Prediction of survival rate in European white fish (*Coregonus lavaretus)* fry on three different feeding regimes

Ahmadi M. R.[1]; Mahmoudzadeh H.[2*]; Babaei M.; Shamsaei Mehrjand M.

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Abstract

During the early stages of white fish, survival rate is a very effective parameter on future fry's sustainability, especially when they are going to be released into the natural habitat for restocking. The aim of this study is to define relationships between morphological and functional traits and the survival rate of the European white fish fry (*Coregonus lavaretus*) when they were fed with different feeding regimes. One hundred and fifty newly hatched white fish fry stocked into nine rearing cages, under a flow-through rearing system, were distributed randomly in a natural pond, and nourished for twelve weeks with three kinds of food, the rotifer (*Brachionus plicatilis*), a compound Salmonidae commercial diet and a mixture of 50% of each , all treated in triplicates. The backward method of regression procedure was used for statistical analysis. Combinations of independent variables to multiple linear regression models were based on the changes in the R2 statistics and the F-value were produced by adding or deleting an independent variable. The five variable combinations (individual weight, length, total weight gain, total feeding rate, and feed efficiency) predicted the larval survival rate in multiple linear regression models and supported by partial correlation. This study revealed that; length and weight bear negative signs whenever they appear in these statistical relations. Also the higher the feeding rate, the survival rate increases accordingly regardless of the treatment effect. Finally, in mixed feed, predicting the survival rate is easier and more practical since the only parameters remaining in the equation are the length and weight of the fry. Perhaps this finding is because of the fact that the co feeding (rotifer and compound) can better satisfy the nutritional needs of these larvae than each feed alone.

Keywords:Statistical models, Survival rate, *Coregonus lavaretus*, Fry

1-Department of Health and Aquatic Diseases, Faculty of Veterinary Medicine, University of Tehran. Tehran, Iran.

2-Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran. Tehran, Iran.

3-Department of Genetics and Animal breeding, Animal Science Research Institute Karaj, Iran.

4-Department of Fisheries, College of Agriculture, Islamic Azad University, Research and Sciences Branch. Tehran, Iran.

*Corresponding author's email: mahmodzdh@ut.ac.ir

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Introduction

Using statistical models in predicting important traits in fish culture especially in larviculture does not have a long priority in literature, whereas knowing such information can be useful to make a clear strategy in preservation of resources in water bodies. Survival rate is an important factor for larviculture of an economic fish fry like European white fish (*Coregonus lavaretus*), therefore, in this study, the percentage of survivability was assigned as a dependent variable and growth parameters; individual weight, specific growth rate, length, weight gain, feeding rate, and feed efficiency, as independent variables. Rotifers are highly suitable feed due to their eurythermal and euryhaline characteristics, which allow them to tolerate wide environmental fluctuations (Hagiwara et al., 2001a, b; Kotani et al., 2006; Hagiwara et al., 2007). However this organism can survive in fresh water for at least two hours (Lim et al., 2003), and have been used for feeding larvae of Japanese ornamental carp *(Cyprinus carpio)*, bait fish *(Carassius sp.) (*Lubzens et al., 1987*)*, tilapia *(Oreochromis spilurus) (*Cruz and James, 1989) and gudgeon *(Gobio gobio L.) (*Kestemont and Awaiss, 1989). A compound Salmonidae commercial ration was used as dry food, since the availability, handling, and cost of such diets makes them more popular to feed (Rösch and Appelbaum, 1985; Luczynski et al., 1986; Dabrowski and Poczyczynski, 1988; Harris and Hulsman, 1991 and Ruohonen et al., 2003) and finally a mixture of 50% of each feed was administered as mixed diet. The above variables were evaluated via statistical procedures. These feeds could have intrinsically evident differences in the culture of this fry; therefore, each would demonstrate its role in a statistical model for prediction of survivability and other growth parameters. According to our knowledge there are no data available for the morphologically similar larvae of this fish, therefore this experiment presents a new approach in fish larviculture for the first time, using statistical models for the prediction of survival rate via growth traits in *Coregonus lavaretus.*

Materials and methods

Location and source of fish

European white fish (*C. lavaretus*) brood stocks were collected from a wild population in

mid winter from the Amir Kabir reservoir (the only residence of this fish in the country) in their spawning season. They were transferred to the propagation centre, where fish adaptation and adjustment to the new environment was accomplished for the reproduction process and fry were produced via artificial propagation (Huet, 1986 ; Shepherd and Bromage, 1992).

Experimental set-up

Cages and water characteristics

Nine rearing cages ($30\times30\times40$ cm^3) with 250 µm pore size on metal skeletons were used (three per treatment). They were all

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kept under a flow-through rearing system, flouted by means of plastic pieces, and distributed randomly in a natural pond with supplied water from hyporheic-zone river to ensure uniform Physico-chemicalphysic chemical conditions. The cages were positioned in appropriate distances where the possible leftovers after the feeding were transferred to the lower sections. During the experiment, water temperature was monitored twice daily at 08.00 and 16.00 hours and ranged between 10.2 to 14.6°C. Dissolved oxygen and pH was measured daily and varied from 9.4 to11.0 mgL-1 and 7.3 to 7.8 respectively. Total ammonia-N and nitrite-N were determined weekly, where they were quantified as negligible.

