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3 **Heritability estimation of silver carp (*Hypophthalmichthys molitrix*) harvest traits using**  
4 **microsatellite based parentage assignment**

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## 18 **Abstract**

19 Silver carp accounts for the largest biomass production of any finfish aquaculture species in  
20 the world. In spite of its great importance as an aquacultural species, very little is known  
21 about the genetic parameters of its commercially important traits. As an initial step towards  
22 developing a selective breeding programme, heritability of harvest weight and length was  
23 estimated for a silver carp stock maintained in the NFRDMP (North West Fisheries Resource  
24 Development and Management Project) hatchery in Bangladesh. Three sets of partial  
25 factorial matings were performed (12 sires and 12 dams in each set) to produce full and half-  
26 sib families for this study. Offspring from all families produced in a set were reared  
27 communally for six months and then weighed and measured upon harvesting. Ten silver carp  
28 microsatellite markers were included in two multiplex PCR systems and were used to assign  
29 parentage to the individuals. Out of 331 offspring, 96.3% could be assigned to a single family.  
30 Statistical analyses to partition the variance components for weight and length data were  
31 carried out by the REML (Restricted Maximum Likelihood) method. Heritability for harvest  
32 weight was estimated to be 0.67 (confidence interval: 0.42-0.93) and for harvest length 0.51  
33 (confidence interval: 0.29-0.78). Despite the limited sample size, the moderate to high  
34 heritability estimates suggest that this population should respond rapidly to selective breeding  
35 for increased harvest size. In addition to this first report of quantitative genetic parameters in  
36 silver carp, this paper also describes two novel multiplexes of silver carp microsatellite  
37 markers for parentage assignment and discusses the effects of the partial factorial mating  
38 design in maintaining effective population size in this species.

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41 *Keywords:* Silver carp, *Hypophthalmichthys molitrix*, heritability, microsatellites, multiplex,  
42 parentage assignment, FAP

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49 **1. Introduction**

50 Aquaculture production of silver carp (*Hypophthalmichthys molitrix*) is the highest of any  
51 finfish species in the world; especially important in the Asia-Pacific region, the species has an  
52 annual global production of nearly 4.2 million metric tons and a value of more than 3.5 billion  
53 US dollars (FAO, 2005). Important attributes of this species are its fast growth rate which can  
54 be achieved with very low inputs, and the ability to be reared in polyculture systems (Kohinoor  
55 et al., 2002). In spite of its great significance as an aquaculture species, very little is known  
56 about the genetic architecture of important traits in silver carp. Such parameters are essential  
57 to predict the success of improving traits through selective breeding and therefore the  
58 absence of parameters is a major gap in planning of breeding programmes. This study  
59 reports heritability estimates for growth traits in a silver carp population for the first time.

60 The precision and/or bias of heritability estimation is known to be affected by a large number  
61 of factors such as breeding design, type of relatives used, number and size of the families,  
62 family rearing approach and the method of analysis (Falconer and Mackay, 1996). In general,  
63 factorial designs are considered more efficient than 'single pair' mating and nested designs,  
64 because factorial designs allow greater separation of additive genetic effects (measured by  
65 heritability) and other effects such as dominance genetic and maternal effects (Blanc, 2003;  
66 Gjerde, 2005). Moreover, factorial designs lower correlations among the estimates of  
67 breeding value for individual parents thereby reducing the risk of selecting individuals from the  
68 same full-sib family (Woolliams, 1989; Sørensen et al., 2005). High fecundity of fish and also  
69 the ability to fertilize eggs *in vitro* make factorial mating a feasible option for fish, unlike some  
70 livestock animals (Dupont-Nivet et al., 2002). However, when breeding and rearing space are  
71 the limiting factors, the application of full factorial design may not be feasible. This may, to  
72 certain extent, be overcome by applying partial factorial designs, which allow the use of more  
73 breeders.

74 For an unbiased estimate of heritability it is important to remove sources of non-genetic  
75 variance confounded with genetic variance. Variation associated with environmental factors

76 can inflate the estimates of heritability or other genetic parameters if these are not properly  
77 accounted for in models of analysis, and this may occur, for example, when the families are  
78 reared separately from each other. Most heritability studies in fish have depended on the  
79 separate rearing of families during early life stages prior to communal rearing to retain family  
80 identity until the fish were large enough for physical tagging. Since parentage can be  
81 identified using highly informative molecular markers, their development offers the opportunity  
82 to avoid this initial separation of families and the consequent problems of interpretation.  
83 Pedigree analysis using molecular markers has been used in a number of fishes for  
84 estimation of genetic parameters such as rainbow trout (Mousseau et al., 1998; Fishback et  
85 al., 2002), common carp (Vandeputte et al., 2004), Japanese flounder (Shikano, 2005) and  
86 tropical abalone (Lucas et al., 2006).

87 Therefore, in the present study heritabilities of harvest weight and length were estimated by  
88 applying a partial factorial mating design and by rearing families communally. Parentage for  
89 individual fish was determined retrospectively using microsatellite markers. The family size  
90 variation obtained was also analysed.

