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

Almas A.Gheyas[a,b], John A. Woolliams[b], John B. Taggart[a], Mohammed A. Sattar[d], Topan K. Das[d], Brendan J. McAndrew[a], David J.Penman[a,*]

[a] *Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK*

[b] *The Roslin Institute and Royal (Dick) School of Veterinary Science, University of Edinburgh, Roslin, Midlothian EH25 9PS, Scotland, UK*

[c] *Northwest Fisheries Resource Development and Management Project (NFRDMP-3), Parbatipur, Dinajpur, Bangladesh.*

*Corresponding author: Telephone +44 1786 467901; Fax +44 1786 472133; Email djp1@stir.ac.uk

Abstract

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

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1 Fish stock

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

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

2.2 Breeding design and rearing

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

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

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

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

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

2.3 Parentage and kinship analysis using microsatellite markers

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

For genotyping of microsatellite loci, DNA was extracted from fin samples using the Chelex method (Estoup et al., 1996). PCR amplifications of the microsatellite markers were performed in two multiplex reactions (Table 1). Apart from the variable concentrations of primers which were needed to achieve similar intensity of the amplification products, concentrations of all other reagents in both the multiplex PCRs were identical: 2x buffer II (ABgene), 280 µM dNTP each, 2 mM $MgCl_2$, 1.5 units of *Taq* DNA polymerase, and 100 ng DNA. The thermocycling conditions were set as follows: an initial denaturation step at 95^{o}C for 2 min 30 s followed by 50 s at 94^{o}C, 50 s at 60^{o}C (for both multiplex systems) and I min at 72^{o}C for 30 cycles and a final extension step at 72^{o}C for 35 min. Fragment analysis was performed using semi-automated ABI PRISM 377 DNA sequencer (Applied Biosystem, Perkin-Elmer). Parentage assignment was performed using the exclusion method by the FAP (Family Assignment Programme) programme (Taggart, 2007). Up to two allele mismatches were tolerated for parentage assignment when the offspring were assigned to single families.

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

2.4 Estimation of family contribution

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

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

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

2.5 Partitioning of variance components and heritability calculation

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

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

Here Y_{rijk} is the length or weight of the k^{th} individual of sire i and dam j in the r^{th} set; m is the 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

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

Phenotypic variance was calculated as $\sigma_P^2 = \sigma_S^2 + \sigma_D^2 + \sigma_e^2$ …… (3).

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

From sire variance only (covariance of paternal half-sibs) as $h_S^2 = 4 \times \sigma_S^2 / \sigma_P^2$ …… (4)

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

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

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

the best fit to the available data under H_0. The H_1 was then tested against H_0 by comparing the minimum deviance under H_0 with the deviance obtained under H_1, which is that obtained from fitting the unconstrained model described by equation 2. The difference between these two was then compared to the χ_1^2 (chi-square value at 1df at *P*=0.05). A 95% confidence interval for the combined estimate of heritability was calculated from the deviance profile as the range of *γ* within which the deviance value was within 3.84 (critical value χ_1^2 at *P*=0.05) of its minimum value. For a given value of *γ* the combined estimate of heritability was:

$$h_C^2 = \frac{4\gamma}{2\gamma+1} \quad \text{..... (6)}$$

3. Results

3.1 Parentage assignment

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

3*.2 Family representation*

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

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

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

3.3 Estimation of genetic parameters

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

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

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

4. Discussion

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

4.1 Estimates of heritability and non-additive components

The present study reports high estimates of heritability for harvest weight and total length for the silver carp stock at the NFRDMP hatchery indicating the potential for rapid improvement of the population through selective breeding. The study, however, suffers from certain limitations, the most important of which is a relatively small sample size leading to the moderately wide confidence intervals for the estimates. However the substantial estimates of genetic variance also contribute to the size of these confidence intervals, since for random effects, unlike fixed effects, the magnitude of the effect influences the standard error (Swiger 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*

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

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

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

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

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

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

4.4 Conclusions

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

Acknowledgements

AAG's PhD was funded by a Commonwealth Scholarship. This research was supported by the UK Department for International Development, Aquaculture and Fish Genetics Research Programme (project R7590). We would like to thank the staff of the NFRDMP, Parbatipur, who helped in the breeding, rearing and harvesting of the silver carp.

References

Blanc, J.M., 2003. Comparison of experimental designs for estimating quantitative genetic parameters in fish. Aquacult. Res. 34, 1099-1105.

Blouin, M.S., 2003. DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends Ecol. Evol. 18, 503-511.