Diet

In order to have a more reliable study and collect enough data to control and find the influence of effective factors involved, only feed as a variable was used to determine its implement on survival rate at this stage of fry growth while all environmental parameters like water quality, fry density and feeding rate were almost constant, so three different diets were used. As a live feed we used marine rotifer (*Brachionus plicatilis*) which is considered as the most important organism for use in larviculture, the rotifer, *B. plicatilis* mass-produced according to Lavens and Sorgeloos, (1996) as live feed for treatment A. The required mass of rotifers were harvested using 150µm plankton net each day from stock tanks after 7 days of culture, a satisfactory size for *C. lavaretus* fry initial feeding for first two weeks, followed by 200-300 µm for the rest of the experiment. They were thoroughly washed with tap water prior to feeding. For the artificial diet, a compound commercially available micro particulate which was locally produced as Salmonidae commercial diet (Chine Feed Manufacturing Co., Tehran, Iran) was used in the control treatment (B), and was selected on the basis of widespread availability in the country with 48% crude protein, 4% crude fibre, 12% crude fat, 11% crude ash and 10% moisture on DM basis. The dry pellet crumbles were sieved and particles that passed through a standard sieve (100-200 µm for the first two weeks followed by 200-300 µm for the rest of the experiment) were used for feeding. A mixture of 50% of each of the above diets was prepared and used as mixed diet for treatment C. In order to prevent cold shock, rotifers were acclimated gradually to 14 ºC by keeping them in 10 L. containers before their transfer to the fry's cages, where they reproduced slowly at this temperature maintaining a constant density, thus assuring a continuous source.

Fry, feeding, sampling, and biometry

One hundred and fifty pieces of newly hatched white fish fry were randomly stocked into each cage. The first feeding phase had begun about five hours before they started to eat, when the yolk sacs were still not completely absorbed. Food was added on a daily basis and the rate of exogenous feeding set initially as 7.5% weight of the live fry’s body mass in each

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cage for all treatments, where they were fed every two hours, between 7:00 to 21:00 hours during the day (Troschel and Rösch, 1991). Every week samples of fry (6 specimens, 18 fry per treatment) were taken randomly from each cage. To minimize stress, they were anesthetised with 80 mg L-1 Tricaine methane sulfonate (methyl-amino benzoate, MS222) before feeding on the morning of the sampling day. Their total length measured nearly 0.1 mm using a dial caliper and their individual wet weight was taken using a Mettler balance (0.1 mg sensitivity). All fry returned to their correspondent cages after the biometry. dead fish were removed and recorded daily. During 12 weeks of experiment, required feed intake was carefully calculated based on their expected growth rate in this stage (Koskela, 1992), and it was adjusted after weekly weighing the fish fry samples. In every feeding, measured feed was diluted with 5.0-10.0 ml. of water to achieve an approximately equal distribution and were transferred to the rearing cages via 10.0 ml syringe. Specific growth rate (SGR), weight gain (WG) and length increment (Leng. Inc.), as well as feed efficiency (FE) and survival rate (Surv. Rate) were calculated for all treatments according to the following formulae:

SGR = 100(ln Wt.final-ln Wt.initial) / Days

WG = Wt.final – 180 Wt.initial.

Leng. Inc. = length final – length initial.

F.E. = Weight gain /Feed consumed

Surv. Rate = 100(live fry – dead fry) / Live fry

The survival rate was determined weekly in each cage. In order to eliminate adaptation impacts, stress sensitivity and other irregularities, mortalities were recorded from the second week of the study thus the experiment was divided into two parts of a four-week and a seven-week period.

Data analysis

To define relationships between morphological and functional traits and the survival rate, the linear multivariate regression and partial correlation for metrical data was used. In order to construct appropriate models, analysis of variance was performed to determine the significance of each model. In these models the survival rate was defined as the dependent variable (Y) and the other traits including; individual weight (X1), specific growth rate (X2), length (X3), length increment (X4), individual weight gain (X5), total weight gain (X6), total feeding rate (X7), and feed efficiency (X8), as independent variables. In this study, the backward method of regression procedure was used. Combinations of independent variables to multivariate regression models were based on the changes in the R2 statistic and in the F-value that were produced by adding or deleting an independent variable. The independent variables were entered into the models at $P<0.05$ and excluded at $P<0.10$ levels. Additionally, F-values and R2 values were used to evaluate the fit of the models. Data analyses were separately executed in two parts for each treatment; the first part was comprised of data from the first four

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weeks of the fry's life and in the second part, observations from their last seven weeks were included. Using the correlation and regression results, a path analysis was conducted to separate direct and indirect effects of independent variables on survival rates. Standardised regression coefficients (ß) are direct effects of each predictor on the dependent variable and the indirect effects obtained via multiplying the direct effect by bivariate Pearson correlation coefficients for each path. All significant partial correlations were plotted to visually inspect scattered spots. SPSS 15.0 was applied for data analysis.

