

WINTER SCHOOL
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Course Manual



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SELECTIVE BREEDING FOR GENETIC IMPROVEMENT OF BROOD STOCK

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Introduction

Though one can bring about improvement in the production performance of a population by environmental manipulations as well as through genetic manipulations, any improvement from the former cannot be transmitted to the next generation. The genetic improvement on the other hand is inherited by the next generation and therefore, more important. The wide variations among the individuals in every population provide ample scope for genetic manipulations for developing genetically superior lines and strains.

A number of modern genetic manipulations techniques like chromosomal engineering for production of polyploid, gynogenic and androgenic populations as well as genetic engineering, where a desirable gene or set of genes from one animal can be transferred into another for producing a transgenic animal with desired characteristics are available for genetic improvement. However, the conventional quantitative genetic techniques like selective breeding can be an attractive technique for production of genetically superior brood stock.

Selective breeding has been used for thousands of years to improve all major crops and livestock. Selective breeding, as used in farm animals and crops, is the time-tested genetic manipulation technique, which can play a definite major role in developing genetically improved fish/shellfish also. However, an in-depth knowledge of the genetics of the animals is a pre-requisite for the formulation of the appropriate techniques for their improvement. Breeding is the applied science of genetics. Inbreeding is often combined with hybridisation to improve the results of the crossbreeding programme. To date, much of the selective breeding work in aquaculture has been devoted to Atlantic salmon, Channel catfish, Rainbow trout and Tilapia. Though, there have been some work on the selective breeding of Carps in our country, it still remains a grey area with much to be done.

Very few attempts have been made for the genetic improvement in mariculture species through selective breeding for enhanced growth as disease resistance, the shrimps being no exception. The main reason is that they are either not domesticated or at the very early stages of domestication. However, of late scientists are successfully, breeding and rearing shrimps under captivity paving the way for selective breeding endeavours. For most aquaculture species, selective breeding is difficult because the juveniles are too small to be tagged for later identification and growth evaluation. Frequent moulting of the shrimps compounds the problem.

Use of selective breeding and marker-assisted selection has been recently introduced in crustacean improvement programmes. Scientists of the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia are undertaking programmes for genetic improvement of *P.japonicus* and have reported good response.

The ultimate goal of every selective breeding programme is to improve the breeding value of the population. To accomplish this goal, a breeder selects (saves) those individuals that possess certain desired phenotypes and culls (removes) those that do not. By selecting and mating only the best ones he hopes that the selected brood will be able to transmit their superiority to their offspring, thereby creating a genetically improved population. If this occurs, the next generation will be more valuable and will increase their market value.

Selection is the process in which certain individuals in a population are preferred over others to produce the next generation. Natural Selection is exercised by nature. Survival of the fittest is the main force responsible for selection by nature. Artificial Selection is the one practiced by man, in his effort to increase frequency of desirable genes in the stock. Selection does not create new genes, but increase the frequency of desirable genes in the population leading to increased proportion of homozygous individuals. Selection is classified on the basis of selection criteria as follows.

- 1) Individual Selection : Selection of the breeders based solely on the individuals own phenotypic performance i.e., phenotype is used as the indicator of his genotype.
- 2) Family selection: Family consisting full-sibs and /or half sibs is the unit of selection and the decision to save is based on family mean.
- 3) Pedigree Selection: Selection based on ancestor's performance (i.e. using performance pedigree) Pedigree is the record of individual's ancestors related it through its parents.
- 4) Selection on the basis of progeny testing: Selection of breeders based on the average merit of the offsprings as compared to the average merit of the progeny of contemporary breeders. Generally used for selecting sites in terrestrial animals. But in fish it can be used for even dams.

Genetic gain in quantitative trait from one generation of selection

$$\Delta G = h^2 \times Sd; \text{ where } Sd = (\overline{P_s} - \overline{P})$$

$$\text{Yearly generic gain : } \Delta_{Gt} = \frac{h^2 \times Sd}{I_g}$$

Knowledge about the phenotypic and genotypic parameters of the economically important traits is vital. Estimates of parameters such as genetic variation, heritability, phenotypic and genetic correlations, heterosis, genotype environment interactions etc are essential for planning a proper breeding strategy. Therefore, breeding studies for the evaluation of the genetic and phenotypic parameters are the essential pre-mediated step. Decision about the organisation of breeding programme could be made only after careful consideration of the above parameters. As for example, when there is relatively larger additive genetic variance, simple selection methods like individual/mass selection should yield good progress. On the other hand if nonadditive genetic variance is predominant, special selective breeding schemes are to be formulated to exploit them. When over dominance is important for a trait, reciprocal or recurrent reciprocal selection needs to be employed. If heterosis is found to be high, cross breeding programmes could be given priority. Genotype environmental interactions of high magnitude call for developing different strains to suit each of the environments.

