GENETIC IMPROVEMENT PROGRAMS FOR AQUACULTURE SPECIES IN DEVELOPING COUNTRIES: PROSPECTS AND CHALLENGES

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SUMMARY

Aquaculture in developing countries is largely based on unimproved fish strains. There is ample evidence indicating the potential of genetic improvement programs and a range of selection methods may be used. Examples of the application of mass, cohort, within family, and combined between-within family are given. The methods are discussed in terms of their effectiveness and suitability. It is concluded that in principle all the methods can work well, provided the selection program is started with a population with a broad genetic base and that during its conduct a balance is struck between selection intensity and containment of inbreeding. Limitations to the implementation of genetic improvement programs and the challenges they face are discussed.

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. A range of methods of varying complexity is available for selection purposes, but their suitability for different circumstances is not always clear. In this paper we briefly present the main selection methods that have been used or advocated, and discuss their virtues and shortcomings (a more detailed description of the methods is given by Ponzoni et al. 2006). When possible, we make reference to practical examples of their application. We also present evidence about the economic worth of genetic improvement programs and discuss some of the challenges faced when implementing such programs in aquatic animals.

APPROACHES TO GENETIC IMPROVEMENT

Aquatic animals allow the implementation of several approaches to genetic improvement. These include hybridization and cross breeding, chromosome manipulation, sex control, transgenesis, and selective breeding. These are almost always mentioned in aquaculture genetics reports, papers and meetings without making a judgement about their relative practical value. For instance, it is seldom, if ever, stated that of all the genetic approaches only selective breeding offers the opportunity of continued genetic gain, that the gains made can be permanent, that it is the only approach in which the gain can be transmitted from generation to generation, and that gains in a nucleus can be multiplied and expressed in thousand or millions of individuals in the production sector (Ponzoni et al. 2007, 2008). In the cases they are useful the other approaches result in ‘once off’ expressions of the benefit. They may be applied at the multiplication (hatchery) level, but not at the nucleus level.

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). The presentation could be considered repetitive and unnecessary in a livestock or crops context, but not in aquaculture where the application of quantitative genetics lags decades behind the two former fields.

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. carcase 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. 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.

Overall, the evidence suggests that simple, unstructured, mass selection will result in problems unless the number of parents is large (Gjerde *et al.* 1996; Villanueva *et al.* 1996), 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. We have found that in some developing countries implementation of these practices was not possible 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. 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.

Note that although with this mating scheme the exact number of parents contributing to the next generation is not known and the rate of inbreeding can be calculated for the worst case, that is, only one pair per cohort left offspring. 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). 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 is twice the actual (Falconer and Mackay, 1996). However, not all the additive genetic variance is available for selection, but only a fraction equal to the coefficient of relationship among the family relatives in question (i.e. 0.5 and 0.25 for full and half sibs, respectively) will be available. 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 Uraiwan 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), 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. 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. 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 (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. carcase 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).

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 mentioned 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. Although the program with *O. shiranus* is at an earlier stage than the other two, the strain has now been tested extensively on farm with very positive results.

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.

PROSPECTS FOR GENETIC IMPROVEMENT PROGRAMS

In this paper we focused on selection approaches that in our perception can be managed with the resources that are available in developing countries. 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. In any case, 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.

From an economic viewpoint, investment appraisal studies indicate very favourable benefit cost ratios for genetic improvement programs for both Nile tilapia (Ponzoni *et al.* 2007) and for common carp (Ponzoni *et al.* 2008). This was shown to be so even for situations in which there was genotype by environment interaction (Ponzoni *et al.* 2008) and a single program had to service more than one environment. It is reasonable to think that these results can be generalised to other, similar, aquatic animal species.

It is sometimes suggested that, given the long term implications of genetic improvement programs, the stock to be improved should be part of an already viable aquaculture sector. This argument deposits little faith in the power of genetic improvement which can turn a non viable aquaculture sector into a prosperous one (e.g. due to 100% superiority of improved stock over farmers' strains). What viability and prosperity can one expect in an aquaculture sector based on stock that grows about 40 per cent less that their wild counterparts (Brummett *et al.* 2004)? Commercially viable farms cultivating crops or running livestock are not based on plants or animals that are significantly less productive than their wild relatives.

LIMITATIONS AND CHALLENGES TO AQUACULTURE DEVELOPMENT

Limitations. Lack of resources is at the forefront in this area. The limitations are often both financial and human. Technical staff involved in aquaculture most often come from a fish biology background and have limited (if any) training in quantitative genetics. The limitations in capacity are more important than the financial limitations because well trained personnel can make important achievements with limited material resources, but there is no amount of resources that will compensate for lack of capacity. Hence, training of local staff in quantitative genetics and animal breeding should be a priority.

When a genetically improved strain is available multiplication and dissemination face difficulties due to lack of capacity at the hatchery level. The notion of paying a greater price for stock of greater genetic merit is not frequently part of the culture. In turn, producers most often lack both the understanding and the financial means to acquire fingerlings from hatcheries breeding an improved strain. The sad reality is that we have been more successful at developing improved strains than at achieving impact with them at the producer level.