Results

Treatment A

Growth and Survival of fry reared for first four weeks

In this period a multivariate linear regression model was formulated with major functional and morphological traits as independent variables, the five variables predicted the fry survival with a high statistical significance ($P<0.01$), (equation-1):

$Y=90.518 –0.269X1–1.291X3–0.003X6+0.075X7 + 0.130X 8.$ ($R2 = 0.989$) (1)

In this model the individual weight, length and total weight gain inversely affected the survival rate, whereas total feeding rate and feed efficiency's effects were positive. Direct and indirect effects in path analysis are presented in Table 1. Total feeding rate and feed efficiency owned the highest and lowest direct effects on survival, respectively.

In addition, the highest indirect effect was for individual weight via total feeding rate and the lowest one for individual weight via feed efficiency. Partial relation, scattered dots between survival rate and the independent variables are shown in Fig. 1(a-e).

Table 1: Direct and indirect affects of independent traits for treatment A (live feed-fed fry) on survival rate in first four weeks of life

Independent Traits	Direct effect	Indirect effect via				
		Indv.Wt (g.)	**Leng.**(mm)	**Total WG**(g)	**Total F.I.** (g.)	**F.E.**(%)
Indv.Wt (g.)	-3.932	----	-3.707	-1.802	8.086	0.542
Leng.(mm)	-3.826	-3.810	----	-1.774	8.085	0.662
Total WG (g)	-1.818	-3.897	-3.734	----	8.037	0.596
Total F.I.(g.)	8.094	-3.928	-3.707	-1.805	----	0.544
F.E. (%)	0.833	-2.559	-3.042	-1.301	5.293	----

Indv.Wt. Individual Weight; Leng.: Length; Total WG: Total Weight Gain; Total F.I.: Total Feed Intake; F.E.: Feed Efficiency.

Growth and Survival of fry reared for the last seven weeks

The results were somehow different in this treatment for the next seven weeks of the experiment where a multivariate linear regression model was formulated, the three variables predicted the fry survival with a high statistical significance ($P<0.01$), (equation 2): $Y=113.395–0.350X2–0.978X3+ 0.001X6.$ ($R2 = 0.564$).

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(2) In comparison to the results of the first four weeks of the experiment, in multivariate regression analysis of the last seven weeks, specific growth rate, length, and total weight gain entered the final model, while individual weight was removed. Direct and indirect effects in path analysis are presented in Table 2. The highest and lowest direct effects were for length and total weight gain respectively. In addition, the highest indirect effect was for specific growth rate via length and the lowest one for specific growth rate via total weight gain. Fig. 2 (a-c) shows Partial relation, scattered dots between independent variables and the survival rate.

Table 2: Direct and indirect affects of independent traits for treatment A (live feed-fed fry) on survival rate in last seven weeks of the experiment

Independent Traits	Direct effect	Indirect effect via		
		SGR (%)	**Leng.**(mm)	**Total WG** (g)
SGR (%)	-1.396	----	2.369	-0.449
Leng.(mm)	-2.031	1.168	----	1.042
Total WG (g)	1.366	0.459	-2.160	----

SGR: Specific Growth Rate; Leng.: Length; Total WG: Total Weight Gain.

Treatment B

Growth and Survival of fry reared for the first four weeks

To formulate a multivariate linear regression model, major functional and morphological traits were used and five variables predicted the fry survival with a high statistical significance ($P<0.01$), (equation 3):

$Y=86.696–2.627X1+7.568X2+404X7-1.201X8$. ($R2=0.981$).

(3) In this model individual weight and feed efficiency inversely affected the survival rate whereas specific growth rate and total feeding rate's effects were positive. Direct and indirect effects in path analysis are presented in Table 3. The highest and lowest direct effects were for individual weight and specific growth rate respectively. In addition, the highest indirect effect was for individual weight via total feed intake and the lowest one for total feed intake via specific growth rate. Partial relation, scattered dots between the independent variables and survival rate are shown in Fig. 3(a-d).

Table 3: Direct and indirect effects of independent traits for treatment B (compound feed-fed fry) on survival rate in first four weeks of life

Independent Traits	Direct effect	Indirect effect via			
		Indv.Wt(g.)	SGR (%)	Total F.I.(g.)	F.E. (%)
Indv.Wt(g.)	-3.638	----	2.028	3.167	-2.378
SGR (%)	2.538	-2.906	----	2.491	-2.963
Total F.I.(g.)	3.189	-3.612	1.982	----	-2.316
F.E. (%)	-2.966	-2.917	2.535	2.489	----

Indv.Wt.: Individual Weight; SGR: Specific Growth Rate; Total F.I.: Total Feed Intake; F.E.: Feed Efficiency.