## 91 **2. Materials and methods**

### 92 *2.1 Fish stock*

93 The brood fish used in the present study came from the silver carp population maintained at  
94 the North-West Fisheries Resource Development and Management Project (NFRDMP)  
95 hatchery at Parbatipur, Bangladesh. This broodstock population was established in 1994,  
96 from a pure stock of silver carp fry procured from the Yangtze River in China by the  
97 Department of Fisheries (DOF) of Bangladesh with the support of the Network of Aquaculture  
98 Centres in Asia (NACA) (Sattar and Das, 2002). Since its establishment an attempt has been  
99 made to retain the genetic variation of this stock by breeding large numbers of fish, using  
100 equal numbers of males and females and taking an equal number of hatchlings at random  
101 from each single pair cross for broodstock replacement purposes, to maintain a high effective  
102 population size ( $N_e$ ).

103 The brood fish for this experiment were taken randomly from the broodstock population.  
104 During this process and setting up the crosses, no mechanism was in place to identify their  
105 relationships. Later, however, when the fish were genotyped for microsatellite markers, a pair-  
106 wise kinship analysis was carried out to assess the likelihood of full-sib mating, as described  
107 in section 2.3. All breeding and rearing activities were carried out at the NFRDMP hatchery  
108 and pond facilities.

## 109 *2.2 Breeding design and rearing*

110 Breeding was performed in three sets using a partial factorial design. The sets are referred to  
111 as Set A, Set B and Set C. Each set consisted of 12 male and 12 female parents so that over  
112 the three sets a total of 36 male and 36 female parents were used. The three sets of mating  
113 and spawning processes were arranged sequentially several days apart, so that all of the  
114 twelve available egg incubators could be used for each set.

115 Males and females were induced by injection with pituitary hormone extracts and milt and  
116 eggs were collected by hand stripping. Milt from all 12 males per set was stripped shortly  
117 before the females were ready for ovulation and held in separate containers in a refrigerator.  
118 As each female was stripped, the eggs were split into four sub-batches of equal volume and  
119 each sub-batch of eggs was fertilized with milt from a single male, i.e. eggs from one female  
120 were fertilized with milt from four males. The milt of a single male was used to fertilise eggs of  
121 four different females. The four sub-batches of fertilized eggs from each female were water  
122 hardened and then pooled into a single incubator for hatching. An equal number of hatched  
123 fry from each incubator was taken for communal rearing in a single nursery pond for each set  
124 (i.e. three such ponds in total). Once the first set (Set A) of fry had been removed from the  
125 incubators, Set B crosses were produced using the same design but with different males and  
126 females. Likewise, Set C followed Set B. Across the three sets, a total of 144 full-sib families  
127 were produced from the 36 males and 36 females. The breeding designs for the three sets  
128 are shown in Appendices 1-3 along with the number of offspring at harvest from each mating.

129 At the fingerling stage a random sample of 120 fish was taken from each nursery pond and  
130 stocked in one of the three grow-out ponds until harvesting at 6 months. Random sampling  
131 gave an *a priori* expectation of 10 offspring per parent. To mimic general commercial

132 conditions the fish in this experiment were also reared in polyculture with other carp species,  
133 and all three ponds had the same mix of species in the same proportions. The weight and  
134 total length of each silver carp were recorded at harvesting for heritability analysis.

135 Fin samples from the parents were collected during the stripping process and from offspring  
136 of all three sets during the harvesting process. Samples were preserved in 95% ethanol for  
137 future DNA analysis.

### 138 *2.3 Parentage and kinship analysis using microsatellite markers*

139 Microsatellite profiles of the 72 parents bred in the present experiment (along with another  
140 eight individuals from same population) were used to characterise 16 new silver carp  
141 microsatellite markers (see Gheyas et al. 2006). From these markers, ten loci (*Hmo11*, *13*,  
142 *25*, *26*, *33*, *34*, *36*, *37*, *39* and *40*) were selected for parentage and kinship analyses based on  
143 their polymorphism, repeatability and ease of amplification in multiplex PCR. The number of  
144 alleles in these 10 loci varied between 5-16 with an average of 8 alleles/locus and the  
145 expected heterozygosity varied between 0.46 and 0.90. All these markers were in Hardy-  
146 Weinberg equilibrium in the sampled population.

147 For genotyping of microsatellite loci, DNA was extracted from fin samples using the Chelex  
148 method (Estoup et al., 1996). PCR amplifications of the microsatellite markers were  
149 performed in two multiplex reactions (Table 1). Apart from the variable concentrations of  
150 primers which were needed to achieve similar intensity of the amplification products,  
151 concentrations of all other reagents in both the multiplex PCRs were identical: 2x buffer II  
152 (ABgene), 280  $\mu$ M dNTP each, 2 mM MgCl<sub>2</sub>, 1.5 units of *Taq* DNA polymerase, and 100 ng  
153 DNA. The thermocycling conditions were set as follows: an initial denaturation step at 95°C  
154 for 2 min 30 s followed by 50 s at 94°C, 50 s at 60°C (for both multiplex systems) and 1 min at  
155 72°C for 30 cycles and a final extension step at 72°C for 35 min. Fragment analysis was  
156 performed using semi-automated ABI PRISM 377 DNA sequencer (Applied Biosystem,  
157 Perkin-Elmer). Parentage assignment was performed using the exclusion method by the FAP  
158 (Family Assignment Programme) programme (Taggart, 2007). Up to two allele mismatches  
159 were tolerated for parentage assignment when the offspring were assigned to single families.

