total cost of commercial aquaculture; however, harvesting rainbow trout prior to 23 mo could possibly evade these problems.

Gonadosomatic index

GSI assessment indicated that rapid egg growth occurred primarily from 20-26 mo (Figure 3), coincident with and likely affecting changes in many of the aforementioned trout performance traits (growth, TGC, FER) and subsequent fillet attributes. GSI was positively correlated with age (r=0.73). In addition, the decline in VSI (excluding gonad weight from the viscera weight) occurred in unison with increasing GSI (Figure 3) from 20-24 mo, consistent with lipid mobilization from the viscera to support egg production. Although VSI stabilized after 24 mo, GSI continued to increase suggesting that nutrients were derived from other sources, feed or possibly muscle lipid and protein. The GSI for fish in this study was lower than typically observed. Female rainbow trout can develop ovaries that account for over 20% of total body weight prior to ovulation [15], but fish in this study reached a maximum GSI of only 7.0% at 26 mo. A lower GSI could indicate that a lower fraction of whole body nutrient and energy resources were expended for gonadal growth than ordinarily occurs in maturing female rainbow trout. In addition, larger rainbow trout size, greater than that of previously reported comparisons to gonad mass, could also have been related to reduced GSI.

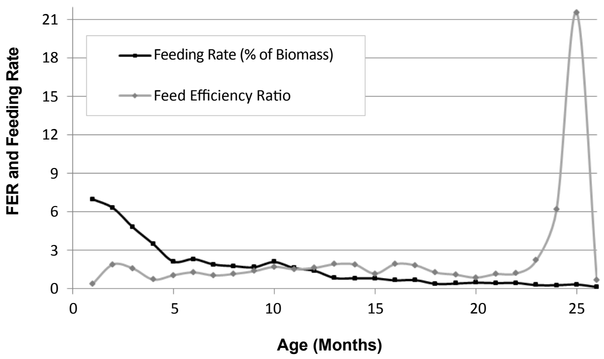


Figure 2: Monthly feeding rate expressed as percent body weight feed and feed efficiency ratio (FER) for rainbow trout throughout the life cycle.

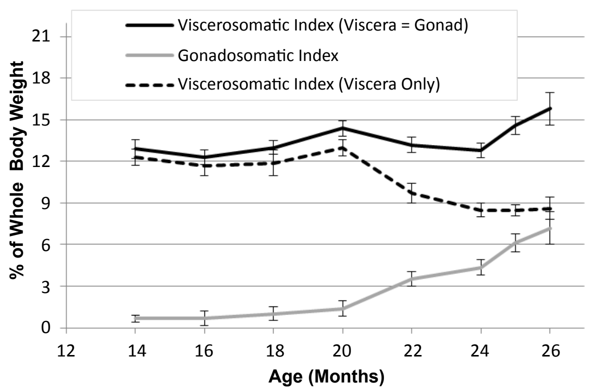


Figure 3: Effect of age on rainbow trout gonadosomatic and viscerosomatic index from 14 to 26 months.

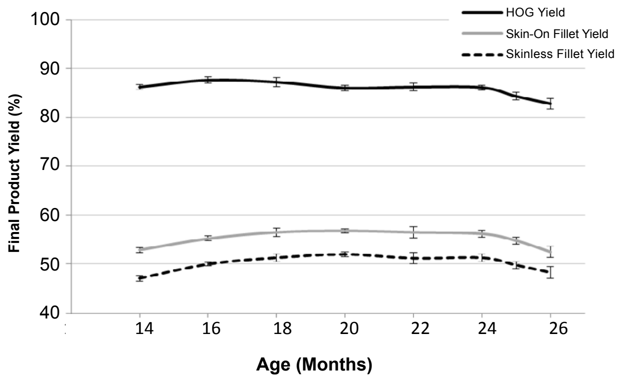


Figure 4: Effect of age on mean product yield percentage including head-on-gutted (HOG) yield, skin-on fillet yield, and skinless fillet yield from 14 to 26 months.

Fillet yield

Head-on-gutted yield ranged from 86.2-87.6% from 14 to 24 mo, but declined to 82.8% by 26 mo (Figure 4). Skin-on fillet (butterfly) yield increased from 52.9% at 14 mo to a maximum of 56.8% at 20 mo and then declined to 52.5% by 26 mo (Figure 4). Skinless fillet yield followed a similar trend, beginning at 47.1% at 14 mo, peaking at 52.0% at 20 mo, and declining to 48.3% by 26 mo (Figure 4). Separable muscle (skinless fillet) continued to increase through 25 mo; however, when expressed as a percent of whole fish weight, separable muscle peaked at the 20 to 22 mo interval (Figure 5). Percent separable muscle declined from 51.9 ± 0.5% at 22 mo to 48.3 ± 1.2% at 26 mo (Figure 5). Critical changes in final product yield were also related to the timing of egg growth and reproductive development. Previous studies have also reported a maximum fillet yield at roughly 20 mo of age and a corresponding increase in GSI thereafter [6,9].