Growth and Survival of fry reared for the last seven weeks

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In this period, the four variables predicted the fry survival with a high statistical significance (P<0.01), (equation 4):

Y=65.907–0.499X1+0.967X4+ 0.089X7+0.071X8. (R2=0.959)

(4) The results show individual weight, length increment, total feeding rate, and feed efficiency entered the final model, while the other measured factors were removed. Direct and indirect effects in path analysis are presented in Table 4. The highest and lowest direct effects were for total feeding rate and feed efficiency respectively. However, the highest indirect effect was for individual weight via total feeding rate and the lowest one for individual weight and total feed intake both via feed efficiency. In Fig. 4 (a-d), Partial relation, scattered dots between survival rate and the independent variables are shown.

Table 4: Direct and indirect effects of independent traits for treatment B (compound feed-fed fry) on survival rate in last seven weeks of the experiment

Independent Traits	Direct effect	Indirect effect via			
		Indv.Wt(g.)	**Leng.Inc.(**mm)	**Total F.I.** (g.)	**F.E.** (%)
Indv.Wt(g.)	-9.017	----	-0.205	9.089	-0.107
Leng.Inc.(mm)	0.283	6.528	----	-6.632	0.134
Total F.I.(g.)	9.098	-9.008	-0.206	----	-0.107
F.E. (%)	0.142	5.131	0.208	-6.887	----

Indv.Wt.: Individual Weight; Leng. Inc.: Length Increment; Total F.I.: Total Feed Intake; F.E.: Feed Efficiency

Treatment C

Growth and Survival of fry reared for the first four weeks

In this part, only two variables predicted the fry survival with a high statistical significance (P<0.01).

Y=92.670+0.569X1–1.806X3. (R2=0.727)

(5) In this model individual weight positively and length negatively affected the survival rate. Direct and indirect effects in path analysis are presented in Table 5. The highest and lowest direct effects were for individual weight and length respectively. In addition, the highest indirect effect was for individual weight via length and the lowest one for length via individual weight. Partial relation, scattered dots between survival rate and the independent variables are shown in Fig. 5 (a and b).

Table 5: Direct and indirect effects of independent traits for treatment C (Mixed feed-fed fry) on survival rate in first four weeks of life

Independent Traits	Direct effect	Indirect effect via	
		Indv.Wt (g.)	**Leng.**(mm)
Indv.Wt(g.)	2.149	----	-2.747
Leng.(mm)	-2.772	2.125	----

Indv.Wt: Individual Weight; Leng.: Length.

Growth and Survival of fry reared for the last seven weeks

In this treatment, only one variable predicted the fry survival with a high

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statistical significance (P<0.01), (equation 6):

Y=91.907–0.290X3. (R2=0.444)

(6) Therefore, only the length entered the final model, while the other parameters were removed. The direct effect of the independent trait on survival rate in the last seven weeks of the experiment was -0.666. Fig. 6 shows Partial relation, scattered dots between survival rate and the independent variable of length.

Discussion

The survival rate of larvae reared to release in natural water resources is a vital criterion, since otherwise they cannot have enough strength and solidity to sustain in their environment and attain desirable market size. After depletion of the yolk, larval growth and survival will depend on the success of starter feed to uphold its nutritional needs yet under larviculture conditions, problems with starter feeding in earlier stages are the major sources of mortality (Puvanendran and Brown, 1999). Numerous researches disclose that the bigger and heavier fry show higher survivability (Parra and Yúfera, 2000 -Enz et al., 2001 and Mahmoudzadeh et al., 2009) but considering the fry population, these individuals are fewer in number than the rest of the fry. In other words, fry with high ability to survive appear differently in their community, i.e. the smaller and lighter fry constitute higher proportion of the population. In general, in our study, difference in various stages of the experiment, affect the survival prediction differently and do not follow a steady trend in separate treatments. In the first four weeks, the impact of length with negative coefficient in both live and mixed feed treatments are similar and with comparable results. In the last seven weeks, the negative effect of fry length persists as a strong predictor of survival rate, but its influence is much less in mixed diet treatment. For the rotifer-fed treatment in both stages of the experiment, individual weight shows a negative correlation with survival of the fry so the combination of the length and weight can be used as powerful 337 l prediction indices. In this treatment, other traits appear much less important in the multivariate linear regression equations and thus exhibit less functionality as significant predictors. The same situation holds true for the mixed diet treatment with a negative coefficient for length in survival prediction equation. In compound feed treatment, individual weight contributes in the multivariate linear regression model as a negatively correlated trait with survival rate throughout the experiment. In all treatments, total feeding rate exhibits its profound positive impact on the survival rate. In direct relationships it can be inferred that the higher the feeding rate, the lower the feed efficiency, a fundamental base in fish larviculture. Examining the indirect effects, in most cases, weight gain is controlled via feeding rate. In the Partial relationship between survival rate and length in all treatments and both phases of the

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experiment, it can be said that in general, whenever length shows its influence, its negative sign indicates that smaller fry live longer. Also in mixed feed treatment survival rate shows negative relation with weight except in the first four weeks. This section of statistical analysis again reveals that increasing share of food in different treatments, causes higher survival ability.