160 The microsatellite markers were also used for pairwise sib-ship analysis among the 72  
 161 parents using the KINGROUP (v1.050513) programme (Konovalov et al. 2004). A null  
 162 hypothesis of “Unrelated” was tested against the primary (alternative) hypothesis of “Full-sib”  
 163 using the Pairwise Likelihood Ratio approach. Under this approach, the programme  
 164 calculates the likelihood of the relationship for both the null and alternative hypotheses for  
 165 each pair of individuals using the population allele frequency and the genotypes of the pair.  
 166 The programme then tests the likelihood ratio and assesses its statistical significance using  
 167 simulation of pairs generated according to the null hypothesis. In the present analysis,  
 168 significance of the ratios was tested at  $P=0.05$  using 10,000 simulated unrelated pairs.

169 *2.4 Estimation of family contribution*

170 The number of observed offspring per male and per female was compared to an expected  
 171 binomial distribution with Pearson’s  $\chi^2$  (chi-square) goodness-of-fit test by GenStat (ver. 8). To  
 172 investigate the possible impact of variation in family size on effective population size ( $N_e$ ),  
 173 relevant for random selection only,  $N_e$  was calculated by the following formula taken from  
 174 Vandeputte et al. (2004):

175 
$$N_e = \frac{4(N - 2)}{(K_s + \frac{V_s}{K_s}) + (K_d + \frac{V_d}{K_d}) - 2} \dots\dots\dots (1)$$

176 Where  $N$  is the total number of offspring,  $K_s$  and  $K_d$  are the mean numbers of offspring per sire  
 177 and per dam, and  $V_s$  and  $V_d$  are the variances of sire and dam family sizes.

178 *2.5 Partitioning of variance components and heritability calculation*

179 The harvest length and weight data of the offspring from all three sets were combined for  
 180 partitioning of variance components. Analysis was performed by REML method (in GenStat  
 181 ver. 8) by fitting the following linear mixed model:

182 
$$Y_{rijk} = m + p_r + s_i + d_j + e_{rijk} \dots\dots\dots (2)$$

183 Here  $Y_{rijk}$  is the length or weight of the  $k^{\text{th}}$  individual of sire  $i$  and dam  $j$  in the  $r^{\text{th}}$  set;  $m$  is the  
 184 overall mean;  $p_r$  is the fixed effect of pond (or set)  $r$  for  $r = 1, 2, 3$ ;  $s_i$  is the effect of sire  $i$ , for  $i$

185 = 1, 2..... 36;  $d_j$  is the effect of dam  $j$ , for  $j = 1, 2.....36$ ; and  $e_{ijk}$  is the residual term for the  
 186 observation. Note that all nursery and rearing pond effects are confounded with Set together  
 187 with effects of spawning date and any genetic sampling deviations from the larger base  
 188 population. The term  $s_i$  was assumed to be independent and randomly distributed as  $N(0,$   
 189  $\sigma_s^2)$ ; similar assumptions were made for the  $d_j$  and  $e_{ijk}$  but with variances  $\sigma_D^2$  and  $\sigma_e^2$   
 190 respectively. To test for dominance variance, a sire by dam interaction was also introduced in  
 191 the random model, but was not found to be statistically significant and hence was dropped in  
 192 further analyses.

193 Phenotypic variance was calculated as  $\sigma_p^2 = \sigma_s^2 + \sigma_D^2 + \sigma_e^2$  ..... (3).

194 Heritability and associated standard errors were calculated using the VFUNCTION procedure  
 195 (GenStat ver. 8) as follows:

196 From sire variance only (covariance of paternal half-sibs) as  $h_s^2 = 4 \times \sigma_s^2 / \sigma_p^2$  ..... (4)

197 And from dam variance only (covariance of maternal half-sibs) as  $h_D^2 = 4 \times \sigma_D^2 / \sigma_p^2$  .....(5)

198 In estimating heritability, the sex of the offspring was not taken into account mainly for two  
 199 reasons. First, it is not possible to identify the gender of silver carp at six months of age (when  
 200 the fish were harvested) without carrying out histology. Secondly, the authors are unaware of  
 201 any sexual growth dimorphism in this species at this stage.

