

IMPORTANCE AND IMPLEMENTATION OF SIMPLE AND ADVANCED SELECTIVE BREEDING PROGRAMS FOR AQUACULTURE SPECIES IN DEVELOPING COUNTRIES

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INTRODUCTION

Aquaculture is predicted to play a major and ever increasing role in meeting human needs for protein. In terrestrial animal and plant species genetic improvement programs have made a substantial contribution to productivity and viability. By contrast, most aquaculture stocks in current use in developing countries are genetically similar or inferior to wild, undomesticated stocks. Hence, there is ample justification for the planning, design and implementation of genetic improvement programs for aquatic animal species. A range of methods of varying complexity is available for selection purposes, but their suitability for different circumstances is not always clear. Bentsen and Gjerde (1994) give a very lucid account of the situation and their diagnostic and recommendations remain valid to date. In this paper we briefly present the main selection methods that have been used or advocated, and discuss their virtues and shortcomings. When possible, we make reference to practical examples of their application.

SELECTION APPROACHES

General. We present the different selection approaches in increasing order of complexity, beginning with the simplest one. In each case, we refer to specific requirements that may constitute a limitation for their implementation in developing countries. Note that we assume that there is genetic variation for the trait(s) of interest in the population undergoing selection and that it does not suffer from problems (e.g. bottlenecks, inbreeding) created by earlier genetic mismanagement. Such problems could undermine the effectiveness of any selection program (e.g. Teichert-Coddington and Smitherman, 1988; Huang and Liao, 1990).

Individual or mass selection. The terms 'individual selection' and 'mass selection' are often used interchangeably, and they refer to selection solely based on the individual's phenotype. It has been a common strategy with fish because of its simplicity. It does not require individual identification or the maintenance of pedigree records, hence it may be considered the least costly method. In principle, it can produce rapid improvement if the heritability of the trait(s) under selection is high. Under those circumstances, however, there is risk of inbreeding due to inadvertent selection of progeny from few parents producing the best offspring, especially if progeny groups are large. For growth rate and morphological traits (easily assessed, expressed in both sexes) it can be quite suitable. By contrast, individual selection is not suitable for situations in which the estimation of breeding values requires slaughter of the animals (e.g. carcass and flesh quality traits) or challenge of some sort (e.g. selection for salinity tolerance or for disease resistance).

Hulata *et al.* (1986) carried out two generations of mass selection for growth rate with Nile Tilapia (*Oreochromis niloticus*) and observed no improvement over the original base population. They attributed the lack of response to selection to a number of possible factors, including inbreeding and genetic drift. They concluded that mass selection was not a promising method unless measures could be taken to control inbreeding. Rezk (2004) reported selection responses in harvest weight of 35 and 14 per cent in *O. niloticus* and *O. aureus* respectively, after four generations of selection. There were no clear reasons for the difference in response between both species since the selection protocol applied in either case was the

same (140 individuals of each sex were selected each generation). WorldFish (unpublished) records indicate that the experience with Silver Barb (*Barbonymus gonionotus*) in Bangladesh and Thailand and Common Carp (*Cyprinus carpio*) in Vietnam has been of satisfactory response to selection in early generations up to the fourth or fifth, declining sharply thereafter.

