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Evaluation of Growth Performance of a Genetically Characterized F1 Rainbow Trout, *Oncorhynchus mykiss* in a Recirculating Aquaculture System

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Abstract

The growth performance of a selected F1 rainbow trout genetically characterized as polymorphic with locus Omy207UoG (NA = 13, PIC = 0.891, H_E = 0.9003) was evaluated in a recirculating aquaculture system (RAS). A set of 157 rainbow trout of 37.8 g of mean body weight and 14.6 cm mean total length per tank, were introduced in each of six fish tank of 5 m³ connected to a recirculating aquaculture system (RAS). The trout was rearing during 317 days. Growth performance indicators and water quality were evaluated during the experiment. The genetically selected F1 rainbow trout showed a tendency towards homogeneity in growth performance along the culture period, reaching a mean total weight of 552.2 g. The length-weight relationship indicated an isometric growth (>3.0). This study presents the basis to establish a long-term marker-assisted selection program for rainbow trout culture in a subtropical region.

Keywords

Rainbow Trout, *Oncorhynchus mykiss*, Genetically Characterized, Growth, Performance, Water Quality

1. Introduction

Rainbow trout *O. mykiss* Walbaum, 1792 was introduced in Mexico in 1886 from South California, USA [1] [2]. Since then, the rainbow trout farming in

Mexico had generated important economic earnings, and is one of the fifth most important species for aquaculture in Mexico nowadays [3].

Mexican trout farming had experimented serious difficulties due to problems related to a deficient genetic quality of fingerlings and the presence of bacteria and virus infectious diseases, affecting the economic income. Bacterial and viral infections in imported eggs from other countries have been reported throughout the country, particularly the Infectious Pancreatic Necrotic Virus (IPNV). This disease was confirmed in 2000 after the introduction of eggs from USA and was identified as a Buhl strain of IPNV, a West Buxton (A1) serotype of the aquatic birnavirus serogroup A [4] and confirmed in asymptomatic rainbow trout in the State of Mexico as IPNV genogroup 1 VP2 residue (221) [5].

In 2006, the Instituto Nacional de la Pesca (INAPESCA) and the Universidad Autónoma Metropolitana (UAM) Campus Iztapalapa conducted a project to set up a Reference Center of Rainbow Trout in Mexico, with the main objective to produce genetically characterized fingerlings, free of IPNV and adapted to the subtropical areas of Mexico.

Information of rainbow trout founder strains genetically characterized and adapted to subtropical conditions under intensive aquaculture systems is scarce. Available information is restricted to comparison of growth performance under experimental conditions in different types of tanks, with commercial foods, in floating-cages, with experimental diets, in small bodies of water and evaluated under different water quality [6]-[20].

The goal of the present study was to evaluate the growth performance in a recirculating aquaculture system (RAS) of a genetically characterize a selected F1 rainbow trout free of IPNV.

2. Materials and Methods

2.1. Genetic Characterization of the F1 Population

The F1 founder population (n = 6,000) conformed by nine families was generated in September 2006 by means of perfect diallelic crosses of three genotypified ♀ (H089*296*526, H093*104*065, H093*126*322) and three ♂ (M078*049*639, M092*876*117, M093*363*327), rainbow trout brooders were certified as free of IPNV. The brooder stock (n = 150) was genetically and reproductively characterized elsewhere. A random sample of the F1 (n = 388) was genotyped with the most polymorphic locus resolved for the brooder stock Omy207UoG (NA = 12, H_E = 0.8571, which showed no allele dropout, nor stuttering, nor null alleles) in order to characterized and diagnosed the polymorphism of the F1.