202 The fixed effect of Set was tested using a Wald statistic with 2 degrees of freedom (df) while  
 203 the statistical significance of the random terms in the model (i.e. sire and dam components)  
 204 was tested by likelihood ratio test (LRT) using the change in deviance (- 2 log likelihood) that  
 205 resulted from dropping the corresponding term from the full model. The statistical significance  
 206 of the difference between the sire and dam components was also tested with a LRT by the  
 207 following iterative procedure. The null and alternative hypotheses were defined as  
 208  $H_0: \sigma_s^2 = \sigma_D^2$  and  $H_1: \sigma_s^2 \neq \sigma_D^2$  respectively; following  $H_0$ , the ratio of the sire and dam  
 209 variance components to the residual variance was constrained to be equal to  $\gamma = \sigma_s^2 / \sigma_e^2 =$   
 210  $\sigma_D^2 / \sigma_e^2$ . The iteration was carried out by varying the  $\gamma$  values over a sufficient range to observe  
 211 the minimum deviance, since the value of  $\gamma$  associated with the minimum deviance provided

212 the best fit to the available data under  $H_0$ . The  $H_1$  was then tested against  $H_0$  by comparing  
 213 the minimum deviance under  $H_0$  with the deviance obtained under  $H_1$ , which is that obtained  
 214 from fitting the unconstrained model described by equation 2. The difference between these  
 215 two was then compared to the  $\chi_1^2$  (chi-square value at 1df at  $P=0.05$ ). A 95% confidence  
 216 interval for the combined estimate of heritability was calculated from the deviance profile as  
 217 the range of  $\gamma$  within which the deviance value was within 3.84 (critical value  $\chi_1^2$  at  $P=0.05$ ) of  
 218 its minimum value. For a given value of  $\gamma$  the combined estimate of heritability was:

$$219 \quad h_c^2 = \frac{4\gamma}{2\gamma + 1} \dots\dots\dots (6)$$

220

## 221 **3. Results**

### 222 *3.1 Parentage assignment*

223 A summary of the parentage assignment with 10 microsatellite markers is presented in Table  
 224 2. From a preliminary prediction analysis by FAP, the 10 markers used in the current study  
 225 were expected to achieve 98.7% single-family assignment under the present family structure.  
 226 Out of 331 offspring from all the three sets, a total of 319 offspring (96.4%) could actually be  
 227 assigned to single parental pairs. From the pair-wise kinship analysis of the parents, the  
 228 “unrelated” null hypothesis was rejected in favour of full-sibs in 4.1% of the cases (after  
 229 adjustment for type I and type II error).

### 230 *3.2 Family representation*

231 The expected number of offspring from all sires and dams in a set was the same. The sires  
 232 were represented by 5-17 offspring in Set A, 3-17 offspring in Set B and 1-16 offspring in Set  
 233 C. Chi square tests within each of the three sets rejected the null hypothesis that observed  
 234 variation arose from simple binomial sampling ( $P<0.05$  in each). In comparison, dams were  
 235 represented by 0-20 offspring in Set A, 1-17 offspring in Set B and 2-20 offspring in Set C:  
 236 likewise the variance in observed distribution of progeny across dams within each set was  
 237 also greater than expected from simple binomial sampling ( $P<0.001$  in each). The exact  
 238 sources of the extra-binomial variation cannot be identified; however sampling errors would

239 have accumulated through sampling of hatchlings from incubators and of fingerlings from  
240 nursery ponds, and differential survival rates cannot be excluded. The very high rate of  
241 success in parentage assignment suggests assignment errors will not be a major source of  
242 this additional variance. Taking all the sires and dams from the three sets, the average half-  
243 sib family size was calculated to be  $8.86 \pm 4.17$  (mean  $\pm$  SD) for sires and  $8.86 \pm 5.42$  for  
244 dams, corresponding to coefficients of variation (CV) of 47% and 61% for sires and dams  
245 respectively.

246 Each set of breeding was performed in a way such that 48 full-sib families would be created.  
247 However, due to the fact that only a small number of fingerlings (120 fingerlings per set) were  
248 finally retained for rearing in grow-out ponds (due to the size of the ponds available) along  
249 with the variance in family size, none of the sets contained offspring from all the full-sib  
250 families. Only 36, 39 and 32 families produced surviving, allocated offspring at harvest in Sets  
251 A, B and C respectively. Considering the variability of the family size, the overall  $N_e$  was  
252 estimated as 60.40, i.e. there was a 16.11% reduction in the actual  $N_e$  from the expectation of  
253 equal representation of the offspring of 36 males and 36 females.

### 254 *3.3 Estimation of genetic parameters*

255 Since a total of 319 offspring from the three sets could be assigned to single families, the  
256 harvest weight and total length data from only these individuals were analysed for heritability  
257 estimations. The means ( $\pm$  SD) of harvest weight and length were found to be  $405.3 \pm 79.6$  g  
258 and  $33.25 \pm 2.06$  cm respectively. Coefficient of variation of weight was 19.6% while that of  
259 total length was 6.2%. The length and weight traits were highly correlated with  $r$  values  
260 ranging between 0.79 - 0.93 in different sets ( $P < 0.001$  for all). The Wald test indicated that the  
261 effect of Set had a highly significant influence ( $P < 0.001$ ) on the harvest parameters. The best  
262 growth values were observed for fish in Set A and the lowest growth values in Set C, with a  
263 difference of 43% greater average weight and 11% higher average length in Set A compared  
264 to Set C.

