

CMFRI

Course Manual

*Winter School on
Recent Advances in Breeding and Larviculture
of Marine Finfish and Shellfish*

30.12.2008 -19.1.2009

Compiled and Edited by

*Dr. K. Madhu, Senior Scientist and Director,
Winter school*

&

*Dr. Rema Madhu, Senior Scientist and Co-ordinator
Central Marine Fisheries Research Institute*



Central Marine Fisheries Research Institute
(Indian Council of Agricultural Research)
P.B.No.1603, Marine Drive North Extension,
Ernakulam North ,P.O.
Cochin, KERALA – INDIA - 682018



P.C. Thomas,

Principal Scientist, Central Marine Fisheries Research Institute, Kochi- 682 018.

E -mail : palahanict@yahoo.com

Introduction

Success in aquaculture depends on the use of animals with high genetic potential and application of sound management techniques. 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 can not be transmitted to the next generation. The genetic improvement on the other hand is inherited by the next generation and therefore, more important. In farm animals and plants application of genetic principles for breeding programmes have played an important role in increased productivity in accordance with the demands of the nation. Of late genetics has acquired an important place in fisheries and aquaculture with the following objectives.

1. Production of high yielding strains of fish and shellfish.
2. Development of disease resistant strains.
3. Conservation and management of natural resources.

The theory of inheritance provide the mathematical basis for predicting results of mating and testing the laws in animals and plants. Quantitative genetics deals with the inheritance of quantitative traits. Quantitative traits are those measurable/ quantifiable traits which are controlled by many genes. Most of the economic traits are quantitative and continuous variation is its characteristics. In contrast, qualitative traits show discrete variation and controlled by one or a few genes. Quantitative genetics is the theoretical basis for all the breeding programmes in animals and plants. It is the logical development of the basic principles of inheritance.

Tools available to the breeder for improvement

Variation

Variation among individuals within the population is the basic and most important pre- requisite for any genetic improvement programme. With out variation no improvement is possible. In order to study the genetic properties of the population we have to partition the phenotypic variation in to component parts attributable to different causes. The phenotypic variation can be divided in to components attributable to the genotype and to the influence of environment. The genotype is the particular assemblage of genes possessed by the individual and the environment is the non-genetic effects.

Variation is quantified and expressed as variance (σ^2). Variance is defined as the average of the squared deviations of individual's value from the population's mean value. The two components associated with the genotype and environment is the genotypic variance and environmental variance.

$$V_p = V_G + V_E + V_{G \cdot E}$$

Where, V_G = Genotypic variance, V_E = Environmental variance, $V_{G \cdot E}$ = Genotype and environmental interaction

The partition of the variance in to its components formulates the question of importance of individual portion in determining its phenotype. The relative importance of cause of variation means the amount of variation it contributes to the total variation. So the relative importance genotype as the determinant of phenotype is the ratio of genotypic variance to phenotypic variance (V_G / V_p). This is termed as the heritability (h^2).

Genotypic variance can be further divided in to additive genetic variance (breeding value) and non-additive genetic variance (Dominance and interaction deviations).



$$V_G = V_A + V_D + V_I$$

Where,

V_A = Additive genetic variance

V_D = Dominance variance

V_I = Interactions (Epistatic) variance

Additive genetic variance:

It is the sum of the effects of all the additively acting alleles that help to produce a phenotype. It is the breeding values. It is an important component because it is the chief cause of resemblances between the relatives. This is the portion of the variance which is definitely and surely being transmitted to the offsprings from the parents.

Non-additive genetic variance:

These variations are due to the dominance and epistatic effects of the alleles, and therefore, consists of the following.

Dominance variance:

This is due to the dominance effects of the alleles at the loci. V_D may not be transmitted to the next generation from the parents since the alleles are disrupted during the meiosis.

Interaction (Epistasis) variance:

It occurs due to the interaction of the alleles between two or more genes. These interactions may be two factor or three factor or so on. The amount of variation due to interaction is rather small; therefore, the breeder can omit it also.

Environmental variance:

It is the variance due to all non-genetic effects starting from feeding to environmental conditions. Environmental variance cannot be eliminated in a population. It plays a major role in determining the phenotypic variance of the population.