412 Brown, C., Woolliams, J.A., McAndrew, B.J., 2005. Factors influencing effective population
413 size in commercial populations of gilthead seabream, *Sparus aurata*. Aquaculture 247,
414 219-225.
415 Dupont-Nivet, M., Vandeputte, M., Chevassus, B., 2002. Optimization of factorial mating
416 designs for inference on heritability in fish species. Aquaculture 204, 361-370.
417 Dupont-Nivet, M., Vandeputte, M., Haffray, P., Chevassus, B., 2006. Effect of different
418 mating designs on inbreeding, genetic variance and response to selection when applying
419 individual selection in fish breeding programme. Aquaculture 252, 161-170.
420 Estoup, A., Largiadr, C.-R., Perrot, E., Chourrout, D.,1996. One-tube rapid DNA extraction for
421 reliable PCR detection of fish polymorphic markers and transgenes. Mol. Mar. Biol.
422 Biotechnol. 5: 295-298.
423 Falconer, D.S., Mackay, F.C., 1996. Introduction to Quantitative Genetics. 4th edn. Longman,
424 Harlow, 464 pp.
425 FAO 2005. http://www.fao.org/fi/statist/statist.asp.
426 Fishback, A.G., Danzmann, R.G., Ferguson, M.M., Gibson, J.P., 2002. Estimates of genetic
427 parameters and genotype by environment interactions for growth traits of rainbow trout
428 (*Oncorhynchus mykiss*) as inferred using molecular pedigree. Aquaculture 206, 137-150.
429 Gheyas, A. A., Cairney, M., Gilmour, A.E., Sattar, M.A., Das, T.K., McAndrew, B.J., Penman,
430 D.J., Taggart, J.B., 2006. Characterization of microsatelllite loci in silver carp
431 (*Hypophthalmichthys molitrix*), and cross-amplification in other cyprinid species. Mol.
432 Ecol. Notes 6, 656-659.
433 Gjerde, B., 2005 Design of breeding programs. In: T. Gjedrem (Ed.) Selection and Breeding
434 Programs in Aquaculture. Springer, Netherlands, pp. 173-196.
435 Henryon, M., Jojumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht,
436 W.J., 2002. Genetic variation for growth rate, feed conversion efficiency, and disease
437 resistance exists within farmed population of rainbow trout. Aquaculture 209, 59-76.
438 Kohinoor, A.H.M., Islam, M.S., Mia, M.Y., Rahman, M.A., Hussain, M.G., Mazid, M.A.,
439 McAndrew, B.J., Penman, D.J., 2002. Evaluation of different stocks of Chinese carps in
440 Bangladesh: design and preliminary results. In: Penman, D.J., Hussain, M.G., McAndrew,
441 B.J., Mazid, M.A., (Eds.) Proceedings of a Workshop on Genetic Management and

Improvement Strategies for Exotic Carps in Bangladesh. 12-14 February 2002, Dhaka, Bangladesh: Bangladesh Fisheries Research Institute, Mymensingh, pp. 59-67.

Konovalov, D.A., Manning C., Heneshaw, M.T., 2004. KINGROUP: a program for pedigree relationship reconstruction and kin group assignments using genetic markers. Mol. Ecol. Notes 4: 779-782.

Liao, M., Yang, G., Wang, X., Wang, D., Zou, G. Wei, Q., 2007. Development of microsatellite DNA markers of silver carp (*Hypophthalmichthys molitrix*) and their cross-species application in bighead carp (*Aristichthys nobilis*). Mol. Ecol. Notes 7: 95-99.

Lucas, T., Macbeth, M., Degnan, S.M., Knibb, W., Degnan, B.M., 2006. Heritability estimate for growth in the tropical abalone *Haliotis asinina* using microsatellite to assign parentage. Aquaculture 259, 146-152.

Mousseau, T.A., Ritland, K., Heath, D.D., 1998. A novel method of estimating heritability using molecular markers. Heredity 80, 218-224.

Perez-Enriquez, R. Takagi, M., Taniguchi, N., 1999. Genetic variability and pedigree tracing of a hatchery-reared stock of red sea bream (*Pagrus major*) used for stock enhancement, based on microsatellite DNA markers. Aquaculture 173, 413-423.

Sattar, M.A., Das, T.K., 2002. Broodstock management of Chinese carps and dissemination strategy at NFEP, Parbatipur. In: Penman, D.J., Hussain, M.G., McAndrew, B.J., Mazid, M.A., (Eds.) Proceedings of a Workshop on Genetic Management and Improvement Strategies for Exotic Carps in Bangladesh. 12-14 February 2002, Dhaka, Bangladesh: Bangladesh Fisheries Research Institute, Mymensingh, pp. 43-50.

