



## Selection of Fast Growth and Disease Resistance Traits in Brood Stock Candidates of Silver Pompano (*Trachinotus Blochii*)

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### ABSTRACT

Provision of superior broodstock silver pompano (*Trachinotus blochii*) is important in supporting the sustainability of seed production activities, one of which is by selecting superior traits from prospective parent seeds. This study aims to select prospective silver pompano broodstock based on superior traits such as fast growth and disease resistance. The method used to select the weight of fish from spawning and hatching eggs in the same cycle. Fry size based on fast-growing group at 1.5 g and control at 1.0 g with a density of 5 fish/l were reared in fiber tanks for 56 days with a running water system. Commercial feed was fed ad satiation, and growth performance was observed every 14 days. The results showed that the absolute weight of the fast-growing fish group was  $23.51 \pm 1.28$  g which was significantly different ( $p < 0.05$ ) from the control which was  $18.75 \pm 0.78$  g. The daily growth rate of fast-growing fish and control group were  $5.29 \pm 0.18$  and  $5.40 \pm 0.06$  (g/day), respectively. FCR values in fast-growing and control group fish were  $1.15 \pm 0.06$  and  $1.39 \pm 0.08$ , respectively. The fish survival rate of the fast-growing and control groups were  $98.78 \pm 1.35\%$  and  $97.45 \pm 4.14\%$ , respectively. Post-challenge survival for fast-growing fish was  $80 \pm 26.46\%$  while the control was  $76.7 \pm 11.55\%$  where this value was significantly different ( $p$  value  $< 0.05$ ). The fast-growing fish group was superior in the parameters of absolute weight and survival of the challenge test compared to the control fish.

**Keywords:** Silver Pompano, Disease Resistance, Selection, Superior Traits, Fast Growing

### 1. Introduction

Silver pompano (*Trachinotus blochii*) is a marine fish farming commodity that needs to be developed for export activities. Silver pompano has been sold under the Pompano trademark, star pomfret cultivation technology has been mastered at a high production level (Syukra, 2020) and has high economic value. Silver pompano is in great demand because it has good taste, is adaptable and has fast growth, namely seed rearing to consumption size takes less than 12 months (Weirich, *et al.*, 2021). Seed maintenance from 80 grams can reach 500 - 600 grams within 6 months with a selling price range of IDR 60,000 - 90,000/kg.

Silver pompano aquaculture is still dependent on wild-caught broodstock, which is unpredictable. High-quality and disease-free broodstock are few and far between. The availability of sustainable silver pompano seeds and broodstock candidates has experienced many obstacles, especially in terms of the preparation of quality broodstock candidates. Good quality seeds are only produced from good quality broodstock and using good fish hatchery methods. The provision of superior silver pompano parents in supporting the sustainability of seed production activities is needed, one of which is by selecting superior traits from prospective fish parents (Ma, *et al.*, 2017). The genetic quality of a group determines the phenotypic appearance of the group. Fish seed groups with high quality and supported by a good environment will provide maximum production results (Tave, 1996). Seed groups with high genetic quality can be obtained through a breeding program. Freshwater fish breeding techniques that are widely developed are selection techniques by exploiting the potential and genetic characters, especially the variety of additive genes (VA). Selection does not create new genes, but exploitation of VA will change the frequency of genes to improve genetic quality qualitatively and quantitatively with the goal of obtaining superior parents (Hardjosubroto, 1994).

Individuals or groups are selected based on their superior traits, such as fast growth, high resistance to disease and others (Weirich, *et al.*, 2021). Fish selection based on growth rate and disease resistance is most often done because it is easy to do and the superior traits will be passed on to the next generation (Luo, *et al.*, 2017; Xie, *et al.*, 2014; Yi, *et al.*, 2013). The provision of seed candidates that have fast growth characteristics plays an important

role in the production of superior parent candidates, therefore it is necessary to carry out "growth selection" observations that will separate and compare silver pompano seeds that have fast growth with average growth or control.

