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ESTIMATION OF RESPONSE TO WITHIN-FAMILY SELECTION FOR GROWTH IN NILE TILAPIA (*Oreochromis niloticus*)

by

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Submitted in partial fulfillment of the requirements for the degree of

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ABSTRACT

Within-family selection approach was undertaken to improve the growth at 16-weeks in Nile tilapia derived from locally adapted strains. The focus was the development of a selection strategy that will be applicable in conditions with limited facilities. Twelve generations of within-family selection have shown that this approach can effectively improve the growth of farmed tilapias as demonstrated by the selection response that was apparent up to the current generation covered by this study. The genetic trend showed a continuous linear response for body weight at 16 weeks. The regression of mean breeding values on generation number indicates that the expected genetic gain would be about 12% per generation. Based on mixed model methodology, the estimate of the heritability in the base population was 0.38. Genotype-environment interaction under the conditions that were examined in this study was of minor importance to the total variation for final body weight. Although the selection was done in a tank environment, substantial response was also observed in hapas and ponds. Routine selection activity can therefore be based on small facilities like tanks while the production of stock and the grow-out can proceed normally in ponds.

On a managerial perspective, it has been found that within-family selection is easy to manage and inbreeding can be kept to a minimum if a structured mating scheme like a rotational mating plan is used. Rotational mating has proven to be easy to apply in association with the within-family selection scheme where a complete pedigree is maintained. The within-family selection approach does not require extensive facilities as would be needed for a presumably more efficient selection approach like combined

selection. The choice of a selection procedure, particularly for tilapia aquaculture, is a matter to be decided not only on genetic but also on economic grounds given the prevalent scale of the tilapia industry in Asia, which is highly diverse and small-scale. On-farm selective breeding using a simple, low-cost within-family selection scheme can be practiced by small-scale farmers to manage and improve fish stocks. This will empower farmers to use strains of their choice and not be continually dependent on commercial hatcheries.

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Chapter 1

GENERAL INTRODUCTION

The application of breeding and genetics has made a substantial contribution towards increased productivity of farm plants and animals. Dickerson (1970) stated that at least 30 per cent of the increase in rate and efficiency of protein production in agriculture animals was the result of genetic research and comprehensive industry breeding programs. Perhaps the primary difference between yield in agriculture plants and animals and aquaculture species lies in the fact that the production of farm plants and animals is based on genetically improved breeds derived from varieties and stocks which have been domesticated for countless generations whereas the genetic improvements of aquaculture species is a very recent endeavour. Until now, the farming of many species of fish and shellfish such as milkfish (*Chanos chanos*), rabbit fish (*Siganus* spp.), some marine and freshwater shrimps/prawns, and bivalves is still dependant on capture of wild fry or brood stocks.

Compared to terrestrial plants and animals, aquaculture species are still largely undomesticated and less genetically improved (Wilkins, 1981; Bentsen, 1990). With the exception of the common carp (*Cyprinus carpio*), the breeding history of most aquaculture species spans only a matter of generations. In many species, there is still a paucity of information on the various determinants of their phenotypes and genotypes. However, a number of reviews on fish breeding and genetics cite the high potential for genetic

improvement to improve the productivity of important aquaculture species (Wilkins, 1981; Kinghorn, 1983; Gjedrem, 1983; 1985; Newkirk, 1980; 1983).

There are only a few applied fish breeding programs. Gjedrem (1993, 1997) mentioned the operation of breeding programs for Atlantic salmon (*Salmo salar*) and rainbow trout (*Onchorhynchus mykiss*) in Norway, Canada, and Sweden. Crossbreeding and selection programs with common carp are also in existence in Israel and Hungary (Hulata, 1995). In Asia, from which the majority of total aquaculture production comes, most aquaculture genetic improvement attempts are still at the research level (see Main and Reynolds, 1993). At present, the Genetic Improvement of Farmed Tilapia (GIFT) breeding program in the Philippines is perhaps the only organized breeding program for Nile tilapia (*Oreochromis niloticus*), with an established multiplier system to disseminate the genetically improved tilapia fingerlings to the farmers.

Gains and problems of fish breeding

According to Falconer (1989), the expected genetic gain per generation or round of selection depends on the product of three parameters; the intensity of selection, the accuracy of selection and the genetic standard deviation. In fish, it has been demonstrated that it is possible to increase growth rate up to about 15% per generation (Kincaid *et al.*, 1977; Bondari, 1983; Dunham and Smitherman, 1987; Hershberger *et al.*, 1990; Gjerde, 1986; Jarimopas, 1986; Dey and Eknath, 1997). These estimates are much higher than what is commonly found in farm animals.

Gjedrem (1997) stressed that a prerequisite for an efficient breeding program is the determination of genetic variation in important economic traits, but only a few fish species had been subjected to such studies. Nevertheless, relatively large genetic variation for some production traits has been found in Atlantic salmon, rainbow trout, tilapia, catfish (*Ictalurus punctatus*), and common carp (Gjedrem, 1983; Tave 1993: 132-147). The high fecundity in fish allows greater genetic gains to be obtained by applying high selection intensities. Although this can be as an advantage, this can also accelerate the rate of inbreeding because a very small number of individuals can make a large contribution to the genetic make up of the succeeding generations. The harmful effects of inbreeding include reduced fitness, depression for economic traits and loss of additive genetic variance (Falconer, 1989). If the inbreeding rate is not kept to a minimum, then these factors would provide less scope for further genetic improvement of aquaculture species. In fish populations, depression in growth traits, survival rates, and increased deformities have been observed due to inbreeding (Aulstad and Kittelsen, 1971; Kincaid, 1976, 1983; Gjerde *et al.*, 1983).