Sekino, M., Saitoh, K., Yamada, T., Kumagai, A., Hara, M., Yamashita, Y., 2003. Microsatellite-based pedigree tracing in a Japanese flounder *Paralichthys olivaceus* hatchery strain: implications for hatchery management related to stock enhancement program. Aquaculture 221, 255-263.

Shikano, T., 2005. Marker-based estimation of heritability for body color variation in Japanese flounder *Paralichthys olivaceus.* Aquaculture 249, 95-105.

Sørensen, A.C., Berg, P., Woolliams, J.A., 2005. The advantage of factorial mating under selection is uncovered by deterministically predicted rates of inbreeding. Genet. Sel. Evol. 37, 57-81.

Swiger, L.A., Harvey, W.R., Everson, D.O., Gregory, K.E. 1964. The variance of interclass correlation involving groups with one observation. Biometrics 18, 818-826.

Taggart, J.B., 2007. FAP: an exclusion–based parental assignment program with enhanced predictive functions. Mol. Ecol. Notes 7, 412-415.

Vandeputte, M., Kocour, M, Mauger, S., Dupont-Nivet, M., de Guerry, D., Rodina, M., Gela, D., Vallod, D., Chevassus, B., Linhart, O., 2004. Heritability estimates for growth-related traits using microsatellite parentage assignment in juvenile common carp (*Cyprinus carpio* L.). Aquaculture 235, 223-236.

Woolliams, J. A., 1989. Modifications to MOET nucleus breeding schemes to improve rates of genetic progress and decrease rates of inbreeding in dairy cattle. Anim. Prod. 49: 1-14.

Woolliams, J.A., Bijma, P., 2000. Predicting rates of inbreeding in populations undergoing selection. Genetics 154, 1851-1864.

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

Multiplex 1	Primer concentration (pmol/µl)*	Multiplex 2	Primer concentration (pmol/µl)*
*Hmo*25	0.06	*Hmo*11	0.05
*Hmo*26	0.25	*Hmo*13	0.045
*Hmo*36	0.07	*Hmo*33	0.05
*Hmo*37	0.09	*Hmo*34	0.20
*Hmo*39	0.09	*Hmo*40	0.06

See text for further information about PCR conditions.

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

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

	Set A	Set B	Set C	Total
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.**

Calculation method	Weight h^2	Total length h^2
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 iteration with equal sire and dam components)	0.67 (Confidence interval: 0.42-0.93)	0.51 (Confidence interval: 0.29-0.78)

List of Figures

Fig. 1: The deviance profile of various heritability estimates for (a) harvest length and (b) harvest weight from manual iteration considering equal sire and dam components. The horizontal lines indicate the minimum deviance (-2 log likelihood) and the threshold for the deviance with χ^2 significance test at 0.05. The vertical arrows indicate the maximum likelihood estimate of heritability and its 95% confidence interval.

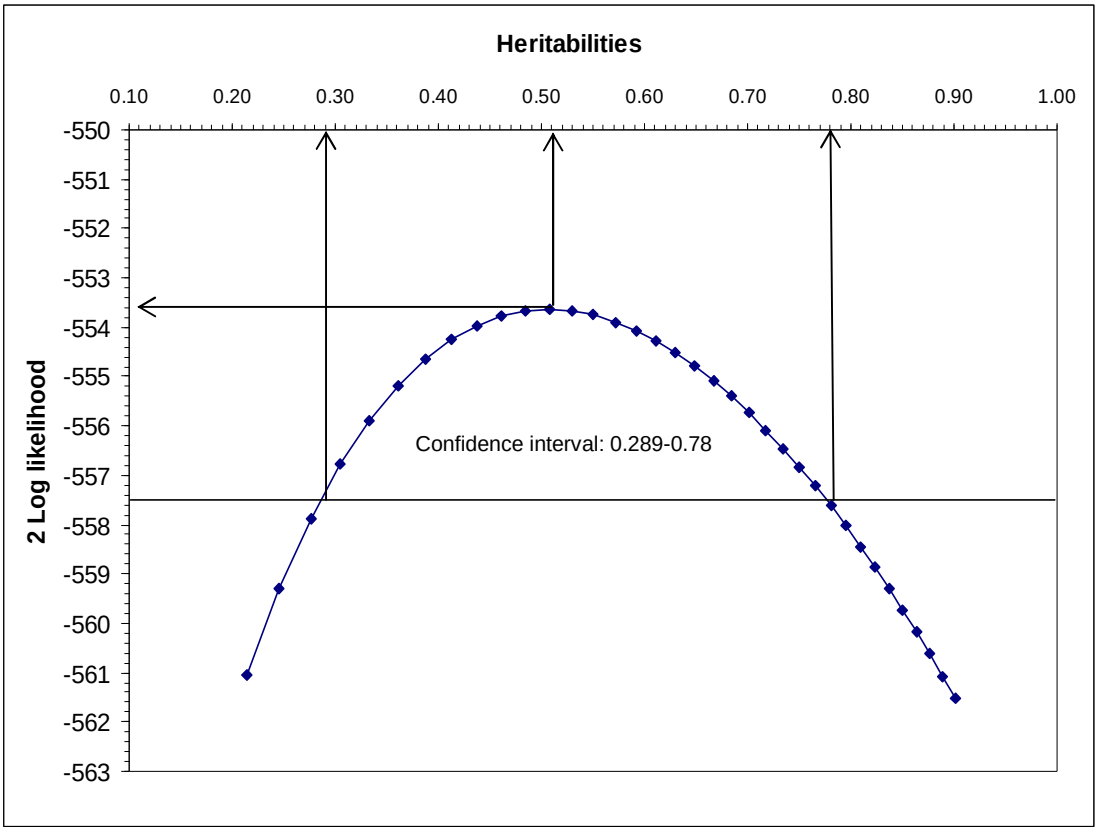
Heritabilities
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
2 Log likelihood
-550
-551
-552
-553
-554
-555
-556
-557
-558
-559
-560
-561
-562
-563
Confidence interval: 0.289-0.78