In rearing of *Coregonus lavaretus* larvae, there were different factors involved in its survival and sustainability and as our results, sometimes they have contradictory messages, but it can be concluded that;

1. Length and weight bear negative signs whenever they appear in these statistical relations, and since survival ability in a population is a numerical trait, thus small size larvae with lower weight showed more liveability.

2. The more feeding rate, the survival rate increases accordingly regardless of the treatment effect.

3. In mixed feed, predicting the survival rate is easier and more practical since the only parameters remaining in the equation are the length and weight of the fry. Perhaps this finding is because of the fact that the co-feeding (rotifer and compound) can better satisfy the nutritional needs of this larvae than each feed alone. As Fletcher et al. (2007) documented that combining live and manufactured feed, from an early developmental stage has been shown to greatly improve the growth and survival as well as the cost.

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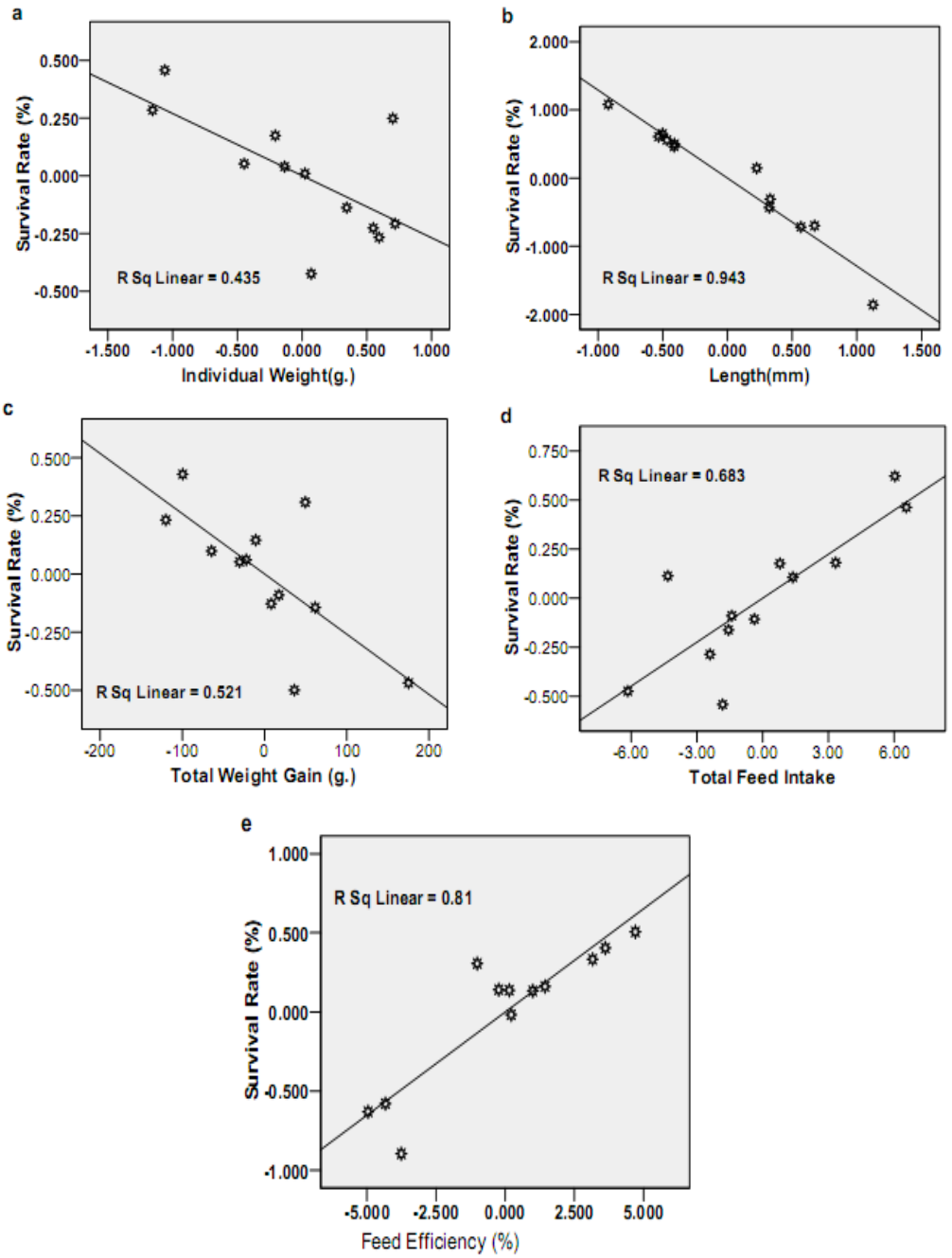


Figure 1: Scatter plots for white fish fry on rotifer base diet in first four weeks of experiment. Data used in the multiple linear regression models demonstrating the partial relationship between survival rates and a growth trait, each display excludes effects of all other traits.