Gjerde *et al.* (1996) and Villanueva *et al.* (1996) provide procedures to determine the optimum design of mass selection programs in fish with constrained levels of inbreeding. The latter authors combine expected rates of genetic progress (G) with expected rates of inbreeding (F) in a linear objective function ($\Delta = G - F$). Maximization of the function for appropriate values of Δ gives the optimum number of sires and dams when specific constraints on the level of inbreeding are imposed. For $\Delta=0$ no restriction on inbreeding is imposed. Using this approach the authors show that for a population size of 300 and no restrictions on inbreeding the optimum design is eight sires and 16 dams, and inbreeding increases at a rate of 3.9 per cent per generation. When inbreeding is restricted to 0.25 per cent then the optimum design is 85 sires and 85 dams, and the rate of genetic gain is halved due to the inbreeding constraint. These design considerations have to be carefully applied in species such as Tilapia. Fessehaye *et al.* (2006) found that in *O. niloticus* under mass spawning in large hapas there was a large variance in male reproductive success, with one third of the males siring more than 70 per cent of the offspring. This led to a rate of inbreeding of approximately twice of that predicted from the effective population size. Overall, the evidence suggests that simple, unstructured, mass selection will result in problems unless the number of parents is large, and even so, chance could have a negative effect. Some form of structuring to control the parental contribution to the next generation appears necessary. If controlled pair matings can be carried out, the results of Bentsen and Olesen (2002) can be used to formulate the design of the breeding program. These authors investigated the effect of number of parents selected and of number of progeny tested per pair for a range of population sizes and heritability values. They show that inbreeding rates can be kept as low as one per cent per generation if a minimum of 50 pairs is mated and the number of progeny tested from each pair is standardized to 30 to 50 progeny. Note that although not requiring individual identification of the fish, the schemes suggested by these authors entail the conduct of pair matings, initial maintenance of the progeny of such pair matings in separate enclosures, and controlled contribution of each full sib family to the next generation at the time the fish are assigned to communal rearing. The guidelines provided by Bentsen and Olesen (2002) can be very valuable if they can be put into practice. However, we have found that in some developing countries the conduct of a large number of pair matings, the subsequent containment of the full sib groups, and the sampling of a standard number of progeny to contribute to the next generation were tasks beyond the available resources, and we had to change the strategy to that described in the following section.

Selection within cohorts and exchange of breeders. Eknath (1991) reports the genetic deterioration taking place in hatcheries in India due to poor brood stock management. Among other measures to remedy the situation he suggested that brood stock could be arbitrarily divided into several groups. Mating could then be performed between individuals from the different groups on a rotational basis to avoid inbreeding. In this section we develop that notion further, based on the mating design used by McPhee *et al.* (2004) for weight selection in redclaw crayfish (*Cherax quadricarinatus*). These authors divided the population into cohorts, namely, groups sampled from a previously established foundation population. A selection line was created, consisting of 20 cohorts, where each cohort had 15 female and 10 male foundation parents. A control line of eight cohorts of the same size was also established. One hundred individuals were measured per cohort. Offspring of cohorts were hatched and grown in separate pens within a pond. At harvest time individuals of the heaviest weight in each cohort were chosen as parents of the next generation in the selection line, whereas

individuals of average weight were chosen in the control line. In either case, selection was based on the difference between the harvest weight of an individual and its cohort mean. This within cohort selection aims to eliminate the environmental effect of cohorts on growth differences among individuals. The same number of individuals was selected from each cohort. Animals selected in one cohort were mated with those selected in another one to avoid mating related animals. After four generations of selection harvest weight in the selection line was 1.25 times greater than in the control line. In this mating design the importance of pen effects can be judged by computing the ratio (e) of the between pen variance component and the total variance (sum of between and within pen variance components). Based on the cohorts' family structure, the knowledge about the reproductive biology of the species and the number of individuals measured it is possible to calculate the expected genetic relationship (g) between any two individuals drawn at random from the same cohort. Then, the expected genetic gain from within cohort selection relative to across cohort selection can be estimated as (after McPhee 1975):

$$G_w / G_b = [1 - e + h^2 (e - g)]^{0.5} / (1 - g)$$

where h^2 is the heritability of the trait under selection. Note also that although the exact number of parents contributing to the next generation is not known the rate of inbreeding can be calculated for the worst case, that is, that only one pair per cohort left offspring. Generally, inbreeding at generation t , would be calculated as:

$$F_t = 1 - (1 - F)^t \text{ where } F = 1/(8f) + 1/(8m)$$

where f and m are the number of females and males leaving progeny in each generation. With only one pair leaving progeny per cohort the equation becomes:

$$F = 1/(8c) + 1/(8c) = 1/4c \text{ where } c \text{ is the number of cohorts. We may then write:}$$