Small differences in fillet yield can have a substantial economic impact on production and processing economics [5,39]; therefore, prediction and selection of a harvest endpoint to obtain peak fillet yield is important. Based on yield data alone, an optimal harvest endpoint of 20-22 mo (mean weight=3.8-4.8 kg) could be targeted under similar conditions and depending on marketing objectives for the final product.

Fillet and belly flap thickness

Fillet thickness increased from approximately 2.7 ± 0.1 cm at 14

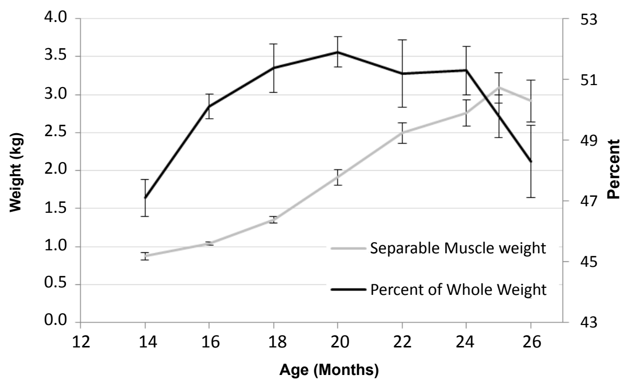


Figure 5: Effect of age on separable muscle weight and percent separable muscle from 14 to 26 months.

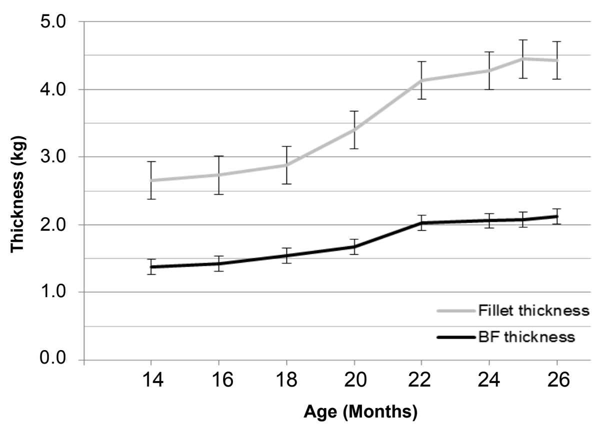


Figure 6: Effect of age on fillet and belly-flap thickness from 14 to 26 months.

mo to 4.4 ± 0.2 cm at 26 mo (Figure 6). Fillet thickness was positively correlated with age (r=0.91). Specific data for optimal fillet thickness as it relates to endpoint cooking rate and temperature is lacking for large rainbow trout. Endpoint cooking temperature has been shown to impact quality characteristics such as cook yield and moisture for rainbow trout [40]. The fillet thickness data obtained during this study could provide a reference for trout producers and food industry stakeholders in selecting a harvest endpoint that meets the qualifications of the desired end-product.

Belly flap thickness increased in unison with fillet thickness from 1.4 ± 0.1 cm at 14 mo to 2.1 ± 0.1 cm at 26 mo (Figure 6). Thus, belly flap thickness was also positively correlated with age (r=0.76). An increase in abdominal wall (belly flap) thickness of rainbow trout was also positively correlated with age and gonadal development during a previous study [6]. The belly flap of fish is generally trimmed from the fillet because of the adiposity of the tissue. Therefore, increased belly flap thickness generally correlates with increased carcass loss and a decrease in product yield.

Fillet quality

Fillet crude fat content increased from 11.9% at 14 mo to 18.1% at 24 mo, but then decreased to 13.5% and 11.7% at 25 and 26 mo, respectively (Figure 7). The timing of egg growth and development was a critical determinant of changes in fillet composition. In the context of an energetically demanding physiological process, such as egg growth and development, changes in adipose tissue are a reflection of the mobilization of stored energy (lipid) to support the development of eggs [6,9,14,17,18]. The timing of changes in fillet lipid content could influence the selection of harvest endpoint because intramuscular lipid represents an important quality parameter that significantly impacts the palatability, flavor, and nutritional value (fatty acid content) of the product [41-43].

