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## Genetic selection for harvest body weight in the Malaysian giant freshwater prawn (*Macrobrachium rosenbergii*)

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**Abstract** A breeding programme of giant freshwater prawn *Macrobrachium rosenbergii* is conducted in Malaysia to improve growth performance. The harvest body weight (HBW) data of 6,159 individuals from 102 sires and 141 dams produced over three generations of selection was analysed using residual maximum-likelihood methodology. This enabled the estimation of variance components and genetic parameters of the studied trait in the population. The heritability ( $h^2$ ) and common environmental effect ( $c^2$ ) of HBW were  $0.165 \pm 0.153$  and  $0.043 \pm 0.013$  respectively. The selection response per generation estimated by comparing the difference in mean breeding value of HBW between generations was 18.01%. It was similar with the average selection response estimated by comparing least square mean (LSM) between generations (17.78%). These results indicated that improvement of the studied trait has been achieved in this population.

**Keywords:** *Macrobrachium rosenbergii*, Genetic selection, Harvest body weight, Heritability, Selection response

### Introduction

One of the major freshwater species for aquaculture that gain good demand and price in Malaysia is *Macrobrachium rosenbergii*. Hatchery technique to produce and rearing of the giant freshwater prawn post larvae (PL) in captivity was developed in Malaysia over the past five decades (Ling, 1962). Since then, the hatchery and grow-out technology of this species were developed all over the world after it was introduced into many countries for aquaculture. Annual production of *M. rosenbergii* from 1,500 ha grow-out pond area in Malaysia ranges from 300 to 350 tonnes, worth of RM17 million (Department of Fisheries [DOF], 2018).

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Currently, there are ten hatcheries producing an average of 50 million fry a year using wild caught brood stocks. However, the trend of decreasing pond culture production was observed since 2013. The total production in 2013 was 456.6 tonnes but decreased to 213.4 tonnes in 2018 (DOF, 2013, 2018). This trend is likely due to the limited supply of wild brood stocks and low quality fry for grow-out. Therefore, a breeding programme of the species was initiated in 2016 to address the problem of lack supply of good quality brood stocks and seeds. The programme aimed to improve growth traits (i.e harvest body weight). To date, three generations of selection have been produced at Fisheries Research Institute (FRI) in Pulau Sayak, Kedah, Malaysia.

It has been proven that selective breeding programmes have successfully improved production traits of tilapia (Hamzah *et al.*, 2017; Nguyen, 2015; Ponzoni *et al.*, 2011), salmon (Gjedrem, 1999; Lhorente *et al.*, 2019; Symonds *et al.*, 2019; Thodesen and Gjedrem, 2006), catfish (Dunham, 1995; Vu *et al.*, 2019) and shrimp (Argue *et al.*, 2002, Castillo-Juarez *et al.*, 2007; Cock *et al.*, 2009; Gjedrem, 2005; Hetzel *et al.*, 2000; Kenway *et al.*, 2006; Krishna *et al.*, 2011; Perez-Rostro and Ibarra, 2003a, 2003b). For giant freshwater prawn, breeding programmes have been implemented in Vietnam (Hung *et al.*, 2013; Thanh *et al.*, 2009, 2010), India (Pillai *et al.*, 2009, 2011, 2014, 2017), China (Luan *et al.*, 2012) and Thailand (Kitcharoen *et al.*, 2011; Uraiwan *et al.*, 2005) to improve economically important traits especially growth as well as survival. Results of the studies indicated heritability of the studied traits ranging from 0.10 to 0.60 and significant responses to selection were achieved (Hung *et al.*, 2013; Kitcharoen *et al.*, 2011; Luan *et al.*, 2012; Pillai *et al.*, 2017).

In Malaysia, the slow growth, low production yield and high size variations of this species during grow-out has affected operational cost which leads to low economic returns. Therefore, the breeding program is conducted to improve growth trait, mainly harvest body weight. Previously, there has not been a systematic breeding programme of *M. rosenbergii* being conducted in Malaysia until a base population for genetic improvement was established in 2016 by FRI. In this report, the genetic parameters for harvest body weight (HBW) as well as the selection responses of the population were estimated.