Another constraint in the development of breeding programs for aquaculture species is the lack of efficient technology to identify individual fish (Kinghorn, 1983). In general, young fish cannot be marked until they reach a certain size. In a program that use family information in the selection decision, this means that family groups must be reared separately until individual fish can be large enough for marking or tagging. Rearing of family groups separately is costly both in space and resources and contributes a level of

environmental variation that reduces the efficiency of family selection schemes.

The currently available marking/tagging methods for fish include branding, fin clipping, and various external tags. Recently, the use of internal PIT (Passive Integrated Transponder) tags has also become common. However, these methods of physical tagging can be size-selective and their long term legibility and retention can be real problems in field studies. In tilapia, for example, the Floy fingerling tags (Floy Tag Co., Seattle, Washington) can only be used when the fish size is about 3-5 g. In terms of the early growth period, this means that families must be reared in separate tanks/net enclosures (hapas) for about 2-3 months before the fish can be individually tagged. Fish can also become entangled on weeds or nets once they are tagged with external tags.

Microsatellite DNA profiling techniques for family identification purposes represents an important development in the selective breeding of aquaculture species. These techniques eliminate the need for physical tagging and enable families to be kept in a common pond/tank from birth onwards, thus eliminating environmental variability. Large numbers of families can be tested and higher selection intensities can be imposed without rapid accumulation of inbreeding (Doyle and Herbinger, 1994). Moav *et al.* (1976) suggested the use of protein polymorphism to "mark" families such that some mixing of stocks can be carried out after fertilization is completed. However, the level of allozyme polymorphism available for marking is relatively low which, according to Doyle and Herbinger (1994), can allow no more than 2 or 3 different genotypes to be distinguished in pooled populations. Herbinger *et al.* (1995) used genetic profiling data from microsatellite

markers to assess the feasibility of establishing pedigrees in mixed populations of rainbow trout under commercial aquaculture operations. The results showed that about 91% of the fish could be traced to one or two parental couples out of the 100 possible couples.

Cost of breeding program

Breeding programs are often regarded as long-term procedures and expensive to plan, initiate, and run. Gjedrem (1997) stated that investment and maintenance costs can be conservative for a breeding program that uses individual selection for a single trait but the costs can increase substantially when family selection is used as this requires testing more families per generation. The argument that breeding is a long-term operation is only true to a certain extent. The use of species with short generation intervals like tilapia have shown that the genetic gain that is achieved from applying genetic improvement can be made readily available to the industry (Eknath *et al.*, 1991). The current knowledge about phenotypic and genetic parameters for economically important traits in aquaculture species is sufficient to start breeding programs for Atlantic salmon, Pacific salmon, rainbow trout, tilapia, catfish, and several species of carps. Initial emphasis should be on simple breeding programs that focus on improving the growth rate (Gjedrem, 1997).

Methods of selection

The choice of a breeding method depends on the type of genetic variation present in the trait(s) of interest. Crossbreeding is used if non-additive genetic variation is considerable, while pure-breeding is used to exploit additive genetic variation.

Several methods are available for obtaining additive genetic improvement including mass selection, family selection, within-family selection, and combined family selection. The choice of methods is based on the heritability of the trait, the nature of the trait (e.g., normally distributed or binary, and whether records can be obtained on live individuals) and the reproductive capacity of the species (Gjerde and Rye, 1997). The following is a brief description of the selection methods that can be applied to aquaculture species.

Individual selection is a widely used selection method because it is relatively simple and easy to perform. Each individual is measured and the phenotypic value is compared to a predetermined cut-off value (e.g., upper 10% of the population). All fish that are equal to or larger than the cut-off value are selected while those that fall below the cut-off value are culled. The process is repeated in each new generation until the desired change in the mean phenotypic value of the population is obtained (Tave, 1993). Individual selection has been found to be efficient for traits with high heritability (Falconer, 1989). However, the moderate success of this method in actual selection experiments with fish may be caused by large, uncontrolled systematic environmental variation (e.g., age and tank/cage differences) and by adverse effects of inbreeding (Bentsen, 1990).

Family selection differs from individual selection in that the decision to select or reject is conducted at the family level, with individual phenotypic values being ignored except in the calculation of the family mean. Entire families are selected, usually groups of full-sibs or half-sibs, according to their mean phenotypic value (Falconer, 1989).

The conditions that make family selection more efficient than individual selection

are low heritability of the trait, little variation due to common environment, and large family size. Environmental variance among family means can be reduced by raising families in similar environments and by averaging over a large number of individuals in the calculation of the family means. However, family selection is likely to result in fewer families being represented among the selected parents, which could result in higher inbreeding rate unless the intensity of selection is correspondingly reduced. If reasonably high selection intensity is to be achieved at a low rate of inbreeding, then the number of families bred and measured must be increased. This makes family selection more expensive to undertake than either individual or within-family selection. The increased complexity and the resources required to rear a large number of families are the principal limitations of family selection. Detailed records must be kept and families of fish must be maintained separately (Falconer, 1989).