$F_t = 1 - (1 - 1/4c)^t$ and we can use this equation to predict the maximum inbreeding after t generations. By designing the selection program in such a way that even in the case that only one pair from each cohort produced progeny the inbreeding rate was not excessive, then we would be able to ensure that we would not run into problems due to inbreeding. With regards to the exchange of breeders between cohorts, this could be achieved by shifting the males born in one cohort to another one in a pattern as described by Nomura and Yonezawa (1996), following for instance Cockerham's cyclical mating system (Cockerham, 1970). If we have information about the likely number of parents leaving offspring in a cohort (e.g. Fessehaye *et al.*'s, 2006 study) then we can further refine the design. In practice, we have found that, in contrast to single pair matings, selection within cohorts with exchange of breeders between cohorts following a prescribed pattern is a feasible design even with limited resources. Field personnel feel comfortable with it, and will thus rigorously adhere to the instructions provided.

Within family selection. The method requires identification of the families. This may be achieved by maintaining them in separate tanks, cages, hapas or any other means of containment, without necessarily tagging the fish. The criterion of selection is the deviation of each individual from the mean of the family to which it belongs. Within family selection is especially advantageous when there is a large component of environmental variance common to members of the same family. Full sib groups reared in unreplicated hapas or any other form of containment fall into this category (e.g. see estimates in Ponzoni *et al.*, 2005). Under such circumstances selection between families would be misleading from a genetic viewpoint because of the confounding between genetic merit and common environmental effects. The method can make very effective use of facilities. If replacements are chosen so that every family contributes the same number of individuals to the next generation (e.g. choose one

female and one male from each family) the effective population size twice the actual (Falconer and Mackay, 1996). However, not all the additive genetic variance is available for selection, only a fraction r will be available, where r is the coefficient of relationship among the family relatives in question (i.e. 0.5 and 0.25 for full and half sibs, respectively). The heritability of within family deviations is consequently reduced:

$$h^2_w = [(1 - r)/(1 - t)] h^2$$

where t is the intraclass correlation. For example, if for a particular trait the heritability in the population is 0.2 and the families are full sib groups, then $h^2_w = 0.11$. The lower within family heritability can be compensated for by the high within family selection intensity that can be applied without increasing the rate of inbreeding. The selection intensity within families will be limited only by the number of individuals tested per family. The number of families involved in the program will determine the lower limit of inbreeding, which can easily be controlled by applying a rotational mating system such as that earlier suggested for selection within cohorts.

The use of within family selection was recommended for SE Asian countries by Uraivan and Doyle (1986). It was successfully applied in the selection program that resulted in an improved Tilapia strain developed in the Philippines by the Freshwater Aquaculture Center (FAC) of Central Luzon State University. The strain is known by a variety of names, FAC-selected, FaST, and IDRC strain (in recognition of the support received from the International Development Research Centre of Canada). The selection program and the strain's performance have been described by Abella *et al.* (1990), Bolivar *et al.* (1994), Bolivar and Newkirk (2000), Camacho *et al.* (2001) and Bolivar and Newkirk (2002). The selection line started from a base population combining four strains of Tilapia, namely, Israel, Singapore, Taiwan and a 'FAC' strain available at the time. Nineteen full sib groups were established, and the basis of selection was body weight at 16 weeks of age. The heaviest male from a given family was mated to the two heaviest females of another family to avoid inbreeding. After 12 generations of selection the genetic gain in body weight has been estimated at 12.4 per cent per generation. All the selection program was conducted (from spawning to selection) in outdoor concrete tanks, but the strain also performed well in hapas and ponds. Camacho *et al.* (2001) comment that within family selection was easy to manage, and that taking care of inbreeding by means of a rotational mating posed no difficulties. The method reduces the need for tagging large numbers of individuals. They estimate that the implementation of a selection method that entailed the individual identification of large numbers of fish and a period of communal rearing would be more expensive and difficult to implement. Note that Bolivar and Newkirk (2000) and Ridha (2004, 2006) compared FAC-selected with GIFT (Genetically Improved Farmed Tilapia), the latter strain resulting from a program combining individual and family selection (dealt with in a later section of this paper), and found that there were no differences in growth rate between both strains, but in some of the experiments (Bolivar and Newkirk, 2000; Ridha, 2004) GIFT had greater (23 per cent) survival rate. However, both strains were significantly more productive than other, unimproved, strains.