265 Table 3 presents heritability estimates for harvest weight and length based on covariances of  
266 paternal half-sib (sire variance) and maternal half-sib (dam variance) and also a combined  
267 estimate using both the covariances. Using only sire variance, the heritability values for

268 harvest weight and length were estimated to be  $0.75 \pm 0.24$  and  $0.82 \pm 0.26$  respectively.  
269 Using only dam variance, heritabilities were estimated to be  $0.55 \pm 0.25$  for weight and  $0.18 \pm$   
270  $0.16$  for length. Although the heritability estimates from individual sire and dam components  
271 were quite different for both the traits, particularly for length, there was no evidence to reject  
272 the null hypothesis that sire and dam components are equal ( $P > 0.05$ ). Therefore, the  
273 combined estimates of heritability provided the most precise estimates: 0.67 for harvest  
274 weight (95% confidence interval: 0.42–0.93) and 0.51 (95% confidence interval: 0.29–0.78)  
275 for harvest length. The deviance profiles and confidence intervals for the combined estimates  
276 of heritability are shown in Figures 1a and 1b. For both length and weight data the variances  
277 due to the interaction between sire and dam were found to be small and non-significant,  
278 indicating negligible dominance variance or other factors that would be specific to full-sib  
279 families.

## 280 **4. Discussion**

281 This paper highlights a number of important aspects for silver carp genetics. To the best of  
282 our knowledge, this is the first published report of heritability of growth parameters in silver  
283 carp despite the commercial significance of the species. The paper also highlights the  
284 usefulness of microsatellite markers in pedigree analysis in silver carp and reports two novel  
285 multiplex PCR system as tools for rapid genotyping. The paper also assesses the usefulness  
286 of a partial factorial design in silver carp breeding and the impact that differential survival  
287 might have on the effective population size (in the absence of any selection).

### 288 *4.1 Estimates of heritability and non-additive components*

289 The present study reports high estimates of heritability for harvest weight and total length for  
290 the silver carp stock at the NFRDMP hatchery indicating the potential for rapid improvement  
291 of the population through selective breeding. The study, however, suffers from certain  
292 limitations, the most important of which is a relatively small sample size leading to the  
293 moderately wide confidence intervals for the estimates. However the substantial estimates of  
294 genetic variance also contribute to the size of these confidence intervals, since for random  
295 effects, unlike fixed effects, the magnitude of the effect influences the standard error (Swiger  
296 et al. 1964). Unfortunately, due to a lack of pond space, it was not possible to use a larger

297 sample size for the experiment without abandoning the polyculture production system  
298 which is the norm in Bangladesh. It was also not feasible to assess the genetic correlations  
299 among the sets by using common males in all sets; hatchery staff considered that holding  
300 males in confinement for reuse in different sets would be too stressful for the fish and if  
301 alternatively the males were returned to the large broodstock pond after use in one mating  
302 set, it would be difficult to find the same fish again subsequently. Cryopreservation of milt was  
303 also not feasible.

304 The design and analysis in the present study produced standard errors that are similar to  
305 published estimates for other fish species (e.g. Mousseau et al., 1998; Henryon et al., 2002).  
306 There is no evidence of any factor other than chance for the differences (although not  
307 statistically significant) between the estimates from sire and dam variances. Notwithstanding  
308 the size of the study, the confidence intervals for heritability calculated in this experiment  
309 clearly establish that heritabilities are moderate to good for both length and weight  
310 parameters and of a magnitude that would promote good response in mass selection.

311 The variation between sets that was observed was large and, in the view of the authors,  
312 primarily represented the differences in condition of the ponds, and spawning and rearing  
313 times. The grow-out pond for Set C fish suffered from a heavy infestation of filamentous  
314 algae, which possibly reduced the phytoplankton abundance (natural food of silver carp) by  
315 competing for nutrients and in turn reducing the growth of the fish. Moreover, the spawning  
316 dates between the consecutive sets varied by a week. The Set A breeding was initiated first  
317 and the Set C breeding last — a gap of about two weeks between Set A and Set C. The  
318 whole breeding and rearing experiment started at the end of August when the daylight and  
319 temperature were gradually decreasing and hence a gap of two weeks might have an  
320 important consequence. The variation between sets, whilst an important factor to be  
321 considered during implementation, does not preclude estimation of heritability since the set  
322 difference was included as a fixed effect in the model. Neither does it preclude the  
323 implementation of selective breeding since it is always possible to apply mass selection within  
324 each set.

325 *4.2 Use of microsatellite markers for pedigree analysis*

326 Microsatellite markers were used for parentage analysis of experimental samples and also  
327 to assess the pair-wise relationship between parents in the present study. Although the use of  
328 microsatellite markers for pedigree analysis offers a number of benefits such as helping to  
329 remove environmental bias, preventing breeding between close relatives and reducing the  
330 cost of rearing families separately, the cost of incorporating genotyping as routine practice  
331 can be quite high for a breeding programme operated by a small hatchery. Nevertheless,  
332 studies like the present one are important as these generate valuable information regarding  
333 genetic parameters for future breeding programmes (Vandeputte et al., 2004). In the present  
334 study, parentage could be successfully assigned to 96% of the offspring using 10  
335 microsatellite markers. Although the panel of 10 markers provided high family assignment  
336 rates with the 48 families in each set, the assignment success might be reduced if the number  
337 of potential families or parents increased considerably. More polymorphic and informative  
338 markers would, therefore, be required for a greater number of parents.