Within-family selection involves the selection of individuals based on their deviation from the family mean. Those individuals that deviate most are considered to be the most desirable. Within-family selection is useful when phenotypic differences among families are due primarily to environmental factors, rather than genetic differences among families (Uraiwan and Doyle, 1986). The mean phenotypic values of families are ignored for within-family selection. Instead, individuals from each family are selected and used as brood stocks. Consequently, only half as much space is required to maintain a population with a given effective size under a within-family selection program compared to individual selection (Falconer, 1989).

Combined selection is considered to be the optimal selection method. By combining family and within-family selection, the additive genetic variance both between and within families is utilized in an optimal way. Individuals are selected on the basis of an index that appropriately weights the deviation of the full-sib family mean from the population mean and the deviation of individual performance from the mean of the individual's family. These weightings are dependent on the intraclass correlation, the genetic relationship among members of the families, and the family size (Falconer, 1989).

Pedigree records will allow for combined selection strategies, utilizing the performance of relatives to determine individual breeding values through a selection index or by a mixed-model method (Gall *et al.*, 1993; Sorensen and Kennedy, 1983; 1984). The large numbers of full-sibs and half-sibs that may be produced simultaneously will increase the accuracy of the individual breeding value estimates. The use of sib information is also important when selecting for traits that may not be recorded in the breeding candidates (e.g., carcass quality traits) or traits that may only be quantified in frequencies (e.g., mortality, sexual maturation).

Combined selection requires individual tagging to provide complete pedigree records of all selected individuals. Mating may be planned to obtain minimum inbreeding coefficients in the progeny. However, physical tagging of fish is only possible after a certain growth period so families have to be reared separately, a situation that gives large common environmental effects in the full-sib families (Gjerde *et al.*, 1997).

A theoretical comparison of the relative efficiency of family selection and individual selection for 147-day weight in rainbow trout showed that family selection can be much more effective than individual selection (Kincaid *et al.*, 1977). This was confirmed by Gall and Huang (1988) in the estimation of expected response per generation to improve body weight traits in rainbow trout from individual, family, within-family, and combined selection. They found that combined selection is expected to produce a response per generation of about 10%-30% above that of individual and family selection and about twice the expected response for within-family selection. They added that the expected response under within-family selection is very low due to the relatively low intraclass correlations and cannot be recommended as a selection method for body weight traits in rainbow trout.

Selection methods for growth in tilapia

Growth rate is of economic importance for all species used in aquaculture. Usually it is easy to estimate through measurement of body weight or length. Individual selection has been used to improve the growth rate of a number of fish species, including tilapias. Teichert-Coddington and Smitherman (1988) selected for increased 58-day length in the Auburn-Ivory Coast strain of *O. niloticus* but obtained a negative response. The estimate of realized heritability for rapid early growth was -0.10 ± 0.02, which means that selection will not be effective in this particular strain of Nile tilapia. Also, Hulata *et al.* (1986) were unable to improve weight at 4 months of Nile tilapia in the Ghana strain. Huang and Liao (1990) found no response to individual selection for body weight in Nile tilapia. However,

Jarimopas (1986) reported a response to individual selection for body weight in red tilapia of about 16% after two generations of selection. Sanchez *et al.* (1995) undertook five generations of mass selection for weight and condition factor in a commercial strain of *O. aureus* which resulted in an average genetic gain per generation of 25.6 g, 11.2 g, and 17.7 g for males, females and combined sexes, respectively. Brzeski and Doyle (1995) have demonstrated a response of 2.3% measured as deviation in body length of the select from the control line in an on-farm mass selection procedure.

Detailed comparisons are hard to make from these results considering the different strains or species of tilapia used and it is difficult to know to what extent the previous history of selection, or the environmental conditions under which these different mass selection experiments were conducted, may have influenced the results. In general, the lack of response in these fish selection experiments has been attributed to the low heritability of the trait selected, inbreeding depression and to uncontrollable sources of environmental variance such as differences in spawning date and maternal effects.

Heritability estimates for growth in tilapia are low to moderate (Tave, 1996). Tave and Smitherman (1980) used half-sib analysis to obtained heritability estimates for the Auburn strain of *O. niloticus*. The sire heritability estimates were 0.10 ± 0.19 for 45-day weight and 0.04 ± 0.06 for 90-day weight while dam heritability estimates were 0.35 ± 0.19 for 45-day weight and 0.04 ± 0.08 for 90-day weight. The predicted responses for this stock of Nile tilapia were found to be small by using individual selection.

Advantages of within-family selection method

Some recognized sources of environmental variation affecting fish selection studies are maternal effects and differences in hatching time (Wolfarth and Moav, 1970; Hulata *et al.*, 1976, Doyle and Talbot, 1986a). In the maternal mouth-brooding tilapias, such as the Nile tilapia, females incubate their eggs and fry for about two weeks after ovulation which means that the fry are subject to a maternal environment during the early life stages. This contributes environmental variance between families. Similarly, the inability of tilapia to spawn synchronously can result in age-related differences between families. This environmental variance is a source of error that reduces precision in genetic studies (Falconer, 1989).