Combined selection. We use the term 'combined selection' in a broad sense, meaning selection that is based on individual information as well as on information coming from relatives (e.g. full and half sibs, progeny). In this case all of the additive genetic variance is available for selection and the use of information from relatives increases the accuracy of the estimation of breeding values. Furthermore, relatives' records can be used to estimate breeding values for traits that require slaughter of the animals (i.e. carcass and flesh quality traits) or that entail a risky challenge (i.e. disease resistance, tolerance to some environmental component). This is not possible with the other methods (e.g. mass selection or within family

selection). Earlier work with fish (e.g. Eknath *et al.*, 1998) used selection index theory to combine individual, full sib and half sib information. A selection index can be very useful in combining such information, but the approach has limitations that have been overcome with BLUP procedures (see Van Vleck, 1993 for a detailed account). Best Linear Unbiased Prediction (BLUP) procedures rely on mixed model methodology for the estimation of individuals' genetic merit. In the case of aquaculture, all systematic effects (e.g. batch, sex, production environment, age variation) associated with traits of interest can be accounted for in the model fitted to the data. The maternal and common environmental effects due to separate rearing of full-sib families before tagging can also be separated effectively from the additive genetic variance. One particular advantage of BLUP procedures is that genetic gain can be estimated from the mean of the estimated breeding values in each year or generation of selection provided there are genetic links. There are now readily available computer programs (e.g. PEST, Groeneveld, 1990; ASReml, Gilmour *et al.*, 2002) that can estimate breeding values using full pedigree information. A drawback of BLUP selection is that if truncation selection on estimated breeding values is practised, it also results in higher levels of inbreeding than individual selection, especially for lowly heritable traits. This is because BLUP uses family information, leading to co-selection of relatives. Hence, optimal genetic contribution theory should be used with the aid of software such as EVA (Berg, 2004) or TGRM (Kinghorn *et al.*, 2002) in order to maximize genetic gain while controlling the rate of inbreeding.

In order to use combined selection, identifiable families have to be produced. This is usually achieved by pair mating or by external fertilization of ova and sperm of known parental origin. The progeny of the different families must be marked in some way so that they can be communally stocked and tested for genetic evaluation purposes. Preferably, all the individuals to be tested should have a unique identification, and the pedigree will take care of all family relations. Aquatic animals are generally very small at spawning. They are kept in their family (usually full sib) groups until they are large enough to be tagged. This often results in an appreciable common environmental effect in traits such as growth rate. Tagging itself is an issue. The most commonly used tags with fish are Floy Tags® and PIT (passive integrated transponder) tags, the latter being about five times more expensive than the former, but far superior in terms of retention rate. With Floy Tags we have lost up to 60 per cent of the tags in some groups due to fragility of the thread that holds the plastic tag. In the hands of unskilled staff the combined effects of poor reproductive rates, large common environmental effects, and high tag losses can negate the theoretical virtues of the approach. The potential advantages of combined selection cannot be disputed, but before embarking upon it, one must be sure that the physical and human resources at hand are adequate for the tasks that will have to be undertaken.