## **Materials and methods**

### ***Establishment of the base population***

This breeding programme is conducted at Fisheries Research Institute (FRI) Kg. Pulau Sayak, Kedah, Malaysia. The founder stocks used to establish the base population in this programme comprised of a domesticated stock (D)

maintained at FRI (150 males weighing  $59.2 \pm 8.2$  g and 300 females weighing  $35.4 \pm 5.6$  g) and two wild populations collected from Sg. Lundu, Sarawak (S) [2°12'11.0"N 111°28'03.3"E] (100 males weighing  $64.2 \pm 10.5$  g and 200 females weighing  $32.8 \pm 6.5$  g) and Sg. Perak, Perak (P) [4°08'33.3"N 100°58'58.4"E] (100 males weighing  $60.6 \pm 8.3$  g and 200 females weighing  $33.8 \pm 5.2$  g). The wild stocks were quarantined in 10 m<sup>3</sup> fibreglass tanks respective to their origin for three weeks before being selected for mating. During this period, they were fed daily with fresh squids, fresh fish fillet and formulated feeds at 10% of the biomass.

The base population was created by half 'diallel' cross mating of breeders from the three populations (Table 1). Prior to the mating, the males and females were reared separately in 10 m<sup>3</sup> circular fibreglass tanks for two weeks to ensure mature gonad development of the females. Once the females developed orange-coloured ovaries, they were mated with a healthy male. The chosen male was mated with three females in a basket submerged in 40 m<sup>3</sup> tank. A total of sixty mating baskets were prepared for this activity in the tank. Therefore, sixty males and hundred and eighty females were mated at one time. During this phase they were only fed with fresh squids two times daily. To maintain water quality in the tank, water exchange was carried out twice a week. Five days after being mated, the baskets were checked for berried females. Once the female brood stocks bearing grey eggs observed they were transferred to the individual hatching tanks (1 m<sup>3</sup>) containing 12 ppt brackish water. Each tank accommodated one berried female. The hatching tanks were monitored daily until the newly hatched larvae observed.

**Table 1.** Half 'diallel' cross design to establish the base population

		Male parent strain		
		D	S	P
Female parent strain	D	DD	SD	PD
	S	-	SS	PS
	P	-	-	PP

Note. D: Domesticated, S: Sarawak, P: Perak

### ***Larval rearing***

The newly hatched larvae from the hatching tanks were collected and transferred for further rearing at a density of 6 larvae per litre in 500 L tanks containing 12 ppt brackish water. The rearing tanks were labelled according to the respective family or parent identification. Rearing was conducted in the tanks for about 30 days until they became post-larvae (PL). The larvae was fed with *Artemia* for the first ten days four times a day. At day eleven onwards, a

combination of formulated feed and *Artemia* were used to feed them up to the PL stage. During this period, the water salinity has been reduced in every five day interval until the salinity level reached 0 ppt at the end of rearing stage where all the larvae became PL.

### ***Tagging of families***

After the rearing stage in tanks, 500 PL of each family were transferred and reared separately in 3 m × 3 m × 1.2 m hapas (1 mm mesh size) fixed in an earthen pond. They were fed with formulated feed twice daily at 100% of their total body weight. The rearing period in the hapas was about 60 days before they reached a minimum size of 3 g for family identification using 'Visible Implant Elastomer (VIE)' colour tag. 100 PL of each family were randomly picked and tagged by injecting specific colour combination of VIE under their exoskeleton at the last abdominal segment. Over three generations, a total of 13,580 juveniles were tagged for growth evaluation in this study.

### ***Grow-out***

After being tagged, the PL were transferred for communal rearing at 50 individuals per square meter in 3 m × 3 m × 1.2 m of bigger mesh (5 mm) hapas fixed in the grow-out pond. Ten grow-out hapas were used for this purpose where PL from each family were randomly stocked equally in the hapas. They were fed with formulated feed (32% protein content) twice daily at 10% of their body weight. To ensure conducive environment throughout the culture period, water parameters (ammonia, pH and dissolved oxygen) in the pond were monitored once a week. When necessary, clean water was pumped from supply canal into the grow-out pond at 20% exchange ratio of the total pond volume. The pond was also equipped with air blower to provide aeration. After a grow-out period of about 150 days, all individuals were harvested and transferred into aerated conditioning tanks placed close to the grow-out pond where body traits measurement of the prawn was carried out. The same grow-out management and harvest were applied in all generations throughout the study. The production summary and scheduled periods of reproduction over the generations are shown in Table 2.

