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Estimation of repeatability and phenotypic correlation of reproductive traits in zebrafish *Danio rerio*

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ABSTRACT

This study was conducted to estimate the repeatability and phenotypic correlation of reproductive traits of zebrafish *Danio rerio*, in captive condition. Breeding trials were conducted on 10 breeding pairs (2 male:1 female). The data on fecundity, fertility, hatchability and survival rate were recorded. The repeatability values were computed as a measure of consistency using the intra-class correlation coefficient. The repeatability estimates for fecundity, fertility, hatchability of total eggs, hatchability of fertilised eggs, survival rate of total eggs and survival rate of larvae were 0.701, 0.406, 0.336, 0.049, 0.295 and 0.314, respectively. Most of the reproductive traits analysed had moderate repeatability values barring the hatchability of fertilised eggs. The phenotypic correlation was calculated using the Pearson correlation method. The phenotypic correlations of fertility with hatchability of fertilised eggs; hatchability of total eggs; survival rate of total eggs and survival rate of larvae were highly significant ($p \leq 0.01$). This study provided valuable information on the consistency of reproductive parameters which would serve as model for conducting research in other fish species.

Keywords: Genetic parameters, Phenotypic correlation, Repeatability, Reproductive traits, Zebrafish

Introduction

Knowledge of genetic and phenotypic parameters is required for planning efficient breeding programs in aquaculture practices. Heritability and repeatability are the two important genetic parameters which are used in breeding programme (Lush, 1937; Roman *et al*., 2000). Repeatability is the measurement of the similarity between the records (two or more) of the same trait which is taken at different times but from the same individual of a population (Falconer and Mackay, 1996). It is a very useful tool for quantifying or measuring the level of magnitude to which an individual's performance remains in harmony or consistent over a particular period of time (Bennett 1987; Lessells and Boag, 1987; Boake, 1989; Arnold, 1994; Hayes and Jenkins, 1997). The repeatability can be used to evaluate the intensity of the selected trait to be inherited from the parents to the progeny. It is also an important parameter for designing the selection programmes as it measures the variability between the marked individuals which defines the spectrum of performance of the selected traits (Tribudi and Prihandini, 2019). The most important reasons for estimating or recording repeating abilities of breeding traits or behavioural traits in the teleost population are that they set an upper limit for the heritability of that essential trait which combines with the variation in reproduction among the individuals and can give new insights into the life history to be evolved. Moreover, the most basic and important contribution of the genetic improvement is to achieve sustainable aquaculture which has been hampered by the deficiency of basic precise information on the genetic parameters such as the heritability, genetic correlation and repeatability of the traits in the fish or fish population.

Zebrafish (*Danio rerio*) has become an important model organism for biological research in areas of diseases as well as for regenerative and toxicity studies (Khan and Alhewairini, 2018). This species has also been used to study thyroid development (Wan *et al*., 2023) and the pathway in Alzheimer's disease (Kiper and Freeman, 2022). Raising and maintaining zebrafish is also popular among aqua-culturists and ornamental breeders. Despite the fact that zebrafish plays a very important role as model animal in biomedical research and as pet in ornamental industry, studies on the genetic parameters of this species is very scanty especially no repeatability of reproductive traits have been studied so far. Therefore, this study was carried out to estimate the repeatability of reproductive traits of zebrafish in captivity.

Materials and methods

Collection and maintenance of zebrafish

In total, 150 adult zebrafishes were obtained from M/s. Tharun Fish Farm, Manimangalam near Chennai, Tamil Nadu. The fishes were packed in oxygen-filled polythene bags and transported to the wet laboratory, of Institute of Fisheries Postgraduate Studies, Chennai. After reaching the laboratory, zebrafishes were kept in glass aquaria for acclimatisation for a week. After acclimatisation, male and female fishes were identified based on physical appearance (Schilling, 2002), segregated and kept separately in glass tanks (45 x 28 x 30 cm). Before stocking, the glass tanks were cleaned, dried and then filled with dechlorinated water. Fishes were fed *ad libitum* with brine shrimp nauplii (SLA-Salt Lake Artemia) and micro pellet feed (Protein 50%; Fat > 6%; Fibre 4%; Moisture 9%) throughout the study period.