In addition to rapid growth, disease resistance of a fish species is important because the spread of disease in aquaculture systems will have an impact on economic losses. Silver pompano, despite being able to adapt to a new environment, is still susceptible to disease attacks, one of which is bacterial disease. *Photobacterium damsela* spp. was first found to infect *Trachinotus ovatus* species in China in 2008 and had a lethal dose of  $1.1 \times 10^6$  CFU/mL and caused symptoms of damage to the spleen and kidneys (Wang, *et al.*, 2013). Infection with *P. damsela* subspecies *damsela* found in silver pomfret in China in 2015 caused mass mortality with symptoms of photobacteriosis, one of which was bleeding in the fin area of the fish (Tao, *et al.*, 2018). Infection with *P. damsela* spp. also causes pasteurellosis, which is similar to vibriosis, but in some fish there are no symptoms in the external organs, but there continues to be a large number of deaths. Disease resistance of silver pompano broodstock candidates to *P. damsela* spp. bacteria will be tested through challenge test activities at lethal doses. The purpose of this study was to determine the performance of growth and disease resistance to *P. damsela* spp. bacteria between fast-growing and control groups in prospective silver pompano broodstock seeds.

## 2. Materials and methods

The study is quantitative research with observation method (observing the growth performance and disease resistance to *P. damsela* spp. bacteria between the fast-growing group (B) and control (K) in silver pompano seed candidates. The seed group consisted of 2 groups, namely groups B and K with each group being replicated 3 times. This activity was carried out for 56 days at the Balai Besar Perikanan Budidaya Laut (BBPBL) Lampung from June to September 2021.

Silver pompano broodstock seeds were obtained from the same spawning period, each group amounted to 300 fish, namely group B with an average weight of 1.5 g and a total length of 3-5 cm and group K with an average weight of 1.0 g and a total length of 2-4 cm. Fish feed was commercial feed with various sizes adjusted to the mouth opening of the fish. Pure cultures of *P. damsela* spp. bacterial isolates that have been identified biochemically and molecularly were obtained from BBPBL Lampung Laboratory.

Broodstock seeds were reared in 1.5 m<sup>3</sup> fiber tanks with a stocking density of 5 fish/liter. Broodstock seeds are maintained with a running water system that is provided with an airing system. Feed is given ad satiation with a frequency of 3-4 times a day. Feed consumables were weighed and recorded to determine the feed conversion ratio (FCR). Cleaning of debris at the bottom of the tanks was done twice a day every morning and evening. Observations of growth parameters such as total fish weight and length were conducted every 14 days using a random sampling technique for each replicate (30 fish). Observations of water quality parameters were carried out by the water quality laboratory team, BBPBL Lampung, every 7 days with test parameters: temperature, salinity, DO, pH, and nutrient content. Maintenance for 56 days was carried out by always recording the number of fish that died in each treatment.

The disease resistance test was carried out at the end of rearing by testing silver pompano broodstock candidates against pathogenic bacteria *P. damsela* at lethal dose 50/LD<sub>50</sub>. 50 test fish were placed in 5 baskets (10 fish per basket). Determination of LD<sub>50</sub> by making a series of bacterial suspensions consisting of 5 different bacterial doses then each dose was injected intraperitoneally to 10 test fish. Post-injection fish survival for each dose was recorded. The LD<sub>50</sub> value that killed 50% of the fish was calculated using the Dragstedt Behrens method (Hubert, 1980) as follows:

$$m = x_1 + d \left[ \frac{50 - \% x_1}{\% x_{1+1} - \% x_1} \right]$$

Description:

m = log LD<sub>50</sub>

x<sub>1</sub> = log of bacterial dose below LD<sub>50</sub>

d = difference between log dose below LD<sub>50</sub> and above LD<sub>50</sub>.

%x<sub>1</sub> = percentage of cumulative mortality at doses below LD<sub>50</sub>

%x<sub>1+1</sub> = percentage of cumulative mortality at doses above LD<sub>50</sub>

The test fish from each group, totaling 60 fish, were placed in 6 basket containers at a density of 10 fish per basket. The LD50 dose of 0.1 ml per fish was injected intraperitoneally into each fish. After infection, the test fish were not fed and the water flow in the tanks was minimal and aeration was normal. Fish mortality in each treatment was recorded to determine the survival rate. Data were analyzed using one-way ANOVA. Calculation of specific growth rate (SPR), FCR and survival rate (SR) using the formula below:

$$LPS = \frac{(\ln W_t - \ln W_o) \times 100}{\text{time}}$$

$$FCR = \frac{KP}{(W_t + WD) - W_o}$$

$$SR = \frac{N_t}{N_o} \times 100$$

Description:

Wo = fish weight at time 0

Wt = fish weight at time t

WD = dead fish weight

KP = feed consumption

Nt = number of fish at time t

No = initial number of fish

### 3. Result and discussion

The results of the study for growth and survival parameters between the silver pompano broodstock seeds of groups B and K are presented in Table 1.