In search for a proper selection method that can be used for tilapia genetic improvement, especially under condition of limited facilities, Uraiwan and Doyle (1986) have found that within-family selection method would be suitable to improve the performance of Nile tilapia. The rationale behind the application of this selection approach is that it removes the environmental variance due to maternal effects and other environmental causes (e.g., climate, water quality, nutrition), permits high selection intensities, minimizes inbreeding, eliminates the extensive need for individual tagging, and reduces the demand for facilities (Uraiwan and Doyle, 1986).

The use of within-family selection has shown positive selection response in tilapia genetic improvement program. Abella *et al.* (1990) reported a higher growth rate of the selected *O. niloticus* than the random-bred control line after 2 generations of within-family

selection. Uraiwan (1990) obtained an improved growth of the Chitralada strain of *O. niloticus* also by applying within-family selection. After 8 generations of within-family selection, Bolivar *et al.* (1994) reported that the selected Nile tilapia were from 8 to 37% heavier than the random-bred control line.

Within-family selection is usually not predicted to lead to higher rates of response because only one-half of the additive genetic variance is expressed within families (Falconer 1989). However, it can be efficient for short-term selection if there is a very high environmental correlation of sibs, and for long-term selection because the effective population size is double that for random sampling among families, and may be many times larger if selection leads to very unequal family representation (Hill *et al.*, 1996). Under the infinitesimal model, selection leads to a reduction of variance between but not within families (Bulmer, 1971).

Demfle (1975) investigated the effect of within-family selection on selection limits and showed that this method is more efficient than individual selection when the heritability and the selection intensities were high, because of a relatively lower decay of the additive variance during selection. Within-family selection caused lower levels of inbreeding and hence ensured higher maintenance of genetic variance in the long term. Falconer (1973) concluded that within-family selection is likely to be the most useful alternative method of selection because it reduces genetic drift variance.

Genotype-environment interaction

Genotype-environment interaction is most commonly used to describe situations where differences between phenotypes due to differences in genotypes differ in their response from one environment to another. These differences in genotype response not only include changes in mean performance but also include variability in performance of different genotypes (Falconer, 1989). In reference to genotype-environment interaction, the term 'genotype' refers to the genetic differences among individuals or among lines within a breed, among breeds or even subspecies. Similarly, the term 'environment' can mean locations, temperatures, rations, years, management systems or other factors usually thought of as experimental treatments.

In the absence of a genotype-environment interaction, the best genotype in terms of the trait measured is perceived as the best in all environments. This has been the basis for the 'universal' or worldwide distribution of commercial strains, particularly in poultry. On the other hand, if the interaction is substantial, a separate breeding population may be needed for each particular type of environment. Doyle *et al.* (1991) used this argument to propose a selective diversification program for genetic improvement that addresses both the need for genetically improved strains to increase aquaculture production and the genetic conservation of aquaculture species. Their proposal is to generate and maintain strong genotype-environment interaction to develop specific strain or strains for specific environments. This way, a multitude of strains would be made available and maintained. However, the decision on whether and when to develop special strain(s) depends on several

factors which involve both the breeder's and the farmer's perspectives. From the breeder's perspective, these factors include the size of the market associated with a specific environment, the cost of developing a specialized strain for that environment, and the relative competitiveness and market share enjoyed by the breeder's own strain in that environment. From the farmer's perspective, the use of a specialized strain depends on the profitability relative to the other strains available or currently used by the farmer. It is obvious that economic factors will determine the decision to develop such specialized strain(s). Breeders will be discouraged to undertake breeding programs when the economic benefits are low. Doyle *et al.* (1991) presented an economic simulation study that suggests the economic advantage of this breeding policy (multiple-breed development) will accrue to the farmers rather than the breeders. The use of small-scale genetic improvement procedures would be an important element in the development of specialized strains (Uraiwan and Doyle, 1986). It may not be appealing to the breeder to put his investment at risk. Therefore such policy should be undertaken through a government initiative (Doyle *et al.*, 1991).

Animal model in fish breeding

Over the last decade, the restricted maximum likelihood (REML) analysis developed by Patterson and Thompson (1971) has emerged as the method of choice in animal breeding for predicting breeding values and estimating variance components (Meyer, 1989). Advances in computer technology and algorithms that exploit specific

features of the data structure or the model of analysis have facilitated this. The model of analysis is linear and includes a random effect representing the additive genetic value for each 'experimental unit' on which the measurement was taken. In animal breeding, these units are generally animals and the model is referred to as *Animal Model* (Henderson, 1984; Meyer, 1989).

The use of the animal model allows for individuals in the data and parents without records to be included in the analysis so that all known relationships among individuals can be taken into account. This gives the correct correlation structure for animals across many generations to be used in the analysis (Meyer and Hill, 1991).