Genotype and environmental interaction variance:

Apart from the above two components, variance can arise from the interaction between the genotype and environment also. A single genotype may perform differently in two different environments. It may show good performance in one environment but may be poor in second. The performance of a population may be reduced or increased in different environments.

Heritability (h^2)

The heritability is one of the most important properties of the economically important quantitative traits. It expresses the proportion of the total variance attributable to genetic causes. Heritability is defined as the ratio of genetic variance to phenotypic variance in a broad sense.

$$\text{Heritability } (H^2) = \frac{\text{Genotypic Variance}}{\text{Phenotypic Variance}} ;$$

It in narrow sense is defined as the ratio of additive genetic variance to the phenotypic variance.

$$\text{Heritability } (h^2) = \frac{\text{Additive genetic Variance}}{\text{Phenotypic Variance}}$$

Heritability ranges from 0-1 or expressed in percentage. It plays an important role in selection programmes for genetic improvement. An important function of heritability in quantitative genetics/animal or plant breeding is for indicating the reliability of using the phenotypic value as a guide to the breeding value. It can be used to predict the genetic gain from selective breeding. It is the property of the population for the trait under consideration, and not of an individual. It is valid for only the given population in a given environment.

Genetic correlations

Economic traits are correlated; means selection for one trait may lead to increase or decrease in the other traits. These are called correlated responses and the traits are called correlated traits. This is expressed in terms of correlation co-efficient (r) which ranges from -1 to +1. Correlated response may be positive or negative. It is due to the pleiotropic effect of the alleles. This plays an important role in trait selection for improvement.

Selective Breeding for fish stock improvement:

Breeding is the practical aspect of the genetics. Clear and thorough understanding of the genetic rationale and genetic principles will avoid the inadvertent mistakes during the breeding. Therefore, the breeder should have clear understanding of its pedigree records, genetic parameters like heritability, correlations and of course reproductive biology and behavior of the species before planning a breeding programme. Therefore, breeding studies for the evaluation of the genetic and phenotypic parameters are the essential pre-mediated step. Decision about the organization 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 non additive 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 calls for developing different strains to suit each of the environments.

Selective breeding means careful selection of superior performing individuals in a population as parents for the next generation. This is based on the Robert Backwell theory of "like begets like". Two elite/best performing individuals are selected and bred to produce the best performing progeny. In animals and plants this lead to production of pure strains and also to improve the production. Selective breeding of fishes started in recent years only. This has been useful approach for genetic improvement of cultured fish populations. Careful consideration of the species and trait to be improved are before taking up the selective breeding programme.

Selection of species

Selection of the species is very important aspect of selective breeding programme. While selecting a species the breeder should consider two things, the first is it should have well established breeding and seed production technology and the second is its economic importance.

Selection of trait

The trait or traits under selection should be economically important, highly heritable, positively correlated and it should be easily measurable. Some economically important traits in fishes are growth rate, body size & shape, meat quality and disease resistance.

Methods of selection :

Selection of the parents for next generation is based on its additive genetic variance or breeding value. The genetic potential of an individual is judged by the phenotypic performance of the individual in several ways.

Individual selection

The individuals are selected based on their own performance, viz. on the basis of their phenotypic value. This



selection method is very effective when the h^2 of the trait is high. It is usually simple to operate and it yields the rapid response. It is also called as Mass selection.

Pedigree selection

In this method individuals are selected based on the performance of their parents or grand parents. Pedigree records of the ancestors considered for selection of parents.

Family selection

Families are selected or rejected as a unit according to the mean phenotypic value of the family. Within the family variations are not considered. The accuracy of the family selection depends on the heritability of the trait, family size, family type and the variation due to the environment. This is more useful when selection is practiced for less heritable traits like reproductive traits, carcass quality and disease resistant.

With in family selection

In this method, families are considered as a sub population and individuals are selected based on the performance in relation to the mean performance. Better performing individuals from the family are retained and the others are culled. This method is more efficient than individual and family selection. Selection within families would eliminate the non-genetic variation.

Progeny testing

Parents are selected based on the performance of the progeny. This method is reliable, and practicable if cryopreservation of the gametes is possible.