339 The present study also demonstrates how molecular markers can give insight into the level of  
340 full-sib mating when relationships between parents are unknown. The same ten markers were  
341 used for pair-wise relationship analysis, and the conclusion was that the proportion of full-sib  
342 matings was small and unlikely to have had a significant effect on the outcome of the  
343 heritability analysis. Although these ten markers produced good parentage assignment rate,  
344 generally more markers are required to achieve a similar level of power with sib-ship analysis  
345 when the potential parents are unknown. According to Blouin (2003) about 15-20 unlinked  
346 markers are required to distinguish full-sibs from unrelated with high power (e.g. power of  
347 0.9). Recently, Liao et al. (2007) have reported 41 new microsatellite markers from silver  
348 carp. This would allow choosing combinations of more polymorphic markers and improve the  
349 power of parentage assignment and kinship analysis. Despite its distinct benefit, however, the  
350 use of microsatellite markers in pedigree analysis would require justification in terms of cost-  
351 benefit analysis.

352

353 *4.3 Effect of breeding design on family representation and effective population size*

354 Differential representation of families and parents in the progeny group is a common  
355 phenomenon in fish breeding programmes, leading to a negative impact on the  $N_e$ , rate of  
356 inbreeding and genetic variance of the population. The potential reasons for variation in family  
357 representation are the differences in reproductive ability of brood fish, fertilization rates of  
358 eggs, hatching rates and survival of offspring. Breeding strategy and design can play crucial  
359 roles in improving this phenomenon and maintaining genetic diversity. The adoption of a  
360 partial factorial design (Woolliams, 1989) along with some other aspects (as suggested by  
361 Fishback et al., 2002) – such as controlled mating by stripping the eggs and sperm, dividing  
362 eggs from each female into equal aliquots and fertilizing each aliquot separately with milt from  
363 a single male and finally mixing an equal number of viable progeny from each female in  
364 communal nursery - minimized the family size variance in the present study as far as  
365 possible. The loss of putative  $N_e$  in the present study was only 16.11%. In a similar study on  
366 common carp Vandeputte et al. (2004) reported a reduction of 21% in putative  $N_e$ . No report  
367 is available on  $N_e$  from mass spawning events either in silver carp or common carp with which  
368 the above results can be directly compared. Nevertheless, these losses of  $N_e$  are very small  
369 when compared to those reported for other species under mass spawning. For instance, in  
370 gilthead seabream the loss of  $N_e$  ranged from 67-73% (Brown et al., 2005); in red sea bream  
371 it was as high as 75% (Perez-Enriquez et al., 1999) and in Japanese flounder  $N_e$  decreased  
372 by 80% in the first generation (Sekino et al., 2003). It is to be noted, however, that the  
373 offspring were still unselected in studied silver carp population: further reduction in  $N_e$  would  
374 be expected from the process of selecting broodstock based on phenotypic traits (Woolliams  
375 and Bijma, 2000). Moreover no account has been taken of any overlapping generation  
376 structure. For these reasons the value of  $N_e$  given here must be interpreted as addressing just  
377 one component of a more complex whole.

378 A number of studies have demonstrated the advantages of factorial design over other designs  
379 such as hierarchical design, single pair mating etc. in maintaining genetic diversity  
380 (Woolliams, 1989; Sørensen et al., 2005; Dupont-Nivet et al., 2006). For instance, using a  
381 deterministic approach Woolliams (1989) showed that when compared at the same level of  
382 genetic progress factorial designs had greater  $N_e$  than hierarchical designs. Factorial breeding  
383 designs create both paternal and maternal half-sibs and reduce the number of full-sibs which

384 lowers the risk of selecting many individuals from the same full-sib family and hence  
385 reduces the variance in the family size after selection (Sørensen et al., 2005). Although  
386 different studies have predicted a full factorial design to be more effective than partial factorial  
387 designs in achieving genetic progress and in maintaining genetic variation (Woolliams, 1989;  
388 Dupont-Nivet et al., 2006), the latter designs offer more practicability in terms of handling and  
389 hence can be a good alternative (Dupont-Nivet et al., 2006).

#### 390 *4.4 Conclusions*

391 In conclusion it can be said that although the sample size was limited (36 dams, 36 sires and  
392 331 offspring analysed), the present study provides the first estimates of genetic parameters  
393 of growth traits in silver carp. Even using the lower bounds of the 95% confidence intervals for  
394 heritability for length and weight, the estimates were of moderate size and sufficient to  
395 indicate the potential for a good response to selective breeding for harvest size in this  
396 species. This work demonstrated the practicality of the partial factorial mating scheme in a  
397 situation with facilities similar to small commercial hatcheries, and the potential efficiency of  
398 the design in maintaining a high level of  $N_e$  for silver carp. Finally the study also showed that  
399 two novel microsatellite marker multiplexes could be effectively used in pedigree analysis in  
400 this species.

401

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483 selection. *Genetics* 154, 1851-1864.