The application of animal models has been extended to the analysis of data from selection experiments to evaluate selection responses. Sorensen and Kennedy (1984) have shown that mixed-model analyses such as animal models could be used to estimate genetic trends, even after several cycles of selection, if the genetic and non-genetic variances or their ratios are known before selection, if the selection is a linear function of the records, and if the relationship matrix is complete, e.g., all animals involved in the selection decision, regardless of whether they contribute offspring, are used to derive the relationship matrix. Blair and Pollak (1984) used a mixed-model approach to evaluate selection response using an assumed estimate of heritability to predict genetic merit. They further suggested that this approach may reduce the need for a control population when estimating genetic trend.

On theoretical grounds, it is well established that mixed-model methodologies, such as animal models with full pedigree information available on each candidate for selection, should lead to higher genetic progress (Henderson, 1973). While these methodologies are now widespread in animal genetic improvement programs, there are no substantial papers that identify their immediate application in fish breeding.

The adoption of mixed-model methodologies, such as an animal model in fish breeding, would require the demonstration of quantifiable benefits. As in any enterprise, before new methods are implemented and major changes in the breeding program effected, it is desirable to quantify the advantages of alternative methods in terms of improved genetic progress and to gain insight on their computing requirements and cost of implementation. In the case of fish breeding, it remains to be seen how much additional genetic progress is possible in using mixed-model approaches. However, Gall *et al.* (1993) predicted that this could become a powerful tool to the analysis of fish selection programs if data collection can be improved. This means that marking techniques to identify individual fish should be improved to allow inclusion of other relationships among individuals.

Tilapia aquaculture

The tilapias (Family Cichlidae) have gained worldwide recognition as one of the most important species for aquaculture. The culture of this group of fish, or research related to such culture, is now underway in at least 65 countries (Pullin *et al.*, 1994). Although several tilapia species are cultured, the Nile tilapia continues to be the most popular cultured

species. Its fast growth rate, tolerance of a wide range of environmental conditions, disease resistance, and acceptability to consumers makes Nile tilapia a good aquaculture species.

In the Philippines, tilapia culture began in 1950 with the introduction of Mozambique tilapia, *Oreochromis mossambicus* (Guerrero, 1985). But because of the undesirable characteristics of the species, notably its precocious maturity and poor yields, the culture of *O. mossambicus* did not flourish and ultimately the species became a pest in milkfish ponds. The introduction of Nile tilapia revived the interest of farmers in the culture of tilapia and promoted the development of the tilapia industry in the Philippines (Guerrero, 1996). Farmed tilapia production in the Philippines has increased from 30,908 t in 1984 to 94,322 t in 1994, an average growth rate of about 8% per year (FAO, 1996). Globally, tilapia production will continue to grow with a great potential for expansion as the market for farmed tilapia grows in developed countries (Popma and Lovshin, 1996).

For many years, tilapia aquaculture research has focused mainly on the development of culture technology that could improve production. A good deal of research related to improved management such as feeds and feeding practices, disease control, and rearing techniques has been undertaken. However, the full benefits from improved management can be obtained only through the use of genetically improved breeds or strains that are able to respond to these improvements.

Tilapia genetics research in the Philippines

The Nile tilapia is an important species for aquaculture in the tropics but the lack of proper stock management for this species has resulted in poor genetic quality of the earlier farmed breeds, making them unproductive for culture. The available stock had also suffered from inbreeding depression due to small founder populations (Pullin and Capili, 1988). Tilapia farmers have been slow in recognizing the importance of applied genetics. Until recently, there have been no attempts to apply additive selective breeding to farmed tilapias, an approach that has been well-proven in livestock but has been only applied with salmonids among aquaculture species (Gjedrem, 1985, 1992). Little work has been done to improve farmed breeds by genetic means. A majority of the genetic studies on tilapia have focused on sex manipulation (Stickney, 1995, 1996) while early works dealt on hybridization and hormonal sex reversal (reviewed by Tave, 1988).

During the last ten to twelve years, tilapia has been the focus of genetic improvement in Asia, particularly in the Philippines. With varying approaches, three internationally funded research projects were conducted at almost the same time. One of these is the Genetic Improvement of Farmed Tilapias (GIFT) implemented by the International Center for Living Aquatic Resource Management (ICLARM) in collaboration with the Philippine Bureau of Fisheries and Aquatic Resources, the Freshwater Aquaculture Center of the Central Luzon State University (FAC-CLSU), and the Institute of Aquaculture Research of Norway (AKVAFORSK). The objective was to bring tilapia germplasm from Africa for evaluation along with existing cultured stocks in the Philippines in a wide range

of farming systems and the establishment of a synthetic base population. Combined selection approach used to improve the growth of tilapia (Eknath *et al.*, 1991).

From 1986 to 1996, the International Development Research Centre supported a project at the Freshwater Aquaculture Center of the Central Luzon State University with the objective of evaluating a within-family selection method for Nile tilapia to improve its growth performance. The approach was to develop a method appropriate to smaller and less-endowed facilities and to utilize only locally adapted strains of tilapia as a base population for the selection program. The rationale for the selection approach that was applied in this project was based on the findings of Uraiwan and Doyle (1986) that within-family selection would be suitable for small-scale tilapia genetic improvement programs.