**Table. 1 - Growth Observation and Survival Data of Silver Pompano Broodstock Seeds**

No	Parameters	Group B		Group K	
		Beginning	End	Beginning	End
	Weight (g)	1.39 ± 0.05	24.92 ± 1.54	1.04 ± 0.06	19.81 ± 0.68
	Total Length (cm)	4.00 ± 0.05	11.46 ± 0.24	3.60 ± 0.06	10.78 ± 0.52
3.	Absolute Growth (g)	23.51 ± 1.28 <sup>b</sup>		18.75 ± 0.78 <sup>a</sup>	
4.	Daily Growth Rate (g/day)	5.29 ± 0.18 <sup>a</sup>		5.40 ± 0.06 <sup>a</sup>	
5.	Feed Conversion Rate (%)	1.15 ± 0.06 <sup>a</sup>		1.39 ± 0.08 <sup>a</sup>	
6.	Rearing Trajectory (%)	98.78 ± 1.35 <sup>a</sup>		97.45 ± 4.14 <sup>a</sup>	
7.	Post-challenge Survival (%)	80 ± 26.46 <sup>b</sup>		76.7 ± 11.5 <sup>a</sup>	

Notes: same subscript letter means not significantly different ( $p \geq 0.05$ )

At the beginning of the activity, the silver pompano broodstock of group B had a total length and weight of  $4.00 \pm 0.05$  cm and  $1.39 \pm 0.05$  g respectively while group K had a length and weight of  $3.60 \pm 0.06$  cm and  $1.04 \pm 0.06$  g respectively. The final growth data where the fish in group B had a length and weight of  $11.46 \pm 0.24$  cm and  $24.92 \pm 1.54$  g respectively. While group K had a length and weight of  $10.78 \pm 0.52$  cm and  $19.81 \pm 0.68$  g, respectively. Fish weight mainly consists of skeletal muscle mass which plays a role as much as 40-60% of the entire fish body (Xu, *et al.*, 2019). Muscle mass growth is influenced by the quality of feed nutrition and gene expression of insulin-like growth factor (IGF), myostatin (MSTN), myogenic regulatory factors (MRFs), fibroblast growth factor 6 (FGF6) and others. (He, *et al.*, 2015; Johnston, *et al.*, 2011).

Table 1. shows the observation data of several growth parameters of silver pompano seed. The absolute growth of fish in group B was  $23.51 \pm 1.28$  g where this value was significantly different ( $p$  value  $< 0.05$ ) compared to group K at  $18.75 \pm 0.78$  g. This rapid growth trait can be passed on to the next generation. This fast growth trait can be passed on to the next generation. However, if the parents that carry this fast-growing gene are only mated with parents of the same breed (inbreeding), the genetic diversity will be reduced. Crossbreeding local parents with parents from diverse geographical origins can increase the genetic diversity of their offspring. Research has shown that the contribution of each parent in the spawning process in providing genetic contribution is not equal. Tracing of fast-growing genes can be done on the parents and their offspring using the DNA microsatellite marker method so that it can be known which parents contribute greatly to carrying fast-growing genes. Parents with high potential for carrying fast-growing genes will be spawned next so that the superior nature of the parent can be maintained. (Luo, *et al.*, 2017).