Aside from reproductive maturation, many factors can influence the fillet lipid content of cultured salmonids, including lipid content of the feed [41,44,45], age/size of the fish [46,47]; and genetic heritability [16,48]. Crude fat levels in rainbow trout fillets are typically lower than the peak levels reported here. Pan-sized rainbow trout raised at Clear Springs Foods Inc. (Buhl, ID, USA), the largest rainbow trout producer in the U.S., have an average fillet fat content of 12-13% (personal communication Randy MacMillan, Clear Springs Foods Inc.). Another source [46] reported fillet fat content for pan-sized rainbow trout (0.3 kg) to range from 4-7%, and noted a correlation with fish size. In addition, muscle lipid levels of approximately 10% have been reported for 3.1 kg rainbow trout [6]. The present study demonstrated a relationship of increasing fillet fat content with trout size/age, at least prior to changes related to reproductive development. For example, fillet lipid was 11.9% when compositional analyses were conducted at 14 mo (mean weight=1.8 kg), and increased to 18.1% by 24 mo (mean weight=5 kg). It is noteworthy that these large rainbow trout contained fillet fat levels that were similar to farm-raised Atlantic salmon. For example, an average fillet lipid level of 15% was reported for 4 kg salmon harvested in Norway [49]. Large rainbow trout fillets with lipid and fatty acid composition comparable to Atlantic salmon could be perceived as a unique product with appealing marketing traits, depending on the effect of fat content on other quality attributes such as texture or fillet firmness.

Fillet moisture declined from 67.8 to 63.2% from 14-24 mo, but increased to 66.3% over the final two months of the study (Figure 7). Overall, a negative correlation (P<0.02) was observed between age and fillet moisture (r=-0.27). Likewise, cooked fillet texture declined from 460 to 290 g/g from 14 to 24 mo, but increased to 577 g/g over the final two months of the study, indicating an increase in fillet firmness (Figure 7). Increased cooked fillet moisture and firmness coincided with decreasing lipid from 18.1 to 11.7%.

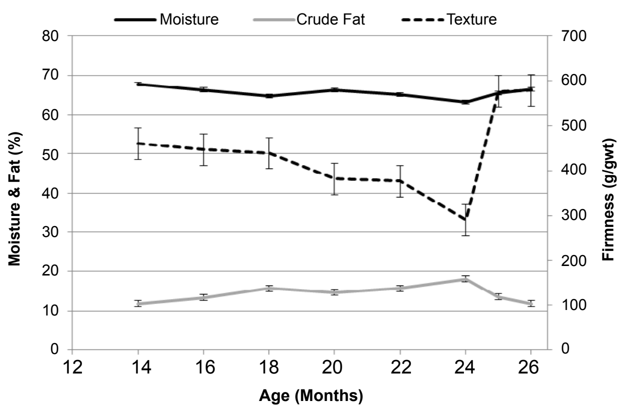


Figure 7: Effect of age on raw fillet composition and cooked fillet texture from 14 to 26 months.

Changes in muscle composition during growth and development did not affect cook yield. Cook yield peaked at 22 mo at 89.3 ± 0.7% and then declined slightly thereafter. Cook yield was positively correlated with age (r=0.57). Typically, greater cook yield indicates increased water and fat retention. It is expected that increased fillet thickness (Figure 6) would slow cooking rate and contribute to greater cook yield. Additionally, increased fat content may have reduced cook yield and firmness after 22 months, as the rainbow trout matured. It appears that raw fillets, when cooked, were not able to retain the increasing intramuscular fat (15 to 18%), as fish aged from 18 to 24 months. Fillet fat content was negatively correlated with cook yield (r=-0.35, P=0.0005).

In addition to the aforementioned quality traits, an important parameter to the processing industry and consumers for salmon and possibly for large rainbow trout fillets is bright red/orange fillet color [4,50]. During the present study, rainbow trout were not fed a diet containing carotenoids and therefore had pale, whitish fillet color. Other studies found that reproductive maturity resulted in a significantly lower red fillet color score in large rainbow trout [4]; thus color is another important quality consideration in determining an optimal harvest endpoint. Further investigation into the relationship of maturation, age, and fillet color is needed for rainbow trout cultured to 4-5 kg.

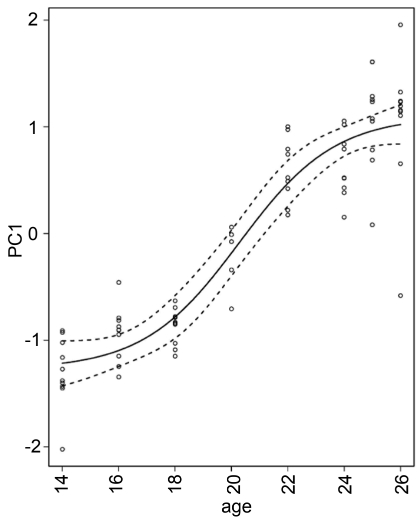
Principal components analysis

In the previous sections, a description of multiple growth and fillet quality responses to age were provided. While this approach was useful in understanding changes in a specific response variable with age, it did not provide a simplified model to analyze and predict the timing of best harvest endpoint for large rainbow trout while considering many variables. Principle components analyses reduced the dimensionality of the data and therefore facilitated identification of best harvest endpoints in terms of overall growth performance and fillet quality.