The research project, Genetic Manipulation for Improved Tilapia (GMIT), investigated the use of genetic manipulation to produce all-male producing brood stocks. This work was also conducted at Freshwater Aquaculture Center of the Central Luzon State University in collaboration with the University of Wales at Swansea with support from the Overseas Development Administration. The research was focused on the sex determination mechanism in different strains of Nile tilapia. The technique for *O. niloticus* was based on the production of large numbers of YY males, which will yield all-male progeny known as genetically male tilapia (GMT) when crossed with normal females (Mair *et al.*, 1997).

Selective breeding experiments with aquaculture species

Selective breeding programs and selection experiments have been conducted to improve growth rate, age at spawning, viability, disease resistance, and sex ratio. Most of these studies are relatively recent and involve only a few aquaculture species such as rainbow trout, Atlantic salmon, coho salmon (*Oncorhynchus kisutch*), channel catfish (*Ictalurus punctatus*), common carp, Mozambique tilapia, Nile tilapia, blue tilapia (*Oreochromis aureus*), red tilapia, European oyster (*Ostrea edulis*), Chilean oyster (*O. chilensis*), and Pacific oyster (*Crassostrea gigas*).

One example of a successful selection program was conducted with coho salmon. Growth was improved an average of 6.7% per generation during the freshwater phase and 10.1% per generation during the saltwater period (Hershberger *et al.*, 1990). This improvement has decreased the time needed to produce marketable-sized fish from 11 months to just 6 months in the selected lines. The program also showed that a long-term selection program could make large improvements in performance without reducing genetic variation.

Another selection program that achieved its goal to improve a production trait was undertaken with rainbow trout (Kincaid *et al.*, 1977). The selection was a combination of between-family and within-family selection for increased body weight at 147 days post-fertilization. The genetic gain during three generations of selection was 0.98 g or 5% gain per year. Bondari (1983) reported strong asymmetrical responses to selection for body weight in a population of *O. aureus*. Moav and Wohlfarth (1976) have shown a selection

response for growth rate of common carp using between family selection. However, selection for fast growth using mass selection yielded a negative response.

Genetic controls in selection experiments

Hill (1972) stated that the separation of observed change into its environmental and genetic components is an important part of the analysis of selection experiments or breeding programmes. Early work in poultry breeding research was conducted without a control as a reference point (reviewed by Hutt, 1949). Similarly, early selection experiments in fish were done without control populations (Lewis, 1944; Donaldson and Olson, 1955). The value of control populations in fish selection experiments has been recognized in more recent years (Kincaid, 1979; Hershberger *et al.*, 1990). It is possible that the reported response is confounded with environmental changes like improved husbandry. When the experiment involves several generations, the genetic treatment cannot be related to the original populations without assuming that the environment has remained constant (Bray *et al.*, 1962). But a constant environment rarely exists except perhaps under very special laboratory conditions and involving small species such as *Drosophila*, *Tribolium*, or mice. In larger species and large populations, it would be very costly to maintain a constant environment. A control population would then be important to separate environmental trends and fluctuations from genetic trends.

Several forms of genetic controls have been designed for selection experiments and breeding programmes: inbred lines, divergent selection, comparison of selected lines,

unpedigreed random bred control lines, pedigreed random bred control lines, frozen embryos, frozen semen, and repeat mating control lines. Gowe and Fairfull (1990) identified the advantages and limitations of each of these methods. Most of the procedures have more utility for poultry and animal breeding, however, Gall *et al.* (1993) discussed the designs which are relevant to fish selection experiments and breeding programs.

Random bred control is the simplest type of control. It is a breeding population sampled from the base population. If the population is effectively large, genetic changes will be very small so the only source of variation in performance of the control line is environmental (Falconer, 1989). The genetic change in the selected line can be estimated as a deviation from the control line each generation, assuming that the environmental changes affect both selected and control populations equally. This type of control has the advantage of being relatively economical to maintain for naturally mated species, particularly if the progeny produced can be used in some practical way between reproductions (Gowe and Fairfull, 1990).

The use of divergently selected lines is efficient if the only objective is to measure the regression of response on the selection differential. The two divergently selected lines will result in the most efficient use of resources (Falconer, 1989). Assessing response through divergent selection requires that two lines be derived from the same base population (Gall *et al.*, 1993). Individuals are selected for increased phenotypic merit in one line and decreased phenotypic merit in the other line under the assumption that the magnitude of genetic change will be equal for both directions. Falconer (1989) pointed out

how a divergent selection method can improve the accuracy of estimation of the specific response being measured. However, he also noted that if the interest is primarily in the change in one direction, the use of an unselected control is preferable since the response in the divergent selection is often not equal in the two directions. Furthermore, it is unlikely that selected lines produced through increased and decreased directions would both be economically valuable.

Repeat mating control, in its simplest form, requires mating all or part of the selected males and females repeatedly so that their progeny can be compared with the progeny of the next generation of selected animals. This was proposed by Goodwin *et al.* (1960) as a method of providing estimates of environmental effects without maintaining a control line. The major disadvantages of this method include the added complication of maintaining populations of different ages and maintaining genetic equality among progeny representing each generation. The genetic differences among progeny sets can arise from random sampling of alleles during repeat matings if the number of progeny is small, and from the loss of parents due to death or infertility (Gall *et al.*, 1993).

Objectives of the thesis

The general objective of this thesis is to evaluate a within-family selection procedure for improving growth of Nile tilapia in limited facilities with the ultimate objective of providing the small- to moderate-scale institutions or farmers with a tool to manage stocks in a more systematic manner than has been practised in the past.