Tissue samples from caudal fins of each one of the sampled organisms from the F1 were obtained and maintained 95% EtOH for further processing. Total DNA extraction (tDNA) was conducted according to the Dneasy™ Tissue (QUIAGEN®, USA) and Wizard SV Genomic DNA Purification System (Promega) protocols. Extracted tDNA were examined in 1.0% agarose gels (1X TAE, Tris-base, glacial acetic acid and 0.5M EDTA, pH 8.0) and electrophorized at 65 V/40 mA for 30 - 60 minutes. Visualization of tDNA was conducted via UV (320

nm) light and photo documented in a Multi Genius SyngeneMR apparatus, using the Gene Snap (ver 6.02.05) software.

To amplified locus Omy207UoG in F1 we used primers 5'-ACC CTA GTC ATT CAG TCA GG-3' and 5'-GAT CAC TGT GAT AGA CAT CG-3 from O'-Connell (1997), with the following amplification conditions: 94˚C –2 min (one cycle), and 35 cycles at 94˚C –15 sec, 54˚C –15 sec 72˚C –30 sec.

Capillar electrophoresis was carried out in the automated sequencer system ABI Prism 3100 Avant at Laboratorio Divisional de Biología Molecular de la División de Ciencias Biológicas de la Universidad Autónoma Metropolitana Unidad Iztapalapa.

The allelic sizes resolved were determined with the Data Collection V 2.0 software and in relation to the size standard LIZ-500 in GeneMapper V 3.5.

2.2. Genetic Characterization and Diversity of the F1

Genetic variability was assessed by determining the following parameters at the intra-specific level: 1) number of alleles observed (NA) and effective number of alleles ($EffeA$) were determined in Geno Dive 2.0b23 [21]; 2) polymorphic information content (PIC), allele frequencies and c) allele direct count (AC) observed and expected heterozygosity (H_O and H_E) were determined in Cervus 3.0.3 [22]. To test Hardy-Weinberg equilibrium (H-W) we used Fisher exact test as implemented in GENEPOP version 4.0.10 [23] and the F_{IS} estimates for each allele were computed following [24]. *P-values* for overall F_{IS} estimate were calculated by the Markov chain method using the following parameter: dememorization 10,000, 1000 batches and 10,000 iterations per batch.

2.3. Experimental Design for Growth Performance of F1

A total of 942 F1 genetically selected juveniles of rainbow trout were introduced in a RAS at Planta Experimental de Producción Acuícola (PExPA), located at 2 240 meters above sea level (masl) in Mexico City (CDMX), in six 5 m³ circular fish tanks connected to recirculating aquaculture system. In each fish tank 157 juveniles were introduced with an initial average total weight (TW) of 37.8 ± 12.05 g and an average total length (TL) of 14.6 ± 1.53 cm. The experiment has a duration of 317 days.

2.4. Feeding

The rainbow trout were fed with commercial Steelhead food (45% of protein and 16% of lipids; Silver Cup, Toluca, Mexico) with different pellets sizes according to the average TW and TL of fishes and were partitioned in two daily rations (10:00 and 16:00 h). The initial food rate was of 2.5% of the total biomass, decreasing according to the TW to 2%, 1.5% and finally 1%.

2.5. Length-Weight Relationship

The length-weight relationship was calculated by the formula TW = aTL^b, where: TW = total weight; TL = total length; a = is the intercept and b = the slope; the b

value was determinate through the regression of the logarithm of TW versus the logarithm of TL, and the slope of the linear adjustment is an estimation of b [25]. TW was measured with a digital balance (Ohaus, model Adventure with a precision of 0.01 g) and TL with a graduated ichthyometer (centimeters). Every three month biometrics samples were conducted (n = 180).

2.6. Growth Performance Indicators

At the end of the experiment the following indicators were evaluated: 1) initial TW (g), 2) final TW (g), 3) gain of TW (g), 4) daily growth rate (g/day), 5) yield (kg), 6) survival rate (%), 7) relative growth rate (RGR), 8) specific growth rate (SGR), 9) feed conversion ratio (FCR) and 10) condition factor (CF) [13] [17] [18] [26].