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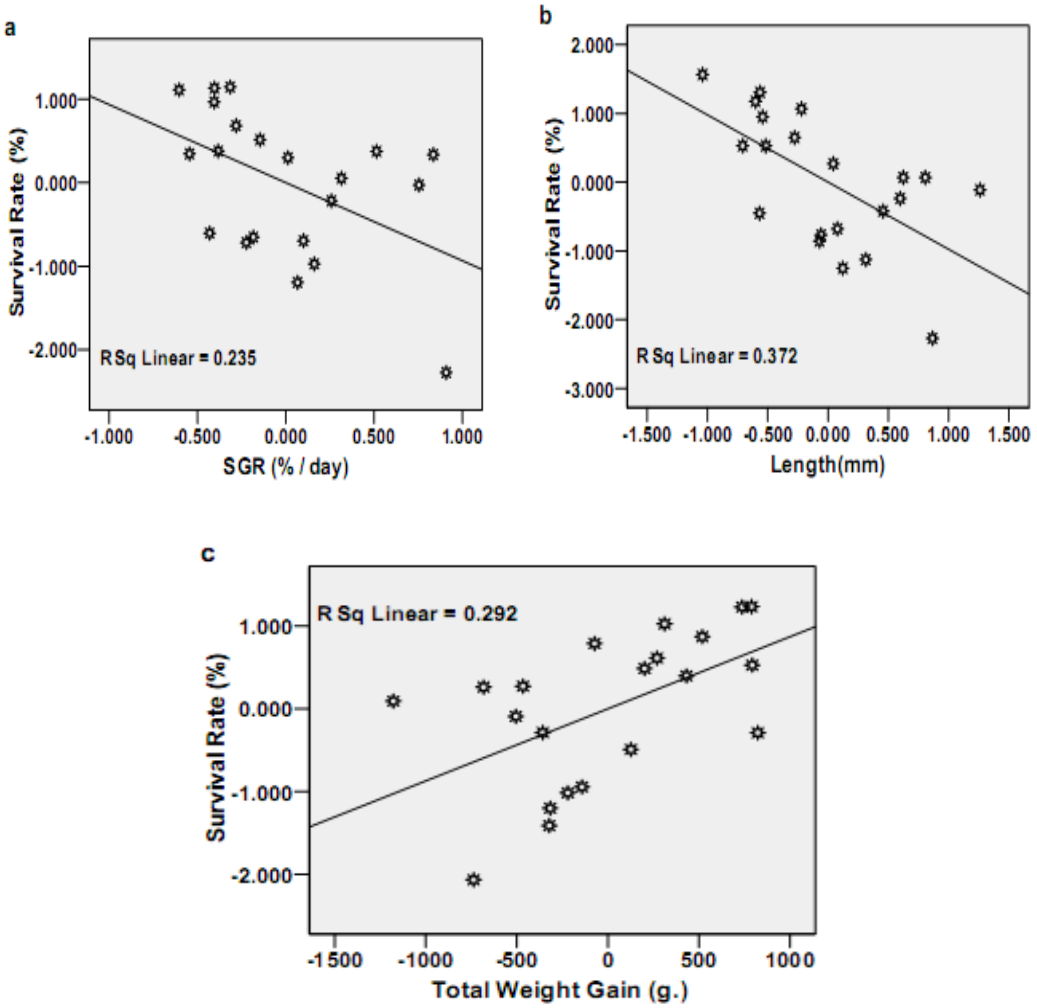


Figure 2: Scatter plots for white fish fry on rotifer base diet in last seven weeks of experiment. Data used in the multiple linear regression models demonstrating the partial relationship between survival rates and a growth trait, each display excludes effects of all other traits.