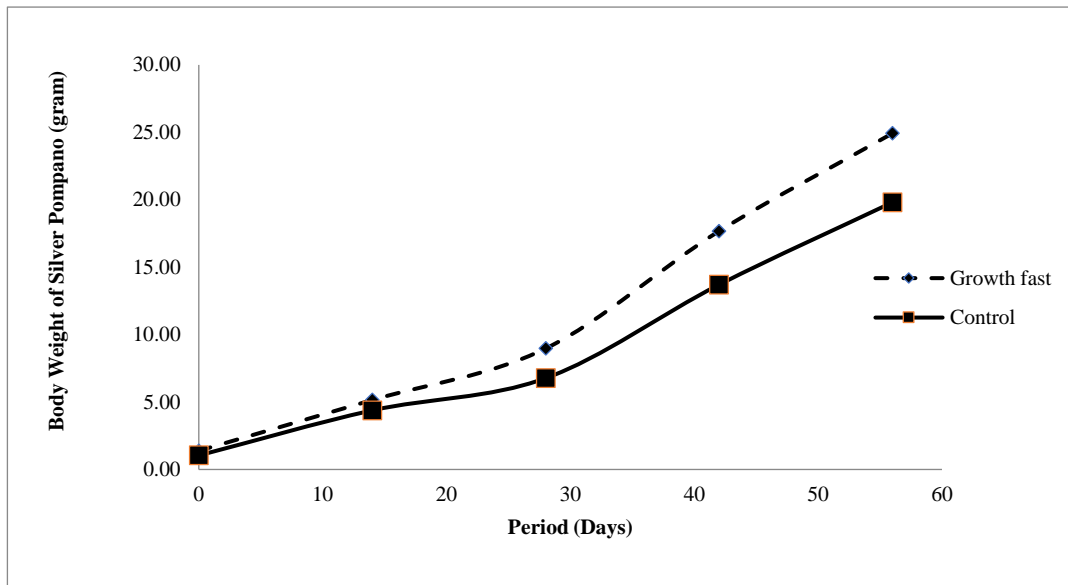


Fig. 1 - Graph of Increased Silver Pompano's Body Weight

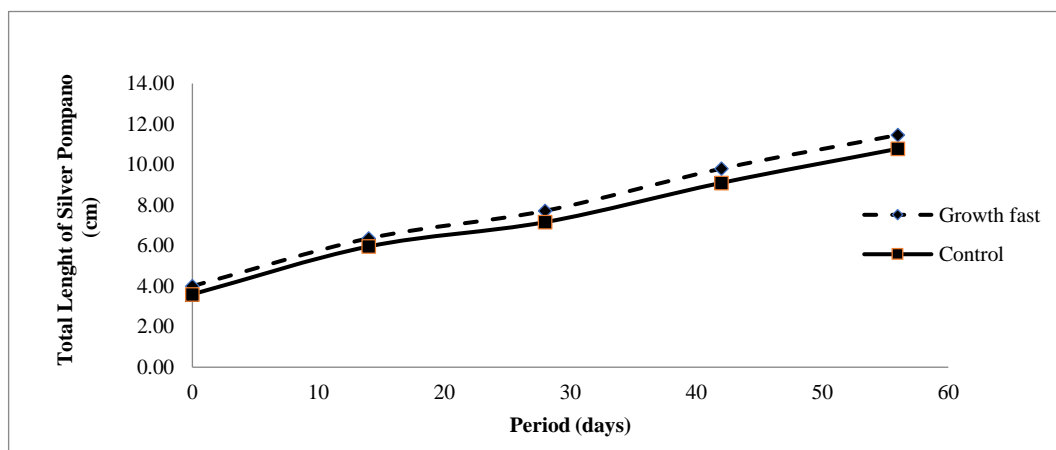
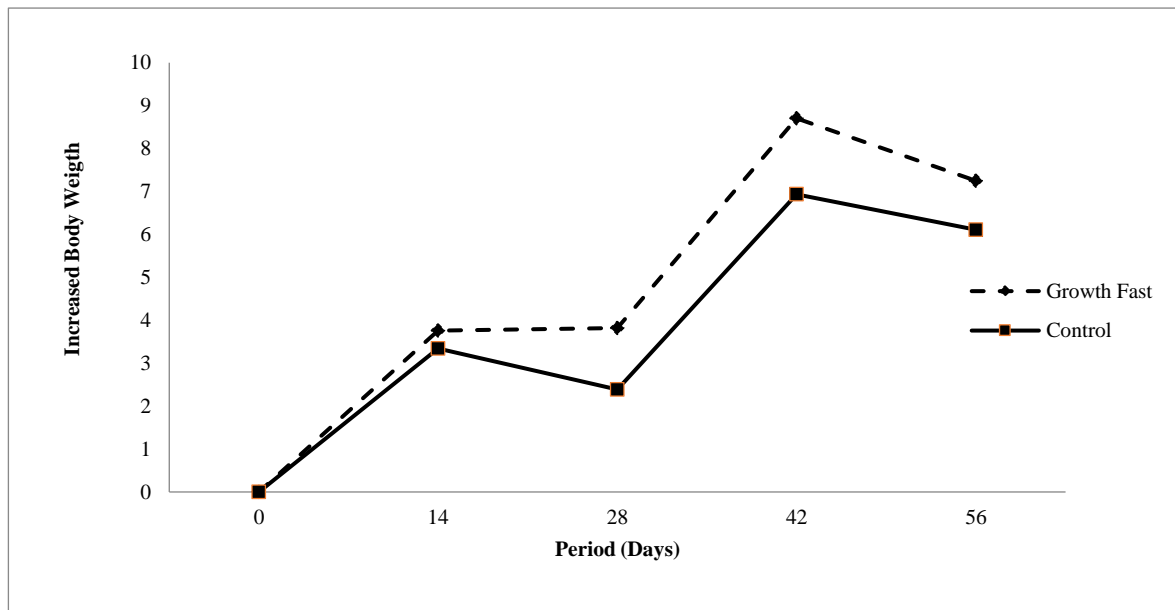