2.7. Water Quality

The water temperature and dissolved oxygen concentration was measured daily, with an oxymeter YSI55, model 55-12FT; pH with a digital pH meter Lauka model 8005, and nitrite (N- NO_2^-), nitrate (N- NO_3^-), and total ammonia nitrogen (TAN) (NH_3 + NH_4) were monitored weekly with a Hach Kit model DR 5000. The non-ionized ammonia (NH_3) was calculated in tables considering TAN values and were adjusted with water temperature and pH values [27].

2.8. Statistical Analysis

Data were emptied in an Excel spreadsheet (Microsoft Office 2003) and central tendency and dispersion statistic measures were calculating. The regression curves between TW vs. TL were elaborated with the same software. Data was adjusted to a potential curve and the equation model and correlation coefficient (R^2) were obtained. In order to determine the growth tendency (isometric or allometric) a t-student test was conducted [28]. The growth rate observed during the experimental period was plotted with the program box plot of SPSS Statistics (IBM, version 18, USA).

3. Results

3.1. Genetic Characterization and Diversity of the F1

The genetic diversity parameters determined for locus Omy207UoG in 388 F1 organisms of *O. mykiss* are summarized in Table 1. The analyzed F1 rainbow trout was resolved as highly polymorphic, with 13 alleles, an effective number of alleles of 9.914, a PIC value of 0.8905 and high observed and expected heterozygosity values (0.8273 and 0.9003, for H_O and H_E, respectively). Fisher exact test indicated an overall low but significant departure from H–W equilibrium at locus Omy207UoG (F_{IS} = 0.0812, P = 0.0001). The significant results were due to deficit of heterozygotes in at least three of the alleles in the F1 analyzed (Table 1). The probability values associated with the F (inbreeding)-coefficients for this sample and locus revealed significant values at alleles 118, 124 and 126. These

Table 1. Shows the resolved allele sizes, their frequencies and direct count number (AC), number of alleles per locus (*NA*), effective number of alleles, polymorphic information content (*PIC*), expected and observed heterozygosity (*HE* and *HO*) detected in locus Omy-207 for 388 F1 rainbow specimens studied.

Alleles	Allele Frequencies (AC)	F_{IS}	NA	EffA	PIC	H_O	H_E
106	0.0425 (33)	−0.0431					
112	0.0735 (57)	0.0735					
114	0.1508 (117)	0.0854					
116	0.0683 (53)	−0.0720					
118	0.0619 (48)	0.2462					
122	0.0258 (20)	−0.0252					
124	0.1198 (93)	0.4025					
126	0.0941 (73)	0.3207					
130	0.1443 (112)	−0.1674					
132	0.0374 (29)	0.1057					
134	0.1198 (93)	−0.0127					
136	0.0335 (26)	−0.0334					
140	0.0284 (22)	−0.0279					
Total		0.0812*	13	9.914	0.8905	0.8273	0.9003

deviations are common when non-random mating and suggest that the sampling design collects cohorts with high relatedness, as it is the case of this group of kin.

3.2. Growth Rate

The growth rate was expressed as increase of mean total weight (TW, g) with respect to the culture time in days. **Figure 1** showed the results obtained in a period of 317 days of culture.

The figure showed a substantial growth performance tendency along the experimental period, and the table size (300 g) was obtained after 247 days of culture.

3.3. Total Length-Total Weight Relationship

The equations obtained in the curve fit model showed an isometric growth (Pauly t-student: $P \leq 0.05$) with a slope of 3.045 and a higher adjusted with 96% with a significance of $P < 0.5$ (**Figure 2**). Higher dispersion of data was registered.