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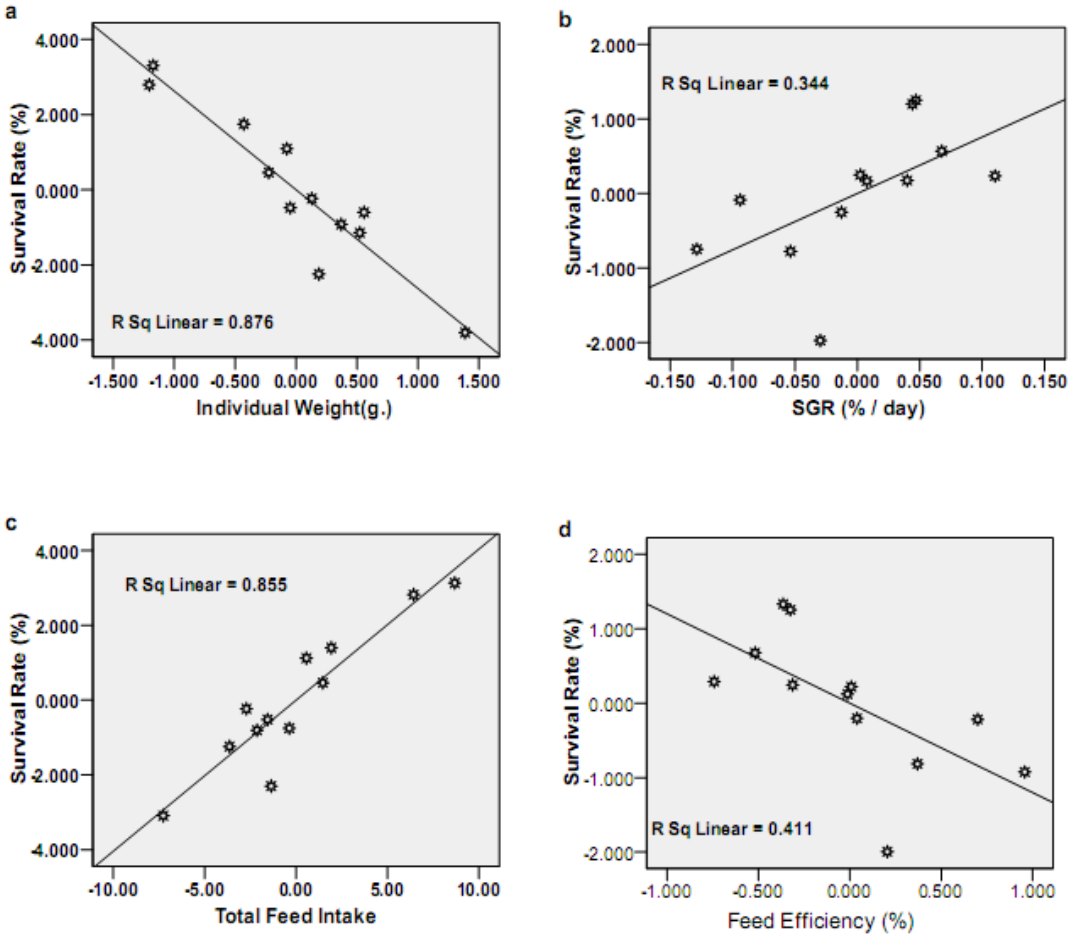


Figure 3: Scatter plots for white fish fry on compound feed in first four weeks of experiment. Data used in the multiple linear regression models demonstrating the partial relationship between survival rates and a growth trait, each display excludes effects

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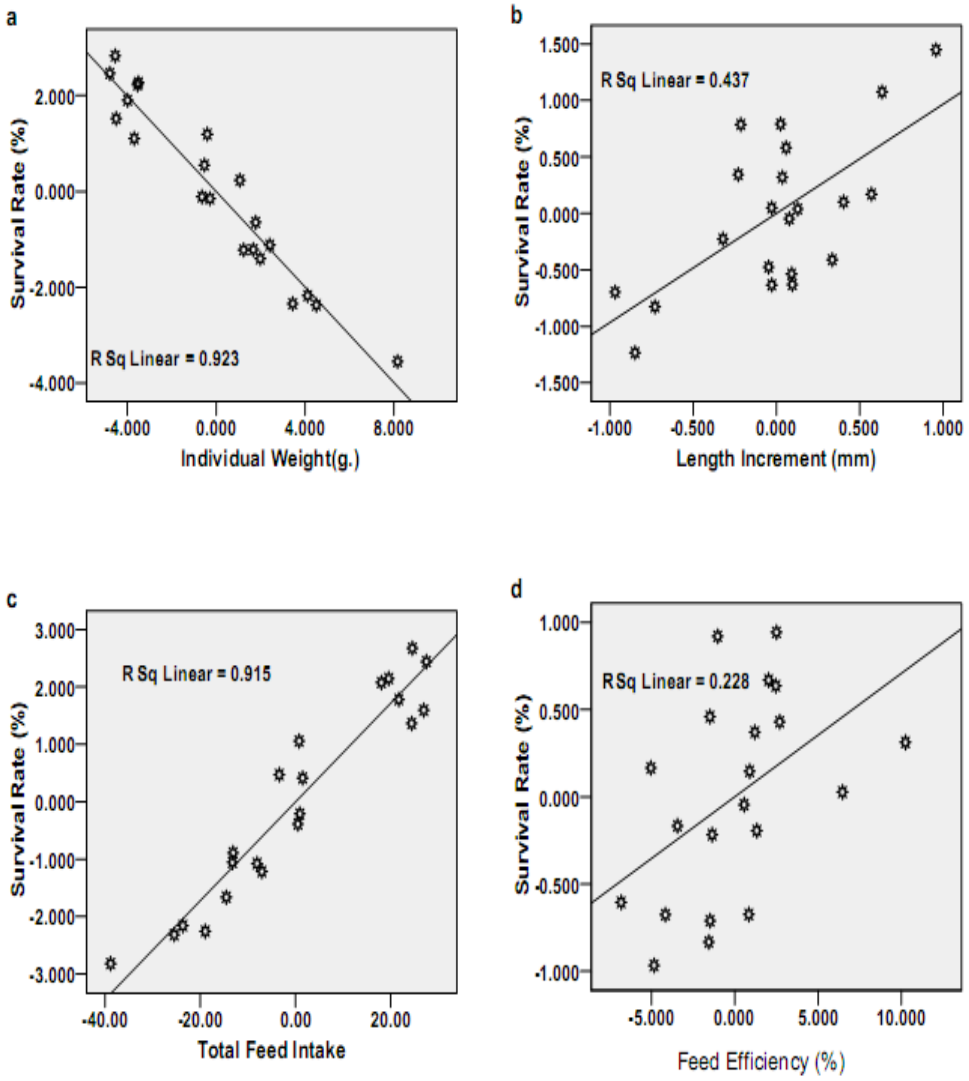