Zebrafish breeding

After a week of acclimatisation, males and females having similar weight (0.45 to 0.50 g) and length (1.75 to 2.0 cm) were selected and kept separately in partitioned glass tanks (25 x 12 x 17 cm) for pairing. The breeding trials were conducted in 2:1 (male: female) ratio. A total of ten pairs were used for breeding experiments. Each pair was allowed for breeding 11 times during the period of study. The duration between successive breeding trials was one week. Breeding was conducted in glass tanks (19 x 12 x 17 cm) and the bottom of the breeding tanks was provided with a mesh (1.0 mm to 1.2 mm mesh size) for separating the zebrafish and eggs to prevent the eggs being eaten by parents. The spent fishes were taken out of the breeding tanks and kept in respective partitioned tanks separately. The eggs which settled at the bottom of the tanks were collected using a strainer having a mesh size of 1 µm. The eggs were given a dip treatment in methylene blue at a concentration of 3 ppm in 1 l water and then transferred to petri-dishes (15 mm height) for hatching and for further observations.

Reproductive traits

The reproductive traits such as fecundity, fertility, hatchability and survival rate were recorded from 6 to 110 h (5 days) post-fertilisation (hpf). The fecundity was counted manually without disturbing the eggs. Fertilisation of the eggs was observed between 18 to 24 h after spawning. The fertilised and unfertilised eggs were counted and monitored for hatching from 48 to 72 hpf. The hatched larvae were kept in petri-dishes for observing the survival until the yolk was completely absorbed and the larvae were ready for natural food. The survival rates were calculated. for eggs and the larvae, which were treated as two sub-traits. The optimum water quality parameters required for zebrafish breeding were maintained throughout the study period (Table 1).

Table 1. Water quality parameters during the study period

Parameters	Mean	Range
Temperature (°C)	26.25	25-27.5
pH value	7.25	6.5-8.0
Oxygen (mg l^{-1})	5.655	4.56-6.75
Alkalinity (as mg $CaCO_3$ l^{-1})	180	150-210
Hardness (as mg $CaCO_3$ l^{-1})	125	100-150
Ammonia (mg l^{-1})	0.005	0.01-0.015
NO_2 Nitrite (mg l^{-1})	0.14	0.12-0.16
NO_3 Nitrate (mg l^{-1})	45.7	40.8-50.6

Data analysis

Fertilisation rate, hatchability and survival rate were calculated using standard formulae as per Pertiwi *et al*. (2018) and Anita and Dewi (2020). Multiple data on the reproductive traits (fecundity, fertility, hatchability and survival rate) were recorded and systemised in an MS Excel sheet. The repeatability was computed as intraclass correlation as a statistical measure of multiple records from the same set of brood fishes (Sokal and Rohlt, 1981; Lessels and Boag, 1987). The method for finding the intraclass correlation was based on the analysis of variance and the estimation of variance components using a two-way mixed model (Bartko *et al.,* 1966).

$$Y_{ijk} = \mu + b_i + p_j + (bp)_{ij} + e_{ijk}$$

where, μ = Overall effect common to all observations; b_i = Random variable common to the i^{th} breeding, p_j = Fixed effect common to the j^{th} pair, $(bp)_{ij}$ = Interaction term, a random variable for observation (i,j) and e_{ijk} = Error associated with observations (i,j); b_i, $(bp)_{ij}$ and e_{ijk} have normal independent distributions with zero means and equal variances. The phenotypic correlation (Pearsons's correlation coefficient) between the reproductive traits of the species was calculated using the formula given by Dabi *et al*. (2016).