3.4. Growth Performance

The results of growth performance indicators are showed in **Table 2**, indicating an isometric growth during the experimental period.
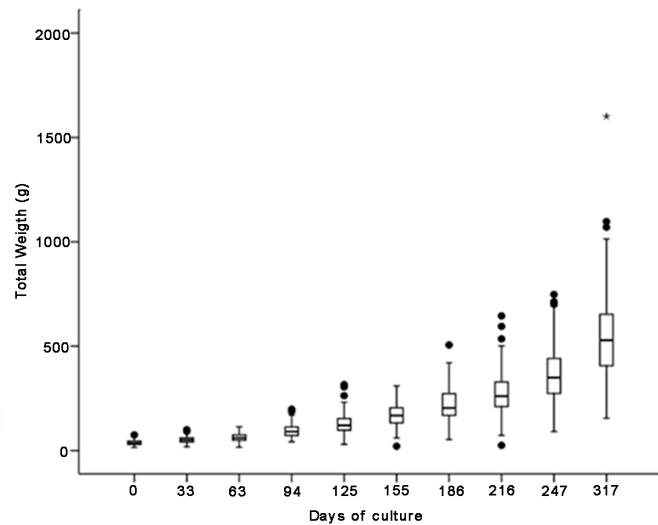
Figure 1. Boxplot graph of growth performance of the total weight (g) during the experimental period in the RAS. Initial fish average TL was 14.6 ± 1.53 cm and average TW, 37.8 ± 12.05 g.
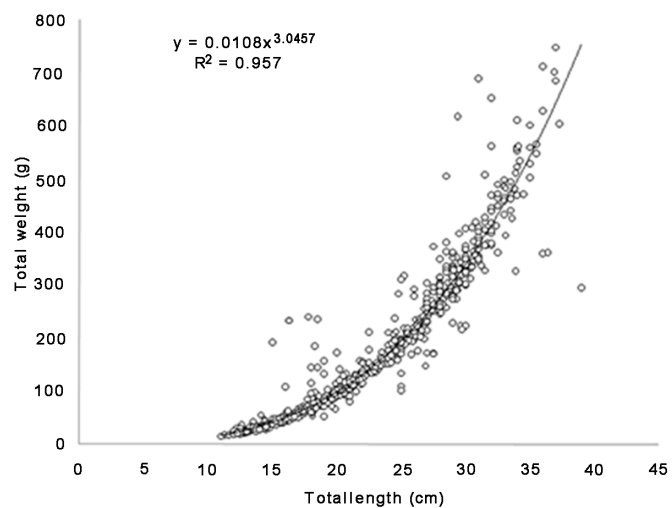
Figure 2. Lineal regression in the RAS. The curve fit model showed an isometric growth during the experimental period for fish with average initial TL 14.6 ± 1.53 cm and TW, 37.8 ± 12.05 g.

3.5 Water Quality

The results of physico-chemical analysis of the systems are showed in Table 3.

4. Discussion

4.1. Genetic Characterization and Diversity of the F1

This is the first study that reports on the genetic diversity of F1 strains of rainbow trout free of infectious hematopoietic necrosis virus (IHNV) and generated by perfect diallelic crosses of genotyped sires and dams with potential use for controlled aquaculture purposes in subtropical regions. Locus Omy207UoG exhibited extraordinary high levels of genetic heterogeneity in the studied stock.

Table 2. Average ± standard deviation results of growth performance indicators of F1 rainbow trout cultured in RAS (n = 6) during 317 days and maintained in RAS at 19.8˚C ± 1.4˚C and pH 8.1 ± 0.4.

Indicators	Average of each tank
Water volume (m^3)	5
Density (org./m^3)	26
Initial total weight (g)	37.8 ± 10.0
Final total weight (g)	552.2 ±112.0
Gain of total weight in g	514.0 ±103.0
Daily growth rate (g/day)	1.6
Initial total length (cm)	14.6 ± 1.0
Final total length (cm)	33.8 ± 2.0
Gain of total length (cm)	19.2 ± 1.0
Daily growth rate in cm	0.1 ± 0.0
Total yield (kg)	260.6 ± 58.0
Yield (kg/m^3)	6.0 ± 12.0
Survival rate (%)	78
Relative growth rate (%)	1 392.0 ± 176.0
Specific growth rate (% day^{-1})	0.9 ± 0.0
Feed conversion factor	1.2
Condition factor	1.4

Table 3. Results of physicochemical parameters registered in the RAS during the experimental period.