Figure 4: Scatter plots for white fish fry on compound feed in last seven weeks of experiment. Data used in the multiple linear regression models demonstrating the partial relationship between survival rates and a growth trait, each display excludes effects of all other traits.

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[DOR: 20.1001.1.15622916.2011.10.2.1.2]

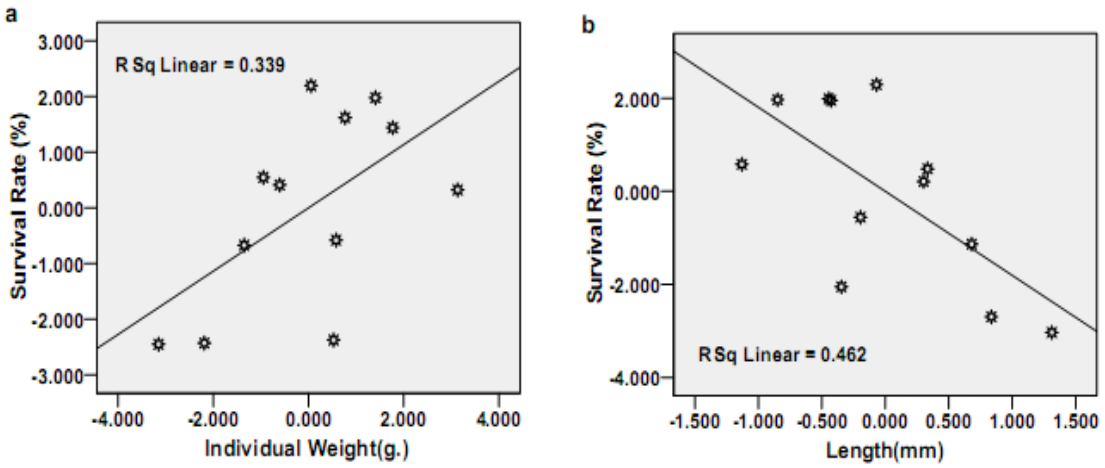


Figure 5: Scatter plots for white fish fry on mixed diet in first four weeks of experiment. Data used in the multiple linear regression models demonstrating the partial relationship between survival rates and a growth trait, each display excludes effects of all other traits.

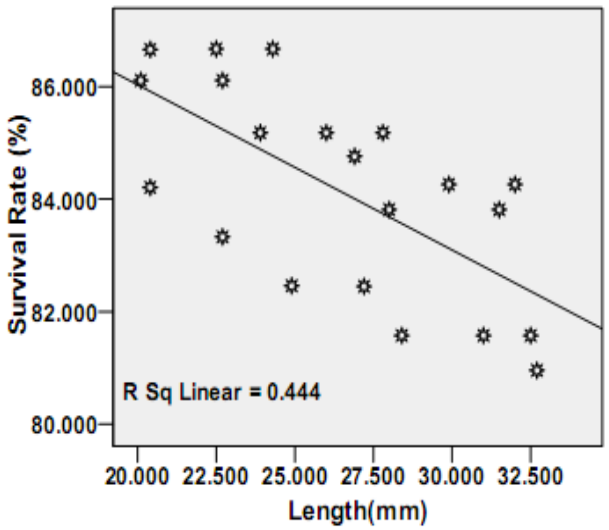


Figure 6: Scatter plots for white fish fry on mixed diet in last seven weeks of experiment. Data used in the multiple linear regression models demonstrating the partial relationship between survival rates and a growth trait, each display excludes

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[DOR: 20.1001.1.15622916.2011.10.2.1.2]