Fig. 2 - Graph of Increased Silver Pompano's Total Length

Body weight in all fish groups, both in groups B and K had varying body weight at each observation but the largest increase was from observation day 28 to 42, which was 8.70 g and 6.93 g, respectively (Figure 3). Meanwhile, the lowest body weight in fish occurred on the 14th day of observation for all group B while for group K the lowest was on the 28th day. This decrease is partly due to the factor of fish density level that is not in accordance with the growth rate of fish, therefore improvements or evaluations are made by reducing the level of fish density. High fish density will affect the hypothalamic-pituitary-somatotropic axis (HPS) where HPS plays an important role in regulating fish growth. The impact of stocking density also varies depending on the fish species, growth phase, and temperature of the rearing medium (Triantaphyllopoulos, *et al.*, 2020).



**Fig. 3- Graph of Increased Pompano's Body Weight every 14 Days**

In Figure 3, the body weight graph for group B is consistently above group K during rearing but has the same pattern of increase. This indicates that the growth acceleration of each fish group has a time that is not different. The specific daily growth rate of group B and K fish were  $5.29 \pm 0.18$  and  $5.40 \pm 0.06$  (% g BW/day), respectively. Fish growth rate is influenced by several factors such as internal factors and external factors. Internal factors such as metabolic and genetic characteristics of fish while external factors such as feed content, water quality, frequency of feeding, fish density level, and others (Kusuma & Putra, 2018; Triantaphyllopoulos, et al., 2020). Fish growth is genetically inherited but influenced by growth hormone (GH) and insulin-like growth factor (IGF-I), both of which are central to the hypothalamic-pituitary-somatotropic axis (HPS). The HPS axis is one of the most important endocrine systems, structurally and functionally regulating the processes of growth and development of soft tissues and bones (Triantaphyllopoulos, et al., 2020).

The FCR values in groups B and K were 1.15% and 1.39%, respectively. Both treatment groups had FCR values that were not significantly different ( $p$  value > 0.05). The value of the feed conversion ratio can also be influenced by the activity of digestive enzymes (Caruso, et al., 2014). Feeding frequency and management play an important role in determining feed conversion ratio and fish growth and size uniformity (Hamed, et al., 2016). Star pomfret growth is also influenced by feed efficiency and protein and fat composition. The observations of fish survival rate in the B and K groups were  $98.78 \pm 1.35$  % and  $97.45 \pm 4.14$  %, respectively.

**Table 2 - Water Quality Data**

Group	Water Quality Parameters					
	Sal (ppt)	Temperature (°C)	pH	DO (mg/l)	NO <sub>2</sub> -N (mg/l)	NH <sub>3</sub> -N (mg/l)
<b>Fast growing</b>	32-33	29.6- 29.7	7.81-8.03	4.11 – 4.33	0.079-0.156	0.238-0.428
<b>Control</b>	32-33	29.7-29.8	7.78-8.11	4.17 – 4.28	0.076-0.122	0.211-0.575
<b>Quality</b>	28-33	28-32	7.5 – 8.5	Minimum 5	Maximum 1	Maximum 1

**Reference Water Quality Standard: SNI 7901.2-2013. Ikan Bawal Bintang (*Trachinotus blochii*) - Bagian 2: Produksi Induk**