Conservationists and biodiversity groups show great concern about the risks involved in using genetically improved strains. Aquatic animals are more difficult to contain than their terrestrial counterparts and escapees may interbreed with wild stock thus eroding or changing genetic variation in those populations. The sentiments guiding conservationists and biodiversity groups are legitimate and noble, but there is sometimes an incomplete understanding of the issues and the genetic risks are magnified relative to others. For instance, the threats to wild populations from pollution and human encroaching may be greater than those from possible escapes of fish from an improved strain, especially if dealing with a non carnivore such as Nile tilapia.

Challenges. The principles used in the genetic improvement of crops and livestock are applicable to aquatic animals but in practice there are many issues that are peculiar to this latter group.

In order to use combined selection, identifiable families have to be produced. The progeny of the different families must be marked so that they can be communally stocked and tested for genetic evaluation purposes. 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. 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. 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 elaborate selection methods. DNA technology is available that enables ascertaining parentage after mass spawning and communal rearing of fry prior to tagging. Communal rearing soon after spawning virtually eliminates the common environmental effect (Ninh 2009). It also enables greater growth rate than rearing in hapas or tanks in full sib groups, it may help reduce the generation interval and increase selection response. The challenge consists of reducing the cost of the DNA technology to the point that it can be applied in practical genetic improvement programs.

There are issues related to animal behaviour, genotype by environment interaction, variability at harvest and animal welfare that can be dealt with newly developed (and developing) quantitative genetic theory. These constitute a fertile ground for research and development with aquatic animals. With the rapid expansion of the aquaculture industry (FAO 2009), we can foresee that fish welfare will become one of the major challenges in fish breeding programs. Welfare can be linked to product quality and quantity and to production efficiency (Ashley 2007). There is no universal definition of fish welfare, but it is commonly defined as representing the physical and mental state of well-being of the animal in relation to its internal and external environment (Ellis *et al.* 2002). Farmed fish are often subject to stress during crowding, handling, transport and controlled reproduction (Kubilay and Uluköy 2002). In developing countries, fish selection programs have been mainly focused on growth rate. Such a breeding objective favours the selection of more aggressive animals (Lopez 1996). Social interactions among individuals can have profound influences on the expression of performance and welfare traits (Muir, 1996;

Brichette *et al.* 2001; Denison *et al.* 2003; Muir, 2005; Bijma *et al.* 2007). They can reduce growth due to competition for limited resources and result in mortality due to cannibalism. The stress effects from high stocking density under intensive culture systems (Ellis *et al.* 2002) coupled with such behavioural change may act against the welfare of genetically improved farmed fish. A strong case can be made for selective breeding programs to include behavioural traits that may lead to reduced aggression, greater uniformity in harvest weight and that are related to fish health and welfare.

In developing countries, virtually always, at best, a single genetically improved line is developed for each species of interest. This poses two obvious problems: (i) Sustainability of the line in relation to effective population size and risk of loss in case of a disaster, and (ii) Requirement to service a range of production environments. The first problem can be easily handled with adequate resources. The second one calls for a breeding strategy that goes beyond common animal breeding practice. The term phenotypic ‘plasticity’ is used more often in aquatic animal literature than in livestock. Phenotypic plasticity is the property of organisms of a genotype to develop systematically different phenotypes in different environments. Genotypes showing highly variable phenotypes across environments are ‘plastic’, whereas genotypes that show little variability are ‘robust’ (de Jong and Bijma, 2002). The concept of robustness has been advocated as a breeding goal (Ellen *et al.* 2008) because it would have a positive effect on welfare and health without loss of integrity. Robust animals are organisms that have the ability to maintain their performance across different environmental conditions (Waddington 1960). Selection for robustness in fish breeding programs would result in animals that have a greater ability to maintain their homeostatic state when exposed to stressors, thus improving fish welfare (Ellen *et al.* 2008). James (2009) discusses this issue in the context of genotype by environment interactions. When a genetic improvement program has to service several environments selection index theory could be applied, using the relative importance of the environments as weighting factors. Because the breeding goal should target the future circumstances the weighting factors should reflect the anticipated future relative importance, rather than the present one. Given the high reproductive rate of aquatic animals and the relatively modest resources (compared to livestock) that would be required for testing in multiple environments it appears that research in this area would be worthwhile. To our knowledge there have been no genetic improvement programs with aquatic animals formally targeting multiple environments.

There are other challenges such as feed utilization efficiency, flesh quality, fitness related issues, and disease outbreaks due to intensive aquaculture that are not dealt in the present review.

CONCLUDING REMARKS

Genetically improved strains are essential to aquaculture development. The application of proven quantitative genetic theory should continue for relevant species. There is ample proof of the success such programs can have. However, greater emphasis is required in the areas of dissemination of the improved stock to farmers in order to ensure impact at the production level. New developments in quantitative genetic theory will help prepare the aquaculture industry for the future. Broadening of the breeding objective to include behavioural traits and selection for multiple environments are areas where much fruitful research and development could be conducted.

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