Both quantitative and molecular genetic tools can be applied in conjunction for improvement of mariculture species. Of late the use of marker assisted selective breeding is being attempted. Combined application of quantitative genetic techniques and molecular level markers can pay rich dividends in the future.

The progress in cultured fish and shrimp genetic research has been slow due to the lack of sufficient knowledge on the basic aspects of their biology. Of late, some of the genes involved in growth reproduction and disease resistance of shellfishes have been identified, cloned and sequenced. The scientists CSIRO, Australia have reportedly produced genetically improved *P.japonicus* which matures three weeks earlier and grows 25% more than the unimproved cultured stock.

The salient results of a selective breeding programme carried out in artemia at CMFRI, Cochin to study the quantitative genetic parameters as well as the response to selection as part of the doctoral programme are presented briefly below.

Materials and methodology:

Artemia franciscana (Kellogg1906) from Great Salt Lake, Utah was used for the study. Method of selection followed was Mass selection (individual selection) and the trait under selection was the naupliar size (length in μm). Bi-directional mass selection was practised in two sub-populations derived from the base generation viz., SNS line & BNS line with the aim of developing two divergent stocks. While selection for reducing the naupliar size was practised in SNS line, BNS was selected for bigger naupliar size. Six selected generations were raised. Intensity of selection (i) common for male and female together was estimated as the mean of two sexes i.e. $i = \frac{1}{2} (i_m + i_f)$ (Falconer, 1981). Intensity of selection for male and female separately calculated as ratio of effective selection differential to the phenotypic standard deviation. The heritability values of the selected trait (naupliar size)

were estimated from full sib data and from regression of offspring on parent as per the procedure given by Becker (1975). The predicted genetic response per generation was calculated for each line separately within sex as per the procedure described by Falconer (1960).

Results :

Heritability of naupilar length:

The heritability estimates of naupilar length, from the regression of progeny on parents, pooled over generations, were 0.2123 ± 0.0766 and 0.3885 ± 0.1108 for males and females respectively in SNS line. The corresponding estimates in BNS were 0.5777 ± 0.1154 and 0.3364 ± 0.1176 respectively. The heritability estimates from full sib data, pooled over generations, were 1.3256 ± 0.0474 and 1.1004 ± 0.0522 for males and females respectively in SNS line, whereas, the corresponding estimates in BNS were 1.2580 ± 0.0583 and 1.4221 ± 0.0479 respectively. While the moderate values of heritability estimated from b_{op} indicated existence of fairly good amount of additive genetic variance which can be exploited through simple selective breeding techniques, the very high estimates of heritability from full sib analysis indicated existence of non-additive genetic variances also.

Selection differential:

Selection differentials, averaged over generations, were slightly higher in females of both SNS and BNS lines. Their mean values were $-16.6780 \mu m$ and $-16.3966 \mu m$ in SNS males, $-19.9266 \mu m$ and $-22.3101 \mu m$ in SNS females, $16.2308 \mu m$ and $15.8700 \mu m$ in BNS males and $17.1180 \mu m$ and $17.0019 \mu m$ in BNS females.

Phenotypic responses :

Phenotypic responses for naupilar length from selection were quite substantial. The naupilar size in SNS line, from six generations of selection for smaller size, could be reduced from $486.99 \mu m$ and $490.58 \mu m$ in males and females respectively to $441.67 \mu m$ and $453.05 \mu m$. The cumulative gain for males and females were $-44.32 \mu m$ and $-37.52 \mu m$ respectively with average gain per generation being $-5.76 \mu m$ and $-4.96 \mu m$. In the BNS line, the naupilar size could be increased to $493.55 \mu m$ and $529.37 \mu m$ in males and females from $486.99 \mu m$ and $490.58 \mu m$ in the first generation, through five generations of selection for bigger naupilar size. The total gain worked out in males and females were $8.59 \mu m$ and $38.80 \mu m$ with mean gain of $0.39 \mu m$ and $5.52 \mu m$ respectively. The mean phenotypic responses were statistically significant except for BNS males.

Realised genetic gain:

The observed phenotypic response is the combined effect of both genetic and environmental factors. Since the environment rarely remains the same over the period of selection, separating out these effects becomes rather difficult. One of the most commonly used methods for removing environmental effect from the phenotypic gains and for

determining genetic gain is the use of an unselected control population, preferably, from the same stock as that of the selected population. Such a control line was used in the present study. Most of the phenotypic responses realized from selection were due to genetic gains. In the SNS line, total genetic gain realized from six generations of individual selection for reduction of the naupliar length was $-41.7244 \mu\text{m}$ in males and $-38.7585 \mu\text{m}$ in females. Whereas in BNS line, the total genetic gain from five generations of selection were $12.6427 \mu\text{m}$ and $39.4836 \mu\text{m}$ in males and females respectively.