### ***Data structure***

Body traits measurement and data collection was conducted at harvest to estimate genetic parameters and selection responses. All the family of the

survived prawns was identified at harvest by visual assessment of the VIE colour code and the individual harvest body weight (HBW), total length (TL), sex (S) and date of harvest were recorded. HBW was measured using electronic scale and TL was measured from the tip of the rostrum to the end of tail (uropod) using measuring board. They were transferred back to their respective hapas after the activity and reared until the estimation of their genetic parameters completed. The age (in days) of each family at harvest was calculated by subtracting the hatching date from the harvest date.

**Table 2.** Reproduction and management schedule of the base population to the 3<sup>rd</sup> generation

Activities	Spawning season (Generation, G)			
	2016 (Base)	2017 (G1)	2018 (G2)	2019 (G3)
<b>Mating</b>	May - June	Mac - Apr	Feb - March	Jan – Feb
<b>Nursing of larvae until post-larvae (PL) stage</b>	June - Jul	May - June	Apr – May	Mac -Apr
<b>Post-larvae (PL) rearing</b>	Aug - Sept	Jul – Aug	June – Jul	May – June
<b>Grow-out</b>	Oct - Jan	Sept – Dec	Aug – Nov	Jul – Oct
<b>Harvest</b>	26 Jan	24 Dec	27 Nov	25 Oct

\* All families were harvested at the end of grow-out period in single day

### Data analysis

Genetic parameters of the trait were estimated using general linear mixed model (GLMM) in ASReml software (Gilmour *et al.*, 2009). The significance of fixed effects (generation, line, sex and their two-way interactions) were analysed before the final model was set to estimate variance components and heritability. In this model, age, line and sex were fitted as linear covariate while the additive genetics of sire and dam, and random maternal common environmental effect of full-sib groups were fitted as random effects. The mixed model is shown below:

$$y = Xb + Za + Wc + e$$

Where  $y$  is the vector of HBW,  $b$  is the vector of fixed effect namely generation, line, sex, environment and their two-way interactions,  $a$  is the vector of the random sire and “and(dam)” effects that use a single matrix in the mixed model where  $A$  is the numerator relationship matrix estimated from the pedigree,  $c$  is the vector of common full-sib effect and  $e$  is the vector of the random effects.  $X$ ,  $Z$  and  $W$  are known design matrices to the level of  $b$ ,  $a$  and  $c$ . In this model, the “and(dam)” option was used in ASReml and the variance of sire and dam ( $\sigma_s^2 = \sigma_d^2$ ) in the population was assumed equal. The

heritability ( $h^2$ ) was computed as  $h^2 = 4\sigma_s^2 / (2\sigma_s^2 + \sigma_c^2 + \sigma_e^2)$  where  $\sigma_s^2$  is the variance component of sire ( $\sigma_s^2 = \sigma_d^2$ ),  $\sigma_c^2$  is the common full-sib variance and  $\sigma_e^2$  is the environmental variance. The analysis enabled the breeding value estimation that was used to estimate selection response of body weight.

### ***Selection of broodstocks***

Selection of brood stocks to produce the subsequent generation was conducted in each generation based on their estimated breeding value (EBV) of HBW. From this estimate, individuals with the greater body weight from families with high EBVs were selected to become parents of the selection line and those from families with average EBVs of the population were used to produce a control line. Mating of full sibs, half sibs or cousins was restricted during this study to avoid inbreeding.

### ***Selection response and production of successive generations***

Genetic gain of HBW was estimated as changes in the EBVs between successive generations and between the selection and control line in the same generation. The gain was also estimated as the difference in least square means (LSM) for HBW between generations and between the selection and control population. Production of the subsequent generations was carried out by mating of the selected brood stocks at a ratio of one male to three females in each generation. For the control line, 20 pairs of the average breeding value brood stocks were chosen for mating to produce full sib families in each generation. Sixty families of the selected line and twenty families of the control were targeted to be produced per generation. This procedure was repeated throughout the programme and undergo the similar assessment in the hatchery and in the grow-out ponds.