The salinity of silver pompano rearing basin water for all treatments ranges from 32 - 33 ppt where this value is in accordance with the required quality standards. The value of water salinity will affect fish osmoregulation activities if the value is too high it will stress the fish. Salinity also affects the expression of fish growth genes that if the value cannot be tolerated, it will inhibit its growth (Bertucci, et al., 2017). The water temperature of fish tanks in all treatments during maintenance was 29.6 - 29.8°C. This value is a value that can still be tolerated by fish so as to support optimal metabolic processes. The pH value range of fish tank water in all treatments during maintenance is 7.78 - 8.11 where this value is still in accordance with quality standards. The pH value will affect the solubility of chemical compounds in water, especially their toxicity. Meanwhile, the value of oxygen/DO solubility in fish rearing basin water in all treatments is slightly above 4 mg/l. In the future, there may need to be improvements such as the addition of an aeration system in the water tank so that it can increase dissolved oxygen more optimally. The content of chemical compounds/nutrients in the rearing basin water such as nitrite and ammonia is still in accordance with the required quality standards. Nitrite is a toxic compound and can increase the risk of death in fish if the value is too high. Similarly, ammonia with the highest value in the control basin was 0.575 mg/l while in the fast-growing basin it was 0.428 mg/l where the value was still within the quality standard value. Ammonia can also be toxic if its content in water is too high. To overcome the high nutrient content in the rearing basin water, the basin can be drained and the water changed after each feeding. This is to avoid the formation of ammonia and nitrite compounds that occur through the degradation of organic matter in the remaining feed and fish feces.

Observation data on the determination of LD 50 of star pomfret broodstock by injecting *P. damsela* bacteria are presented in Table 3.

**Table 3 - Seed Mortality Results of Silver Pompano Broodstock Candidates in the Challenge Test**

Dosage	Fish Mortality					Total
	24 hours	48 hours	72 hours	96 hours	120 hours	
1,2 x 10 <sup>10</sup> CFU/ml	10	0	0	0	0	10
3,0 x 10 <sup>9</sup> CFU/ml	6	4	0	0	0	10
1,5 x 10 <sup>9</sup> CFU/ml	0	0	0	0	0	0
7,5 x 10 <sup>8</sup> CFU/ml	0	0	0	0	0	0

From the calculation, the LD 50 of *P. damsela* for potential pomfret seed is 2.1 x 10<sup>9</sup> CFU/ml. This dose will be used for the challenge test on fish treatment B and K by taking a sample of 10 fish per treatment. Data obtained in Table 4. The survival value in the challenge test for group B was 80 ± 26.46% while group K was 76.7 ± 11.55% (significantly different, p value <0.05).

**Table 4 - Mortality of Challenge Test Fish**

Treatment	Number of Fish Mortality (fish)					Total	Remaining	Synthesis
	24 hours	48 hours	72 hours	96 hours	120 hours			
B1	2	3	0	0	0	5	5	50
B2	0	0	0	1	0	1	9	90
B3	0	0	0	0	0	0	10	100
<b>Average</b>								80 ± 26.46 %
K1	0	1	0	0	0	1	9	90
K2	0	3	0	0	0	3	7	70
K3	0	3	0	0	0	3	7	70
<b>Average</b>								76.7 ± 11.55 %

## 4. Conclusions and suggestions

### Conclusions

The results of selecting seed parents for long-finned silver pompano fish revealed significant differences between the fast growth group (B) and the average growth or control group (K) in terms of absolute weight growth (23.51 ± 1.28 g and 18.75 ± 0.78 g, respectively) and post-challenge survival (80 ± 26.46% and 76.7 ± 11.55%, respectively). Daily growth rate, FCR, and survival rate, however, showed no significant differences. The daily growth rates of the fast-growing fish and control group were 5.29 ± 0.18 and 5.40 ± 0.06 (grams/day), respectively. The fast-growing and control group fish exhibited FCR (feed conversion ratio) values of 1.15 ± 0.06 and 1.39 ± 0.08, respectively. The survival rates for fast-growing and control groups were 98.78 ± 1.35% and 97.45 ± 4.14%, respectively. The fast-growing fish group outperformed the control group in absolute weight parameters and post-challenge survival. Selection of potential broodstock requires a prolonged and sustained period of observation to investigate if the parent fish's performance or quality remains high or has declined. Selection of parents based on genetic traits is necessary to yield improved and informative data.

### Suggestions

Further parent selection based on genetic markers needs to be done to produce complete data.

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