The realized mean genetic gain per generation, estimated from regression of control corrected generation means on generation numbers was $-5.2585 \mu\text{m}$ in males and $-5.2289 \mu\text{m}$ in females of SNS, and $0.9338 \mu\text{m}$ in males and $5.3493 \mu\text{m}$ in females of BNS line. The mean genetic gains were fairly high and statistically significant except in BNS males.

Expected genetic gains :

Expected responses were calculated using heritability estimated from regression of offspring on parent (bop) and also full sib heritability. While, estimates as per former were close to realized genetic gains, those from latter were on the higher side. This result indicates that heritability estimates from full sibs are indeed inflated by non-additive genetic variance, unlike the bop, which includes only additive genetic variance.

Generation wise phenotypic response in naupliar length realized from bi-directional selection for reducing naupliar length in SNS line and for increasing naupliar length in BNS line showed that the response in both the lines were in the desired direction. The total cumulative decrease in naupliar length from six generations of selection for smaller naupliar size viz. $-45.3177 \mu\text{m}$ and $-37.5220 \mu\text{m}$ in males and females respectively, works out to 9.3057% and 7.6486% of the naupliar size of the base population. Similarly, cumulative increase of $8.5923 \mu\text{m}$ and $38.7966 \mu\text{m}$ in males and females of BNS line from five generations of selection for larger naupliar size work out out to be 1.7644% and 7.9084% of the naupliar size in base population. The mean decrease in the naupliar length per generation in SNS line was $-5.7554 \mu\text{m}$ and $-4.9743 \mu\text{m}$ for males and females respectively. The corresponding increase per generation in BNS line was $0.3833 \mu\text{m}$ and $5.5222 \mu\text{m}$ respectively. It can thus be seen that while in SNS line, both the sexes readily responded to selection for decrease in naupliar size, there was a differential response to selection for decrease in naupliar size there was a differential response to selection for the higher size in BNS line. In BNS line, the females showed 14.5 times higher response than males, while in the SNS line both sexes showed comparable response. It is rather difficult to explain whether this low response in males was due to attainment of the genetically pre-set maximum size for that sex or due to any other reasons.

The point to be noted in this context is that the male nauplii were always smaller than females in both SNS and BNS lines as well as in the base population. The smaller size

of males as compared to females may be the nature's provision to enable them to clasp the female quite easily and to maintain buoyancy during copulation. The males might have reached the size limit set by nature and hence have exhibited a lower response, when larger sized nauplii were selected.

The realized response calculated by subtracting the mean control values of each generation from the corresponding selected generation mean is free of environmental effects and therefore, gives the true genetic gain from selection. Comparison of the genetic and phenotypic gains realized in this study point towards the fact that though the environment had played a role in deviating the phenotypic response from the genetic response, its effect was comparatively low and that the genetic gain was quite substantial.

Most of the documented selection studies in the aquatic species have reported the response to selection with out considering the environmental effects and therefore, represents only the phenotypic response and not the genetic response. In this study, response to bi-directional selection was in the expected direction, though the rate of response was relatively of higher magnitude in line selected for reduction of naupliar size. The substantial genetic gains realized from selection indicate the usefulness of selective breeding for developing genetically altered lines. Very few bi-directional selection studies, with reference to growth, have been reported in aquatic animals. While Moav and Wahlforth (1976) observed no response from five generation of selection for high growth rate in common carp, there was a strong response to selection for slow growth rate. In channel cat fish, Bondari (1983) reported response to selection for body weight and length in both upward and down ward directions. Huang and Liao (1990) reported little response to mass selection for high body weight as well as for low body weight in tilapia. Behrends et al. (1987) and Rochetta (1996) could not observe any response to selection for growth in tilapia and guppy, due to the prolonged domestication process in these fishes. No comparable results from *A. franciscana* are available.

Genetic improvement need not be restricted to maximising growth and feed conversion, but can be in survivability and disease resistance. Susceptibility to disease especially viral infections is playing havoc with the shrimp culture and needs immediate attention. Like any other biological traits there is naturally occurring genetic diversity in disease resistance. Also, leading to differential susceptibility of individuals of a population to various pathogens, leading to the survival of some individuals even after a disease out break. Selective breeding for disease resistance, supported by marker-assisted selection is need of the hour, which calls for investigations in the field of genetic markers of enhanced disease resistance.

The decision to conduct a selective breeding programme is a decision that must be made on a case-by-case basis. Because selective breeding programmes require dedication, a certain level of sophistication, record keeping, and the investment of extra labour. Additionally, selective breeding programmes are not free; they also require the investment of

money. Finally, these programmes usually do not produce immediate improvements. Improvements are usually not seen for at least one growing season, so a breeder must be able to incorporate long-term planning into his programme, and he must be patient. A final requirement that must be met for conducting a selective breeding programme is the existence of proper facilities.

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