Three documented examples of the successful application of combined selection to the improvement of fish in developing countries will be cited here (in all cases growth rate was the main focus of selection): (i) The GIFT project in Philippines, which reported genetic gains of 12 to 17 per cent per generation in Nile Tilapia, over five generations (Eknath *et al.*, 1998); (ii) The Jayanti Rohu (*Labeo rohita*) selective breeding project in India, which reported a genetic gain of 17 per cent per generation over five generations (Reddy *et al.*, 1999; Mahapatra, 2005, personal communication); and (iii) The selection project of a Malawian indigenous Tilapia, *Oreochromis shiranus*, where the accumulated gain over two generations was 13 per cent (Maluwa, 2005). GIFT and Jayanti Rohu have been tested extensively on farm and proven to outperform other strains used by farmers. We earlier noted that the GIFT and FaST strains have very similar growth performance, but GIFT has shown greater survival rate, possibly due to the broader genetic basis in the population originally assembled and to the greater effective population size relative to FaST. Because the program with *O. shiranus* is still in its early stages, the strain has not yet been tested extensively on farm.

These three programs (GIFT, Jayanti Rohu, *O. shiranus*) have a number of features in common: (i) They all started with the assembly of a base population drawn from different sources in order to capture genetic variation; (ii) Controlled matings of identified females to identified males were conducted and complete pedigrees were maintained; (iii) Full sib groups were kept together until tagging; (iv) Approximately, 50 to 200 fish per full sib group were tagged and destined to communal rearing in a range of production environments in order to estimate genotype by environment interactions. In the case of GIFT and Jayanti Rohu a selection index combining individual, full sib and half sib information was used to rank individuals on genetic merit, whereas BLUP breeding values were estimated in *O. shiranus*. BLUP procedures are also used in the selection of GIFT in the population that was transferred to Malaysia (Ponzoni *et al.*, 2005). The sound design coupled with rigorous conduct and analysis accounts for the gains achieved in these programs. Furthermore, data sets of this nature, developed over a number of generations, provide great research opportunities in the area of estimation of phenotypic and genetic parameters, as well as of environmental effects and genotype by environment interactions. As a by-product of the genetic improvement program, opportunities for local staff capacity building are created around it. If captured, these opportunities can result in the training of staff to a level that enables them to independently plan and conduct genetic improvement programs. Note that the amount of information that can be extracted from a pedigreed population is much greater than from a non-pedigreed one. This general model, packaged in what has been called 'GIFT Technology' has been advocated by the WorldFish Center for implementation in several developing countries (WorldFish Center, 2004).

CONCLUDING REMARKS

In this paper we focused on selection approaches that in our perception can be managed with the resources that are available in developing countries. We did not comment on more sophisticated ones such as the 'walk-back' (Doyle and Herbinger, 1994; Sonesson, 2005) or the 'PROSPER' (Chevassus *et al.*, 2004) methods. Whereas we recognize their potential value, we believe that due to the resources they require they would be difficult to implement in the developing countries where aquaculture development is most needed (e.g. Sub Saharan Africa).

A rigorous comparison of different methods based on published evidence is not possible. In practice the outcome of a program will be affected by many factors other than the selection method itself. When planning a new program in a developing country, a way of approaching the problem could be to begin thinking about and outlining the most complete one (i.e. full pedigrees and BLUP estimates), and to simplify it gradually until it becomes feasible with the available resources, working backwards through the methods we presented. The final decision before implementation will be a matter of judgment. For instance, a national program developed with a NAR would most likely have a larger component of capacity building than one for a commercial operator. In any case, one should ensure that the program is manageable within the limits of the available physical and human resources, and that it has a high probability of continuing beyond project duration. Note, for instance, that mass, within cohort and within family selection cannot be used to select for traits that cannot be recorded in the live breeding candidates. Hence, application of these methods may render the improvement program unsustainable in the long term. Starting with a population with ample genetic variation is a trademark of successful fish genetic improvement programs. Although this in itself is not a sufficient condition for success, it is indeed a necessary condition. Sophisticated designs and genetic evaluation procedures are no substitute or remedy for a genetically deteriorated base population. The failure of some attempts to achieve genetic improvement with aquatic animals may have been due more to weaknesses in the base population than to the selection method utilized. Irrespective of the method of choice,

continued genetic improvement will hinge upon the adequate balance between high selection intensity and the maintenance of low inbreeding rate.

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