484 **Table 1. Multiplex PCRs used for parentage assignment in silver carp. See text for**  
 485 **further information about PCR conditions.**

<b>Multiplex 1</b>	<b>Primer concentration (pmol/μl)*</b>	<b>Multiplex 2</b>	<b>Primer concentration (pmol/μl)*</b>
<i>Hmo25</i>	0.06	<i>Hmo11</i>	0.05
<i>Hmo26</i>	0.25	<i>Hmo13</i>	0.045
<i>Hmo36</i>	0.07	<i>Hmo33</i>	0.05
<i>Hmo37</i>	0.09	<i>Hmo34</i>	0.20
<i>Hmo39</i>	0.09	<i>Hmo40</i>	0.06

486 **See text for further information about PCR conditions.**

487 \* Denotes primer concentration for both forward and reverse primers

488 **Table 2. FAP based parentage assignment prediction and actual parentage**  
 489 **assignment result.**

	<b>Set A</b>	<b>Set B</b>	<b>Set C</b>	<b>Total</b>
Total number of individuals initially stocked	120	120	120	360
Total number of individuals surviving to harvest	114	117	100	331
Prediction for parentage assignment success	99.6%	97.8%	98.7%	98.7%
Actual assignment to a single family	114 (100.0%)	111 (94.9%)	94 (94.0%)	319 (96.4%)
Assigned to 2 families	--	3 (2.6%)	5 (5.0%)	8 (2.4%)
Assigned to more than 2 families	--	3 (2.6%)	1 (1.0%)	4 (1.2%)

490 **Table 3: Heritability estimates of silver carp harvest traits.**

<b>Calculation method</b>	<b>Weight <math>h^2</math></b>	<b>Total length <math>h^2</math></b>
Based on paternal half-sib	0.76 ± 0.25	0.82 ± 0.26
Based on maternal half-sib	0.55 ± 0.24	0.18 ± 0.16
Based on full-sib (manual	0.67	0.51
iteration with equal sire and	(Confidence interval:	(Confidence interval:
dam components)	0.42-0.93)	0.29-0.78)

**List of Figures**

491

492 **Fig. 1:** The deviance profile of various heritability estimates for (a) harvest length and

493 (b) harvest weight from manual iteration considering equal sire and dam components.

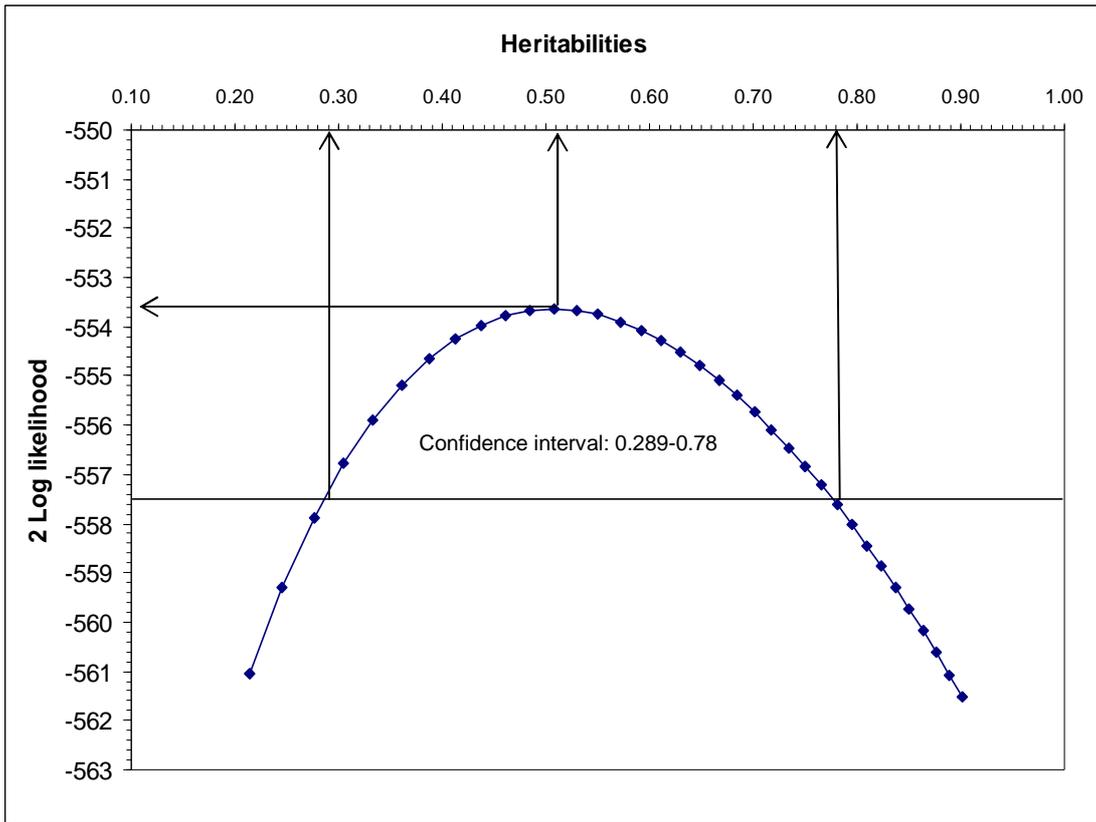
494 The horizontal lines indicate the minimum deviance (-2 log likelihood) and the