Tandem selection

In this method of selection, one trait is considered at one time. The selection is aimed at improving one trait at a time for generations till the goal is reached. Then other trait is selected and it continued for all the traits. It is simple but time consuming, it is inefficient because it takes long time and there is a chance of negative correlation, which decreases the mean performance.

Independent culling levels

Independent culling level is a selection programme employed when two or more traits are considered for selection simultaneously. In this the breeder has to fix the minimum performance level for each trait, and the individual must perform above the minimum level to get selected. Disadvantage of this method is that a fish should be outstanding in all the traits under selection. A fish with best performance in one trait and average performance in the other is liable for rejection. One more disadvantage is when selection is practiced for more traits at a time you may end up with few individuals which satisfy the minimal performance levels of all the traits.

Selection index

Index selection is the best and most efficient method of selection programme. In this method all the traits considered for selection are given due weightage according to their performance and a selection index is formulated based on the linear regression. In this, traits are given weightage according to their relative economic importance.

Breeding plans :

Selective breeding programme intends to exploit the genetic variations existing in the population, so there should be wide genetic base in the foundation stock. For this, different stocks may be inducted from different sources, making sure the presence wide genetic base. Superior individuals selected from the population are *inter se* mated to produce

the next generation. Continuous selection eliminates the undesirable alleles present in the population. It makes the population homozygous. This is very much useful in evolution of different strains and lines. Mating of related individuals can lead to inbreeding depression, which a breeder should be concerned about. To overcome this, crossing between the strains or inbred lines is employed. Out crossing is very useful method in selective breeding wherein unrelated individuals of same species are bred to minimize the effect of inbreeding depression. Crossing of two inbred lines increases the performance because of heterosis. Heterosis is the observed superiority of the hybrid progeny over the parental mean. Hybridization is the rapid method of bringing about genetic modification. Species hybridization is the inter specific crossing. 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 are presented briefly below.

Materials and methodology

Artemia franciscana (Kellogs1906) 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 practiced 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 practiced 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 naupliar length

The heritability estimates of naupliar 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 $-16.6780 \mu\text{m}$ and $-16.3966 \mu\text{m}$ in SNS males, $-19.9266 \mu\text{m}$ and $-22.3101 \mu\text{m}$ in SNS females, $16.2308 \mu\text{m}$ and $15.8700 \mu\text{m}$ in BNS males and $17.1180 \mu\text{m}$ and $17.0019 \mu\text{m}$ in BNS females.

Phenotypic responses :

Phenotypic responses for naupliar length from selection was quite substantial. The naupliar size in SNS line, from six generations of selection for smaller size, could be reduced from $486.99 \mu\text{m}$ and $490.58 \mu\text{m}$ in males and females respectively to $441.67 \mu\text{m}$ and $453.05 \mu\text{m}$. The cumulative gain for males and females were $-44.32 \mu\text{m}$ and $-37.52 \mu\text{m}$ respectively with average gain per generation being $-5.76 \mu\text{m}$ and $-4.96 \mu\text{m}$. In the BNS line, the naupliar size could be increased to $495.58 \mu\text{m}$ and $529.37 \mu\text{m}$ in males and females from $486.99 \mu\text{m}$ and $490.58 \mu\text{m}$ in the base generation, through five generations of selection for bigger naupliar size. The total gain worked out in males and females were $8.59 \mu\text{m}$ and $38.80 \mu\text{m}$ with mean gain of $0.39 \mu\text{m}$ and $5.52 \mu\text{m}$ 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. 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 μm in males and -38.7585 μm in females. Whereas in BNS line, the total genetic gain from five generations of selection were 12.6427 μm and 39.4836 μm 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 μm in males and -5.2289 μm in females of SNS, and 0.9338 μm in males and 5.3493 μm 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. It can 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 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 realized response calculated by subtracting the mean control values of each generations 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. The substantial genetic gains realized in this study indicate the usefulness of selective breeding for developing genetically altered lines.

Genetic improvement need not be restricted to maximising growth and feed conversion, but can be in survivability and disease resistance. Like any other biological traits there is naturally occurring genetic diversity in disease resistance also. 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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