My specific objectives are:

1. To quantify the response to selection for body weight at 16 weeks in Nile tilapia in various culture environments.
2. To evaluate the effect of communal and separate rearing methods in growth performance testings of different test groups of Nile tilapia.
3. To determine the presence of genotype-environment interactions under the range of conditions that were tested in this study.
4. To quantify the selection response from 12 generations of within-family selection experiment using a single-trait animal model.

Structure of the thesis

This thesis is divided into six chapters. Chapter 1 is a general introduction that states the objectives of the thesis, discusses the gains and problems in fish breeding, the importance of tilapia and genetics research in aquaculture and reviews briefly the selection methods and control populations that can be used in selection experiments. Chapter 2 is a description of the general methodology used in the study associated with the within-family selection experiments. Although it does not deal directly with answering the specific objectives of the research, it provides an important methodological chapter. Chapter 3 deals with the testing of response in the selected Nile tilapia in various culture environments, using two variants of control lines, a commercial strain of Nile tilapia, and two other

genetically improved Nile tilapia strains. Genotype-environment interactions are also evaluated in this chapter. Chapter 4 presents the results of testing selection response under communal and separate rearing. Chapter 5 deals with the estimation of genetic trend and genetic parameters using a mixed-model methodology (animal model). At present, very few studies have used an animal model in estimating response in fish selection experiments. Chapter 6 is a general discussion of overall results of this thesis and the potential implication of the methodological work on the design of small-scale tilapia genetic improvement program.

Chapter 2

WITHIN-FAMILY SELECTION: GENERAL METHODOLOGY

This chapter outlines the different procedures that were used in this selection experiment. The method of selection used to improve growth rate on Nile tilapia was within-family selection. This chapter also describes the procedure to establish and maintain control lines.

Base population

The base population in this selection experiment was taken from the second generation of a high growth line of Nile tilapia developed from a separate selection experiment (Abella *et al.*, 1986). Four strains of Nile tilapia, namely Israel, Singapore, Taiwan, and FAC strain were combined to create a founder population for that previous selection experiment. The FAC strain was collected from the breeding ponds of the Freshwater Aquaculture Center and believed to have a record of ancestry from an earlier introduction of the Singapore strains (Lester *et al.*, 1988). Random samples of brood fish from the high growth line were obtained to establish a base population of 19 families in the present study (Abella *et al.*, 1990).

Spawning procedure

Ideally, one male and female are needed to produce the next generation but to ensure the propagation of families, one male was paired with two females in each tank. If the two

females spawned in the same week, the family that came from the heaviest female at the time of selection was chosen. Beyond that period, the family from the female that spawned first was selected. Back-up matings using the 2nd heaviest male and the 3rd and 4th heaviest females were also performed.

The brood fish were observed closely for spawning activity. When a male tilapia showed aggressive behaviour to the point that the female appeared to be severely stressed, the upper jaw (premaxilla) of the male was removed with a pair of scissors. The females were observed for mouthbrooding activity. Brooding females, which can be detected by an enlarged buccal cavity and territorial behaviour, were left in the tanks while the others were transferred to other holding units. The females were allowed to incubate the fertilized eggs for up to 12 days to make certain that yolk-sac fry were collected. However, in cases when the female spat eggs out accidentally, the eggs were transferred to hatching jars for artificial incubation. The brooders were removed from the tanks after spawning.

Rearing of fry

The average number of eggs per female was about 400 but there were females that spawned as many as 800-1000 fry at one ovulation. These numbers can result in high stocking density in the tank and may affect early growth of fry. To solve this problem, the initial number of fry was standardized to 200 randomly sampled fry from each family. (Fry were sampled by scooping out fry in a small net and counting out the first 200.) Each full-sib family was stocked in a separate tank. Thirty randomly chosen fish from each family were individually weighed at stocking to record initial weight.

Management procedures related to the rearing of fry consisted of regular feeding, cleaning, and changing of the water in the tanks. A flow-through water system was maintained. The fish were fed a commercial tilapia diet (40% crude protein) at a rate of 100% of body weight per day during the first two weeks in the tank, 50% on the 3rd to 8th week, 30% on the 9th week, 20% on the 10th week, and 10% thereafter until selection. Feeding occurred twice a day. Thirty fish from each family were weighed every month to adjust the weight of feed accordingly. The fish were blot-dried prior to weighing to remove excess water on the body that may otherwise affect the weight of the fish.

Size-grading technique

Size-grading was carried out when the fish reached an average weight of 0.5 g to reduce the phenotypic and presumed non-genetic variance in size during the early stage of the life cycle of the fish (Doyle and Talbot, 1986a).

Size-grading was done by measuring 30 random samples of fish to determine the mean body weight in each family. The largest and the smallest fish in each family were culled and those fish with body weights closest to the family mean $\pm$ 1 standard deviation were saved. Family size was reduced to 100 fish during this procedure. This reduction in the number of fish was done to keep the family size within the limits of the rearing capacity of the tanks, thereby reducing the possible effects of competition.