Results and discussion

Reproductive traits of zebrafish

The reproductive traits such as fecundity, fertility, hatchability of the total eggs, hatchability of fertilised eggs, survival rate of total eggs and survival rate of fertilised eggs were recorded and analysed. The mean and standard error of the mean (SE) of all the reproductive traits is shown in Table 2. The lowest and highest fecundity observed were 72 and 685, respectively.

Table 2. Reproductive traits of zebrafish

Traits	N	Mean±SE	Min	Max	Confidence interval 95%	
					Lower bound	Upper bound
Fecundity (Nos.)	100	263.42±22.53	195.10	352.40	212.45	314.39
Fertility (%)	100	62.88±2.63	39.50	81.40	56.93	68.84
Hatchability of total eggs (%)	100	59.07±2.41	35.30	76.80	53.61	64.53
Hatchability of fertilised eggs (%)	100	89.13±2.01	72.60	96.20	84.58	93.68
Survival rate of total eggs (%)	100	58.73±2.34	34.40	76.60	53.44	64.02
Survival rate of larvae (%)	100	95.22±2.27	78.30	99.70	90.08	100.36

A few of the breeding pairs did not lay eggs or produced immature eggs. Fertilisation rate was calculated after 24 h from spawning. The highest fertilisation rate (98.7%) was observed from the pair 9 in the 10th breeding trial, while the lowest fertilisation rate (0.76%) was observed from the pair 5 in the 1st breeding trial. The highest number of hatchlings or larvae (590) were obtained from the pair 6 in the 8th breeding and the lowest number of hatchlings (19) were obtained from the pair 5 in the 1st breeding. The hatchability trait was estimated over the total eggs as well as the fertile eggs. Fertilised eggs from most of the zebrafish pairs recorded 100% hatching rate. In case of hatchability of total eggs, the highest and the lowest were 97.2 and 0.45% respectively.

The survival rate was calculated from 72 to 110 h post-spawning. The highest number of hatchlings survived was 587 from pair 6 in 8th breeding and the lowest number was 19 from pair 5 in 1st breeding. The highest survival rate over the total eggs was 97.2% and the lowest percentage recorded was 0.45%. The hatched larvae from most of the breeding pairs showed 100% survival rate.

The reproductive traits such as spawning frequency, fecundity, fertilisation rate, hatchability and survival rate were documented in the present study. The spawning frequency was set once per week. Pertiwi *et al.* (2018) estimated the highest fertilisation rate in Lukas fish (*Puntius bramoides*) as 78.92±1.81%. Anita and Dewi (2020) used the hatchability formula of total hatched larvae over the total number of eggs for evaluating the hatching rate of cantang grouper (*Epinephelus fuscoguttatus x lanceolatus*) and the hatching rate recorded was 53.3%.

Repeatability of reproductive traits

The repeatability values for reproductive traits estimated using the intraclass correlation method are given in Table 3. The repeatability values were significantly higher for fecundity (0.701) and moderate but non-significant for fertility (0.406), hatchability of total eggs (0.336), survival rates of eggs (0.295) and larvae (0.314). The repeatability value for the hatchability of fertilised eggs was found to be lower which indicated major influence of environment over the genetic component. The sub-traits were estimated because they seemed to give a more natural insight on the repeatability of the reproductive traits. The genetic variance or the environmental variance or both might have caused the phenotypic variance, in the sub-traits. It was observed that the environment played a major role in the repeatability estimate of hatchability of fertilised eggs which contributed up to 95% in the trait.

The fecundity was highly significant ($p<0.01$) and can even be said that this trait can be repeatable throughout the life of the brood fish until the loss of expectancy. Ariyomo *et al*. (2017) also stated that these highly repeatable traits implied that these traits are potentially heritable, but the traits which showed an error in the significance level may not be repeatable. Ariyomo *et al*. (2017) reported that both aggression and boldness were highly repeatable in the three strains of male and female zebrafishes.