## **Results**

### ***Descriptive statistics***

The numbers of observations, minimum, maximum, means, standard deviation and coefficient of variation of body weight at harvest (HBW) across generations are shown in Table 3. The mean age of individuals at harvest was 194 days. The average body weight and the coefficient of variation (CV, %) of HBW for males was greater than that in females (26.73 g vs 19.46 g and 78.33% vs 36.22% respectively). Out of 13,142 tagged juveniles (Table 4) stocked in

pond over three generations, 6,001 individuals were survived at harvest (average survival rate of 45.6% per generation) and measured for genetic evaluation. Note that, the survival rate of the third generation was low due to flood during grow-out period which caused some hapas collapsed and the prawn escaped.

**Table 3.** Number of individual (N), minimum (Min), maximum (Max), mean, standard deviation (SD) and coefficient of variation (CV) for harvest body weight

Variable	Sex	N	Min	Max	Mean	SD	CV (%)
Harvest weight (g)	M	3144	1.2	149.5	26.73	20.94	78.33
	F	3015	1.7	64.7	19.46	7.05	36.22
Age at harvest (day)			157	296	194.6	43.6	22.40

**Table 4.** Number of tagged individual (N) and number of individual at harvest in each generation

Generation	Number of tagged individual (N)	Number of individual at harvest	Percentage of survival (%)
1	2082	1028	49.4
2	4854	3052	62.9
3	6206	1921	30.9
<b>Total</b>	13142	6001	45.6

Attempts to produce 20 families of control population in each generation were unsuccessful (Table 5). There were only two families of control out of 20 mating pairs produced in generations one and two respectively, and fourteen families in the third generation. Therefore, the control lines in the first two generations were not used to estimate the genetic gain within generation. Nevertheless, selection response within generation was estimated in the third generation.

**Table 5.** Number of sires, dams and families for each generation

Generation	Line	Sire	Dam	Total families
0	Base population	13	13	13
1	Selection	14	27	27
	Control	2	2	2
2	Selection	41	41	41
	Control	2	2	2
3	Selection	35	43	43
	Control	14	14	14

### *Analysis of variance*

The statistical significance of the fixed effects and age of HBW are shown in Table 6. The main effects except environment were statistically different ( $p < 0.05$ ). The covariate age at harvest was not statistically significant. The significant difference between ‘Selection’ and ‘Control’ lines as well as between generations for HBW suggest that there was response to selection in the trait. The difference between sexes was due to the bigger size of males than the females. Significant sex by generation ( $S \times G$ ) and sex by environment ( $S \times E$ ) indicated that between sex-difference occurred in each generation and environment.

**Table 6.** Analysis of variance (ANOVA) of harvest body weight (HBW) to test the fixed effect

Effects	F-value	Prob. > F
Generation (G)	8.92	<0.001
Line (L)	3.97	0.019
Sex (S)	467.48	<0.001
Environment (E)	1.44	0.181
$S \times G$	24.86	<0.001
$S \times E$	6.15	<0.001
Age at harvest	0.19	0.668
Residual variance	221.64	

### *Variance components and heritability*

Variance components and heritability of HBW are presented in Table 7. There are additive genetic and environmental variances in the population. The heritability for HBW was 0.165 while there was low common environmental effect (0.043) on the trait.

**Table 7.** Genetic parameters and heritability of harvest body weight (HBW)

Parameter	HBW
Sire variance [ $\sigma^2s(s.e)$ ]	10.546 (10.545)
Dam variance [ $\sigma^2d(s.e)$ ]	10.998 (2.964)
Residual environment variance [ $\sigma^2e(s.e)$ ]	223.379 (4.009)
Total variance [ $2\sigma^2s + \sigma^2c + \sigma^2e$ ]	255.47 (19.496)
Heritability [ $h^2(s.e.)$ ]	0.165 (0.153)
Common environmental variance [ $\sigma^2c (s.e)$ ]	0.043 (0.013)

### *Selection response*

The selection response for HBW was measured in actual units (g) and percentage of the gain in relation to the least square mean (LSM) of HBW of



the 'Control' population. Selection response within generation was only estimated in the third generation due to unsuccessful production of control line in the earlier generations. Therefore, the percentage of gain for all generations was only estimated based on the (LSM) of the third generation's control line. The average gain achieved by comparing EBVs between generations was 18.01% per generation (Table 8). Similar magnitude was recorded when the response was measured by comparing LSM between generations (17.78%) (Table 9). The selection response within the third generation indicated lower magnitude (15.51%) either by comparing EBVs or the LSM between the selection and the control line.