495 threshold for the deviance with  $\chi^2$  significance test at 0.05. The vertical arrows

496 indicate the maximum likelihood estimate of heritability and its 95% confidence

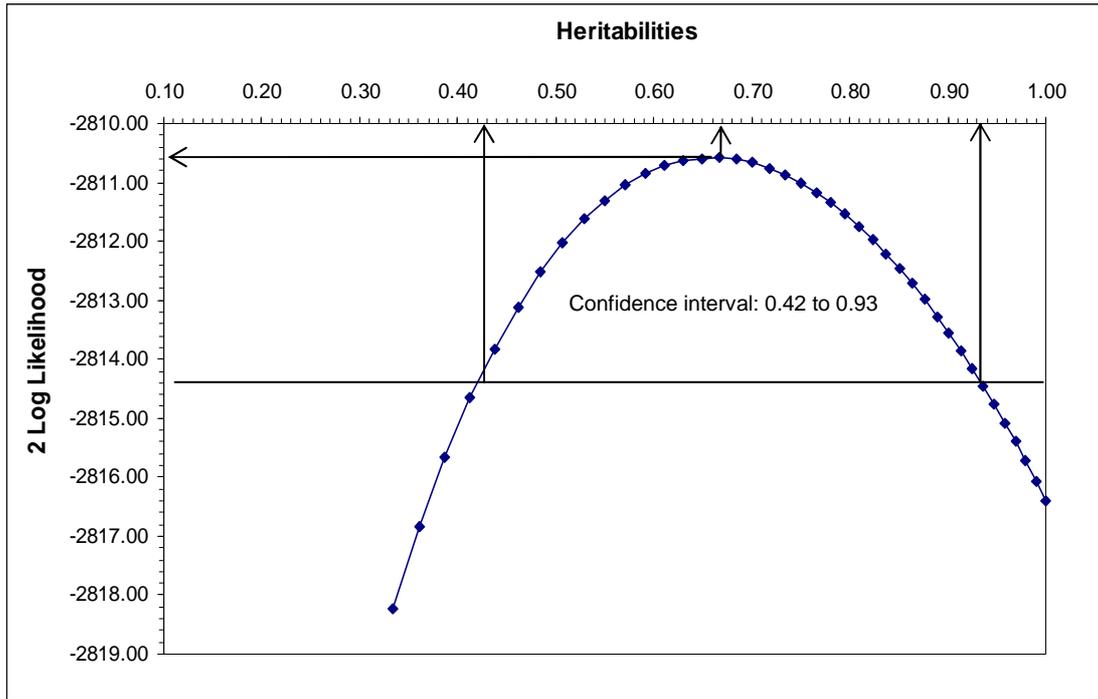
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Figure 1a



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Figure 1b

536 **Appendix 1: Mating structure and number of assigned offspring from sires and dams**

537 **in Set A (the shaded squares indicate allowed matings).**

538

Sire	Dam												Progeny per sire
	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12	
S01	1	2				5				1			9
S02		1	7				0				1		9
S03			0	0				3				2	5
S04	4			0	0				0				4
S05		7			1	6				1			15
S06			1			4	0				0		5
S07				1			0	2				2	5
S08	4				0			2	5				11
S09		3				5			0	0			8
S10			5				0			1	5		11
S11				2				4			7	4	17
S12	2				4				5			4	15
Progeny per dam	11	13	13	3	5	20	0	11	10	3	13	12	114

539 **Appendix 2: Mating structure and number of assigned offspring from sires and dams**  
 540 **in Set B (the shaded squares indicate allowed matings).**

Sire	Dam												Progeny per sire
	D13	D14	D15	D16	D17	D18	D19	D20	D21	D22	D23	D24	
S13	2	0				1				2			5
S14		1	1				1				0		3
S15			1	3				0				3	7
S16	3			4	1				1				9
S17		1			4	1				2			8
S18			2			4	0				2		8
S19				3			3	0				3	9
S20	6				1			0	0				7
S21		12				2			0	3			17
S22			1				4			6	4		15
S23				6				1			4	3	14
S24	6				2				0			1	9
Progeny per dam	17	14	5	16	8	8	8	1	1	13	10	10	111

541 **Appendix 3: Mating structure and number of assigned offspring from sires and dams**

542 **in Set C (the shaded squares indicate allowed matings).**

Sire	Dam												Progeny per sire
	D25	D26	D27	D28	D29	D30	D31	D32	D33	D34	D35	D36	
S25	0	2				7				0			9
S26		0	3				5				1		9
S27			7	3				0				1	11
S28	0			0	0				1				1
S29		0			2	3				2			7
S30			7			0	0				2		9
S31				1			3	2				4	10
S32	0				8			2	6				16
S33		0				1			2	0			3
S34			3				2			1	0		6
S35				2				1			0	0	3
S36	2				5				2			1	10
Progeny per dam	2	2	20	6	15	11	10	5	11	3	3	6	94

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