Phenotypic correlation among reproductive traits of zebrafish

The reproductive traits were recorded from all of the 10 pairs simultaneously and the correlation between reproductive traits calculated using Pearson's correlation method are given in Table 4. Correlation between the fecundity and the hatchability of fertilised eggs was

Table 3. Repeatability of reproductive traits of zebrafish

Traits	Repeatability	F	P	Confidence Interval 95%	
				Lower bound	Upper bound
Fecundity	0.701	3.346	0.002	0.320	0.913
Fertility	0.406	1.685	0.106	-0.351	0.826
Hatchability of total eggs	0.336	1.505	0.160	-0.512	0.806
Hatchability of fertilised eggs	0.049	1.052	0.407	-1.163	0.722
Survival rate of total eggs	0.295	1.419	0.194	-0.604	0.794
Survival rate of larvae	0.314	1.458	0.178	-0.561	0.799

F=F test value; p=Significance

Table 4. Phenotypic correlation between the reproductive traits of zebrafish

Traits	Fecundity	Fertility	Hatchability of total eggs	Hatchability of fertilised eggs	Survival rate of total eggs	Survival rate of larvae
Fecundity	1	0.104 (0.304)	0.070 (0.492)	0.243* (0.015)	0.065 (0.520)	0.359** (0.00)
Fertility	0.104 (0.304)	1	0.978** (0.00)	0.537** (0.00)	0.977** (0.00)	0.527** (0.00)
Hatchability of total eggs	0.070 (0.492)	0.978** (0.00)	1	0.585** (0.00)	1.000** (0.00)	0.515** (0.00)
Hatchability of fertilised eggs	0.243* (0.015)	0.537** (0.00)	0.585** (0.00)	1	0.584** (0.00)	0.908** (0.00)
Survival rate of total eggs	0.065 (0.520)	0.977** (0.00)	1.000** (0.00)	0.584** (0.00)	1	0.515** (0.00)
Survival rate of larvae	0.359** (0.00)	0.527** (0.00)	0.515** (0.00)	0.908** (0.00)	0.515** (0.00)	1

*Significant at 0.05 level (2 tailed); **Significant at 0.01 level (2 tailed)

moderate (0.243±0.015) and significant. Correlation values were moderate between the fertility and hatchability of fertilised eggs (0.537±0.00) and the survival rate of larvae with fertility (0.527±0.00). The values were very high and significant among the hatchability of total eggs and fertility (0.978±0.00), survival rate of total eggs and fertility (0.977±0.00) and the survival rate of total eggs and the hatchability of total eggs (1.0). The correlation between fecundity with fertility, hatchability of total eggs, and survival rate of total eggs were not significant. There was no negative correlation found between any of the reproductive traits. Tan *et al*. (2017) stated that to enhance the overall net productive value, it was very important to understand the genotypic and phenotypic correlation between the reproductive traits and growth traits of *Litopenaeus vannamei*.

In this study, the phenotypic correlation among the reproductive traits in the captive-bred zebrafish was quite significant. The fecundity in relation to the hatchability of fertilised eggs had low significance but both the traits had influence over each other and fecundity towards the survival rate of larvae was moderately significant. Both hatchability of total eggs and survival rate of total eggs were highly significant. Grima *et al*. (2010) measured a group of 50 seabass for phenotypic correlation for residual feed intake. de Verdal *et al*. (2018) estimated the phenotypic correlation in Nile tilapia in which phenotypic correlation for few of the traits were moderate such as feed conversion ratio, body weight gain and thermal growth coefficient during the F1 period.

During the present study, it was observed that female zebrafishes on a few occassions were not able to spawn eggs, the reason for which is not known specifically. However, it is assumed that environmental factor (temperature or illumination) might be the reason for their non-performance. The appropriate laboratory conditions played a major role in the spawning and the egg-laying activity and even in the fertility, hatchability and survival rate. The outcome of this research study provides important information on the consistency of reproductive parameters of zebrafish which would serve as model for conducting research in other fish species.

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