Parameters	Fish tanks
Temperature (˚C)	19.8 ± 1.4
Dissolved oxygen (mg/L)	6.5 ± 0.59
pH	8.1 ± 0.4
N- NO_3^- (mg/L)	17.8 ± 11.56
N- NO_2^- (mg/L)	0.247 ± 0.127
TAN (mg/L)	0.535 ± 0.269
N-NH_3 (mg/L)	0.021 ± 0.010

Slightly lower levels were reported for this locus in rainbow trout stocks derived from introductions to Western Australia (H_E = 0.65) [29]. However, they are high in comparison to what has been reported for rainbow trout for aquaculture purposes, using more microsatellite *loci* and different experimental designs in other countries. For instance, the used of nine microsatellites to characterize the genetic diversity of five strains of rainbow trout *Oncorhynchus mykiss* in USA

reported levels of gene diversity (equivalent to H_E) that range from 0.65 to 0.83 [30], moreover, the genetic variability and differentiation of 13-farmed strains of rainbow trout from northern and Eastern Europe were determined using 10 microsatellite *loci* and the reported H_E values ranged from 0.42 to 0.76 [31]. A study of genotyped farmed rainbow trout was made in Norway to identify the farm of origin for escaped organism captured in a Fjord, using 12 *loci* and found levels of variation that range from 0.65 to 0.79 (H_O) [32]. The high levels of diversity found in the F1 strain are related to the large sample sized analyzed. These genetic data present the basis to establish a long-term marker-assisted selection program for introduced rainbow trout in a subtropical region.

4.2. Growth Performance

In general, the F1 rainbow trout growth tendency in TW was similar to others registered in aquaculture intensive systems. Differences between densities and pond types (concrete and earth ponds) have been reported [7]. Likewise, it was demonstrated that in low densities, growth rate was higher, especially when bigger rainbow trout (98.2 ± 18.9 g and 22.5 ± 0.94 cm) were introduced in RAS in the PExPA, and under those conditions daily growth rate reached 3.6 g [6]. Similar growth rates were obtained with an initial total weight of 85.2 g, obtaining organisms with an average of 250 g in four months of culture [33]. Rainbow trout exhibits different growth rates according to fish density, type of culture system, water quality and food [8]-[13] [16]. The growth rates exhibited in this study in RAS was a response of the density. Although, water quality was maintained in optimal conditions and the commercial food and the rations covered the nutritional requirements of fishes.

4.3. Length-Weight Relationship

It has been found that in an intensive culture TW fluctuates as a potency of length. This expression can be applied better when a fish is measured and weighted in successive phases of their life history [25], this relationship is essential for numerous studies; for example, for the estimation of growth rate, the age structure in populations and other aspects related with population dynamics. The length-weight relationship is also used to calculate the condition factor (CF), and is useful in life history comparisons of certain species [34] [35]. In aquaculture work this relationship has practical approaches because it is possible to estimate weight from length data and vice versa and to calculate other parameters such as growth tendency in time, the biomass contained in ponds, fed rations and the condition factor. When a curve model is fitted, it is possible to estimate the slope value (b) and this value was of >3 indicating an isometric or allometric growth [25]. The results obtained in the experiment showed values from 3.04 indicating an isometric growth (t-student, $P < 0.05$), lineal correlation of data indicated values from 96% being highly significant and indicating an excellent fit model in spite of the dominance effect of some organisms of rainbow trout that growth faster, fed more and were dominant over other fishes.

4.4. Growth and Performance

A great number of scientific papers refer to rainbow trout growth performance data with comparative purposes. In this study were used such indicators. Some authors had expressed that it is only possible to compare some indicators due the differences propitiated between different experimental conditions such as rainbow trout sizes, densities, aquaculture system, food rate, water quality, time of culture and nutritional content of foods. However, for practical purposes the growth performance indicators used in this study were important for future comparisons reasons of the selected F1 rainbow trout.