**Table 8.** Genetic gain for harvest body weight estimated as the difference of average breeding value (EBV) between generations

Generation	Breeding value	Genetic gain	Percentage*
0	0.00	0.00	-
1	0.18	0.18	0.70
2	7.50	7.32	28.50
3	13.88	6.38	24.84
Average		4.62	18.01

*Note.* \*Calculated as a ratio of the genetic gain to the least squares means of the control line (25.68 g)

**Table 9.** Genetic gain for harvest body weight estimated as the difference of least square mean (LSM) between generations

Generation	Least square mean, LSM (g)	Selection response	
		Genetic gain (g)	Percentage*
1	15.96	-	-
2	23.28	7.32	28.50
3	29.66	6.38	24.84
Average		4.59	17.78

*Note.* \*Calculated as a ratio of the genetic gain to the least squares means of the control line (25.68 g)

## Discussion

### *Descriptive data*

Large variability in harvest body weight was observed in the population. This variability is consistent with the range reported for the same species (Hung *et al.*, 2014; Kitcharoen *et al.*, 2012; Pillai, *et al.*, 2017; Wahidah *et al.*, 2017). Males were heavier than females by 37%, consistent with that were reported by Kitcharoen *et al.* (2012), Pillai *et al.* (2017) and Wahidah *et al.* (2017). The high CV of body weight in the population indicates heterogeneous

morphological characters as shown in other fish species (Ferrito *et al.*, 2007). Thus, in giant freshwater prawn culture, farmers are manually selecting males for grow-out due to its higher body weight than the females. Currently, monosex culture of the species had also been applied by producing all male progeny using sex reversed breeders (Aflalo *et al.*, 2012; Sagi and Aflalo, 2005).

### ***Base population***

The synthetic base population in this programme was developed by a ‘half diallel’ cross mating with an aim to broaden genetic variability in the population for future selection. This strategy has been applied in other breeding programmes namely tilapia (Eknath *et al.*, 1998; Eknath *et al.*, 2007) and giant freshwater prawn (Pillai *et al.*, 2011, 2017; Thanh *et al.*, 2009). Selection of the founder stocks was based on our previous growth performance evaluation experiments on five wild populations and one domesticated strain at FRI. The three top ranking stocks specifically the Sarawak, Perak and FRI were selected as parents of the base population. Selection of these stocks that are geographically distant from each other may increase the genetic diversity of the base population as has been reported in tilapia (Eknath *et al.*, 1993; Gjerde *et al.*, 2002). Moreover, previous studies conducted on the wild Malaysian giant freshwater stocks exhibited significant variability in genetic characteristics among the populations (Harun, 2013; Hasnita *et al.*, 2014; Lim and Yong, 2015). The findings provide valuable information to establish the base population for this programme. Furthermore, Thanh *et al.* (2009) has also reported that there were significant differences in growth performance among three stocks of *M. rosenbergii* that originated from geographically separated locations in Vietnam. Note however that, the ‘half’ not ‘full diallel’ cross was applied to create the base population for the current programme due to the limited number of wild male breeders. This has produced sixty-seven families but only thirteen survived due to high mortality rate in nursing period. The limited number could abrupt inbreeding rate in the subsequent generations. Hence, a selected wild population will be incorporated in the production of the fourth generation to broaden the genetic variability of the studied population.

### ***Heritability***

The moderate estimate of heritability across generations for HBW (0.165) indicate that there are genetic variations in the trait. This was likely because the ‘diallel’ cross to create the base population comprises two different wild strains and a domesticated strain which was also assembled previously from four

distinct wild stocks. However, the standard error of the heritability estimate was also high which were possibly due to the limited number of survived individuals per family at harvest and large size variation between males and females in the population.