Figure 1a

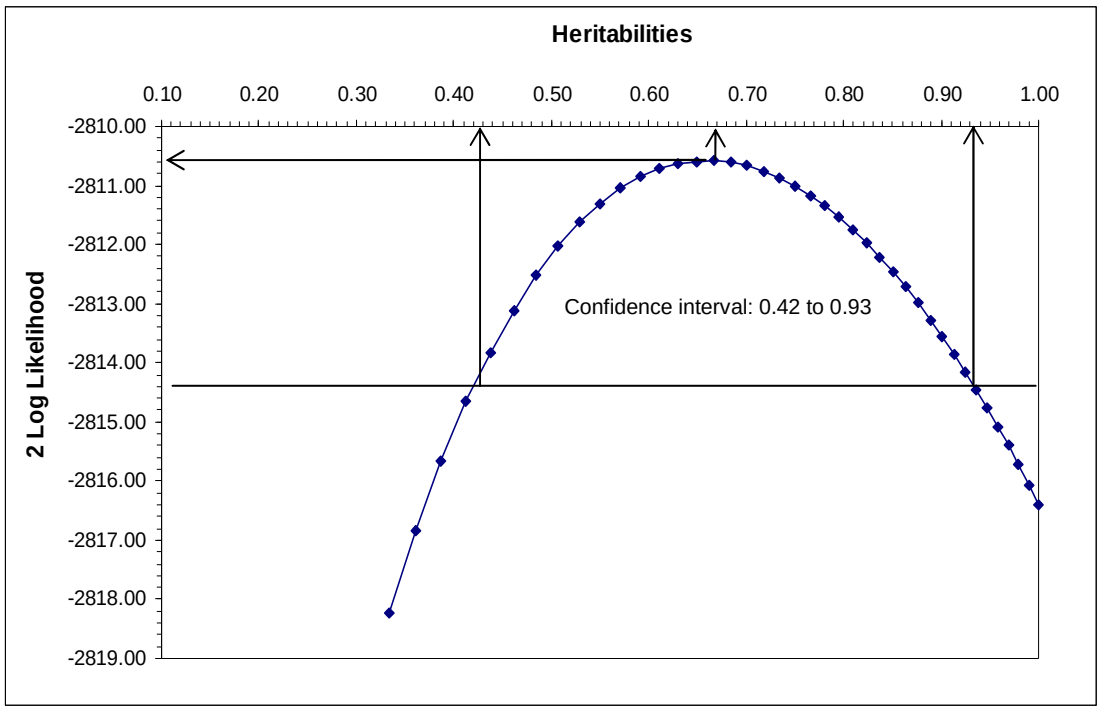
Heritabilities
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
2 Log Likelihood
-2810.00
-2811.00
-2812.00
-2813.00
-2814.00
-2815.00
-2816.00
-2817.00
-2818.00
-2819.00
Confidence interval: 0.42 to 0.93

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

	Dam												Progeny
Sire	D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12	per sire
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

Appendix 2: Mating structure and number of assigned offspring from sires and dams 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

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