The main performance indicators for comparison purposes are feed conversion ratio (FCR), condition factor (CF) and specific growth rate (SGR). An experiment focused for the evaluation growth and survival rate of rainbow trout under different types of tanks and densities showed an average of SGR from 1.52 to 1.81, and a FCR from 1.44 to 2.51 [7]. Likewise, with the use of two types of commercial food was reported a SGR between 1.37 and 2.18, FCR from 1.04 to 1.37 and survival rate up 95% [8]. In net cages culture was reported a SGR from 1.02 to 1.11, a FCR from 1.37 to 1.70 and a CF of 1.39 to 1.44 [9]. With the use of experimental vegetal protein diets a SGR 1.87 to 1.94, a FCR 1.06 to 1.12 was reported [10]. Evaluating experimental diets with different vitamin E quantities, registered a SGR from 1.87 to 1.94 and a FCR of 1.06 to 1.12 [11]. Others authors reported a SGR from 1.4 to 1.5 and a CF of 1.6. and a SGR from 0.30 to 1.21 and FC from 1.18 to 1.52 [13] [15].Testing the effects of natural pigments in rainbow trout, were reported a SGR from 1.3 to 1.6 and a FCR with an average of 1.5 [14], and in floating cage culture were recorder a SGR of 0.07 and 3.3 and a FCR from 1.1 to 1.3 [17], Yoshitomi [18] showed values of SGR from 3.0 to 3.1, with a survival rate around 99%. Finally, in 21 small water bodies with a rainbow trout cage farming system, SGR fluctuated from 1.1 to 2.5 reaching a daily growth rate from 1.4 to 9.0 g/day. The survival rate varied from 50% to 95% [19].

The data obtained in this research confirmed that values of SGR are within the limits reported in different investigations. In RAS daily growth rate reached 1.84 g/day. The FCR presented higher values but was similar to other experiments. Then FCR and the survival rate are considered acceptable. These indicators showed similar performance when compared to other world regions, confirming that F1 rainbow trout maintained along the experimental period exhibited a growth rate, FCR, SGR, CF and survival rate similar to those reported in different experimental conditions.

The yield of RAS was higher. This situation is common in fish culture activities because the fish table size depends on the carrying capacity and a lesser load means a higher weight had been suggested higher load manage to obtain economical profits, however a higher fish biomass requires an expense investment for waste elimination, to maintain toxic metabolites under control and good levels of dissolved oxygen and water quality [36].

Several advantages in this RAS are relevant allowed a better control of the system, less manpower and easy harvest. On the other hand, F1 rainbow trout had

excellent adaptation to this system such as has been showed for growth rate and performance.

4.5. Water Quality

The physico-chemical parameters registered in this study were found between the standard limits reported by [37]. However, the ponds water temperature was over 3°C of the optimum value; the dissolved oxygen (DO) was controlled by different mechanisms implemented in the RAS. There is a quite amount of information on water quality of this PExPA system [6] [14] [33] [38] and it has been probed in several occasions that water quality keeps acceptable levels allowing a good growth performance of rainbow trout. In general, DO was maintained through three main processes: 1) atmosphere interchange, 2) blower air injection and 3) injection by mean of Ventury tubes. Those mechanisms maintained high DO levels all the time, with saturation and oversaturation values that were enough to maintain appropriate conditions for rainbow trout culture, in the spite of high temperatures reached (up 19°C) and high altitude (2240 masl).

The behavior of the physico-chemical parameters was similar to other recirculating aquaculture systems, for example, the water temperature and DO. The nitrite and nitrate values can be considered higher due the nitrification processes are more acute. It was also noted a difference in TAN and toxic ammonia, with an increase tendency with respect the intensification of gain of total weight. However, these chemical species never reached sub-lethal or lethal levels as those reported for rainbow trout [27].

Acknowledgements

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