The moderate heritability indicates that the trait could be further improved. The estimate was higher than those reported by Luan *et al.* (2012) ( $h^2 = 0.056$ ) and comparable with different populations of freshwater prawn in Vietnam ( $h^2 = 0.11$ ) (Hung *et al.*, 2014), India ( $h^2 = 0.22$ ) (Pillai *et al.*, 2017), China ( $h^2 = 0.21$ ) (Sui *et al.*, 2019) and Thailand ( $h^2 = 0.20$ ) (Kitcharoen *et al.*, 2011) but lower than the estimate of 0.35 (Malecha *et al.*, 1984). Meanwhile, the estimated heritability of harvest body weight in marine shrimp (*Penaeus japonicus*), ranging from 0.16 to 0.31 (Hetzel *et al.*, 2000). Apparently, all of the breeding programmes were mainly focused to improve growth that indirectly could reduce the production cost.

It is important to include the maternal and common environmental effects ( $c^2$ ) as the  $h^2$  can be biased upward if the  $c^2$  effect is omitted. The estimated  $c^2$  over the three generations in this population was low (0.043) indicating the slight influence of rearing the post-larvae in separate hapas before being tagged. Note however, that this effect could be minimized by better synchronization in family production stage as well as by communally rearing of the progenies at early stage as has been reported in *Litopenaeus vannamei* (Kong *et al.*, 2020). Families of the giant prawn in this programme were nursed in separate nursing hapas until they attained suitable size for tagging due to unavailable facilities and resources for progeny identification using molecular methods.

### ***Selection response***

Results indicated substantial selection response of HBW was achieved (averaging about 17% per generation). The high genetic gain is consistent with the moderate heritability of the trait. It is also likely due to the broad genetic variability in the population which was established from different wild stocks of *M. rosenbergii*. The response was greater than the previous study on *M. rosenbergii* reported by Hung *et al.* (2013; 2014), Luan *et al.* (2012) and Sui *et al.* (2019). It was also higher than the responses for marine shrimp (Argue *et al.*, 2002; Fjalestad *et al.*, 1997; Goyard *et al.*, 2002; Hetzel *et al.*, 2000; Preston *et al.*, 2004; Sui *et al.*, 2015) but it is in line with other aquaculture species as reviewed by Nguyen (2015), likely due to the large genetic variability and the strong selection pressure applied here.

### *Other issues*

In principle, selective breeding is applicable for *M. rosenbergii* species but a number of issues should be considered. For instance, mating of a same sire with many dams was not always successful to produce a large number of half-sib families. It is resulted in bias estimates of genetic variance component due to the loss of relationship information of half-sib families (Luan *et al.*, 2012). Once the mating succeeds, the female breeders will produce thousands of small larvae. They have to be nursed separately according to their family groups until their size are suitable for tagging and communally reared in ponds. The nursing phase in separate tanks will result in common environmental effect in the studied trait. Furthermore, additional investment cost is required as more facilities have to be established in order to maintain the family groups separately.

Once the post-larvae are ready for tagging, a suitable method must be considered before they could be communally reared in pond. This could minimize common environmental effect to the population. However, physical tagging incurs extra cost and difficult to be applied to this species as they moult periodically at some stage of growth. Alternatively, DNA technology can be used for family identification soon after spawning but the cost is also substantial and laboratory-dependent. Hence, incorporating genomic selection along with the conventional selective breeding of this species is a good option to be explored. It offers essential improvements in selection accuracy over pedigree-based methods as have been applied for yellowtail kingfish (*Seriola lalandi*) (Nguyen *et al.*, 2018), Atlantic salmon (*Salmo salar*) (Tsai *et al.*, 2015) and Pacific white shrimp (*Litopenaeus vannamei*) (Wang *et al.*, 2017).

One of the main issue in giant freshwater prawn farming is their low survival during grow-out. High cannibalism among individuals may cause the reduction of the survival rate at harvest. Thus, individuals with the high breeding values of body weight might be dead before harvest. In another breeding programme of the giant freshwater prawn, similar trend of survival (20 – 46%) was reported (Vu *et al.*, 2017). Therefore, it is crucial to reduce this effect to ensure better selection option of breeders throughout this programme. Prolonged nursery phases in tanks to produce bigger individuals at tagging is an option that could achieve better survival during grow-out. However, this may result in greater common environment effect as families are kept for a long period in separate tanks.

It concluded that there is heritable additive genetic for harvest body weight in the population. Significantly genetic improvement in the trait was observed which indicating the population could be further improved. Moreover,

due to the low survival of the species during grow-out, mitigation plan is crucial for further development of the programme.

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