Two principal components were retained. Principal component 1 grouped length, weight, fillet thickness, belly flap thickness, and cook yield; while principal component 2 grouped moisture content, fat content, and cooked fillet firmness. Examination of these variables indicated that PC1measured size and "Growth" of the fish, with larger fish receiving larger scores; whereas PC2 described the "Quality" of the fillet.

Interestingly, a scatter plot of PC1 scores versus *age* suggested a sigmoidal curve (Figure 8) with growth clearly exhibiting a positive association with *age*, thus corroborating the interpretation of PC1 as measuring fish growth. Based on the 4PL model, the estimated regression function is displayed in Figure 8 along with approximate 95% confidence limits on the conditional expected PC1 score. Figure 8 indicates that harvesting fish at 20-22 mo would coincide with the peak of the linear stage of growth, around the point of inflection on the sigmoidal curve [51].

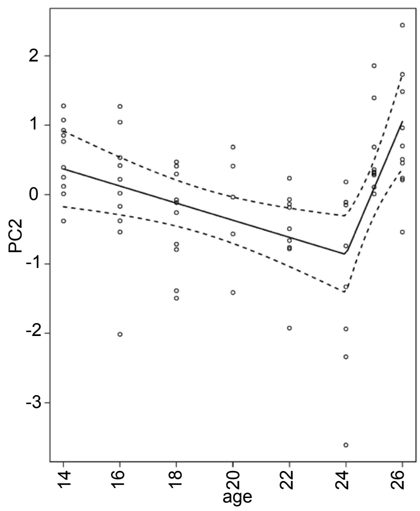
Fillets that were firmer, with less fat, and higher in moisture received higher PC2 scores. The scatter plot of PC2 scores versus age fitted with a segmented regression model is shown in Figure 9 along with the 95% Working Hotelling confidence band for the regression function. A lower PC2 score should not necessarily be interpreted as a lower quality fillet. More research is needed to determine if the fillet fat composition measured in 3.8-4.8 kg rainbow trout during the present study is acceptable to consumers; and if necessary, methods to achieve optimal fat composition and texture, such as optimization of feeding regime or diet composition, should be evaluated. In the meantime, the plot of PC2 scores with age provides a general basis for expected changes in fillet quality for maturing rainbow trout (Figure 9).



[a] The residual standard error was 0.3904 on 69 degrees of freedom.

[b] The asymptotic likelihood-based estimate of the value of Age at the inflection point is 20.2775 with a standard error of 0.4600. A 95% profile likelihood confidence interval for this value is (19.2683, 21.5386).

Figure 8: Estimated regression function of principal component 1 (PC1) versus age with approximate 95% confidence limits.



[a] The coefficient of determination for this fitted model was approximately 0.33.

[b]An estimate of the slope of the regression function for *age* ≤ 24 was -0.1232 (standard error, SE=0.0312) whereas that of *age* > 24 was 0.9578 (SE=0.1652).

[c]The breakpoint of *age* = 24 was subjectively fixed for this analysis and computed for an approximate 95% profile likelihood confidence interval estimate of (23.2, 24.3) [52].

Figure 9: A segmented regression model with Working-Hotelling confidence band with principal component 2 (PC2) regressed with age.

Conclusions

This research provides rainbow trout growth performance and fillet quality results that can be referenced for the development of recirculating system production plans and for selection of harvest endpoints that balance the requirements of fish farmers and food industry sectors. The results indicated that a commercially available strain of all-female rainbow trout can be cultured in freshwater recirculating systems at approximately 13°C, under constant lighting, and with around-the-clock feeding to a mean size of 5 kg in 24 mo. Principle components analysis indicated that a peak in growth performance variables, as a whole, occurred between 20-22 months. If growth performance was the only consideration for determination of best harvest endpoint, then it is reasonable to estimate that harvesting large rainbow trout at 20-22 mo of age or 3.8-4.8 kg in size might be optimal. However, selection of harvest endpoint may vary depending upon the end-product quality desired by value-chain sectors or relative to the form or preparation of the final product. More research is needed to interpret quality preferences for large rainbow trout fillets, particularly with increased fat content (up to 18%), as was measured during this study. Overall, the corresponding growth and fillet quality data provided by this research serve as an initial reference for industry, when culturing large rainbow trout under similar conditions.

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