Selection procedure

The selection was carried out within families, selecting for body weight at 16 weeks post-hatching. This is the time when tilapia attain sexual maturity and when growth rate decreases due to reproductive activities. All individuals in each family were weighed at the time of selection. Selection was done independently in males and females. The heaviest male and female within each family was selected to be the brood fish to produce the next generation. However, back-up fish were also kept in the event of loss or death of the selected fish. Up to 8 and 10 top ranking males and females were individually tagged accordingly in each family. Selected males and females were kept separated in each tank by putting a net screen at the middle of the tank. The fish were kept in the tanks until the next round of mating began.

Tagging of selected fish

In using within-family selection, tagging of the selected fish was necessary because more than one fish of each sex was selected from each family. During the early stage of the project, several kinds of marking and tagging were tried to determine which was suitable for tilapia. Among these were cold branding, use of dye, fin clipping, and tagging. Cold branding and the use of dye did not give satisfactory results to mark tilapia. Clipping of pectoral and pelvic fins provided a longer mark on the fish, but the regeneration of the fins often occurred 2-3 weeks after clipping. Clipping at the base of the fins retarded the regeneration of fins. Fin clipping was used in growth evaluation studies under communal rearing (Chapters 3 and 4).

Improvised tags were used in the earlier generations of selected fish. The tag was made of nylon thread with cut pieces (about 2 mm in length) of telephone wire insulators secured at one end of the thread. The number of pieces corresponded to the ranking of the selected fish in that family (e.g., 1 piece corresponded to the top ranking fish). The availability of various colors of insulators allowed for the creation of additional codes by color combination.

The later generations were tagged with fingerling Floy tags. This tag consisted of an elastic thread with a small plastic disk attached at the end of the thread. The disk contained a combination of numbers and letters. The Floy tags were more effective than the improvised tags in keeping fish identity. However, their availability and cost are constraints because they have to be ordered from the United States.

The Floy tags were placed into the body of the fish using a needle that was passed through the anterior musculature between the lateral line and the dorsal fin. The tag was secured by making a loop on the thread. The fish were anesthetized during this procedure.

Spawning of the selected brood stock

Often sexual maturity was not reached until the fish had been conditioned for a certain period of time in the tanks. Once the brood fish were ready, which can be determined from the condition of the genital papillae of the fish, the next round of mating was set. The priority was the spawning of the heaviest female but in the event that the heaviest female did not spawn, the second heaviest female (based on body weight measured

at the time of selection) was considered. A waiting period of 10 weeks was established to allow as many families to spawn as possible.

Rotational mating scheme

A modified rotational mating scheme was used for family crossings rather than the line-crossing method that was described by Kincaid (1977). This mating scheme was used to produce the succeeding generations. This design avoided mating of closely related individuals, thus the rate of inbreeding was minimized. In the base population, the families were assigned with letters (e.g., A, B, C, etc.). To produce the first generation, female from family A was mated to the male of family B, female from family B was mated to male of family C, etc. In the next generation, the female in family A was mated to the male in family C, the females of family B mated to the male of family D, and so on. This continued with a jump to 4 letters then 8 letters in the next 2 matings (Figure 2.1). The female retained the family letter across generations. The male was moved to the next assigned family. Figure 2.2 shows the pedigree of family A after 5 generations.

Record keeping would be necessary to keep track of the proper mating plan. It is important to at least record which families were mated and when. Keeping a record of the pedigree will be useful for a systematic mating scheme such as a rotational mating.

Labelling of generations

The parent generation was labelled as P_o and the offspring they produced became the first generation of selected fish (S_1). When the selection was done at 16 weeks, the fish that were saved to become brood fish were labelled as S_{1sp} and their offspring were called the

second generation of selected fish and were labelled as S_2, and so on. Twelve generations of selection were completed for this study.

Facilities

One important requirement to initiate a selective breeding program is the availability of facilities. For this particular selection experiment, the spawning of fish, rearing and selection procedures were all done in out-door concrete tanks. This gave better control of the mating and easier monitoring of the condition of the fish at various stages of development. The concrete tanks measured 2.5-m^2. A total of 32 tanks was used to hold the selected families every generation although it was necessary to have additional tanks to hold fish during routine activities. A water pump with an overhead tank supplied water in the tanks.

Hapas or net enclosures were useful for holding brood stock and for fry rearing. (Hapas are small enclosures made of netting materials that are suspended in the pond using bamboo poles.). In general, the facilities that were used in this experiment were typical of those found in small-scale tilapia farms or fisheries research institutions in the Philippines.

Establishment of control populations

The first phase of the selection experiment did not include an unselected control line. Previous estimates of selection response involved comparison of the progeny groups that differed in generation of selection. This meant re-spawning of the fish from the earlier selected generation and the latest generation and then comparing their progeny performance.

The comparison involved females differing in age, so genetic change was confounded with age-of-dam effect. The other problems with this method were the older females proved difficult to spawn naturally and few repeat matings could be undertaken. Also, considering the limited resources and facilities available, it was not easy to maintain the fish in the earlier selected generations without losing some of the selected fish.

To circumvent these problems, two variants of control lines were established from the select line. The select line that had previously undergone two generations of selection for body weight was used to start the random bred control line. Figure 2.3 shows the establishment and maintenance of the two control lines.

Random Bred Control Line (RBC)

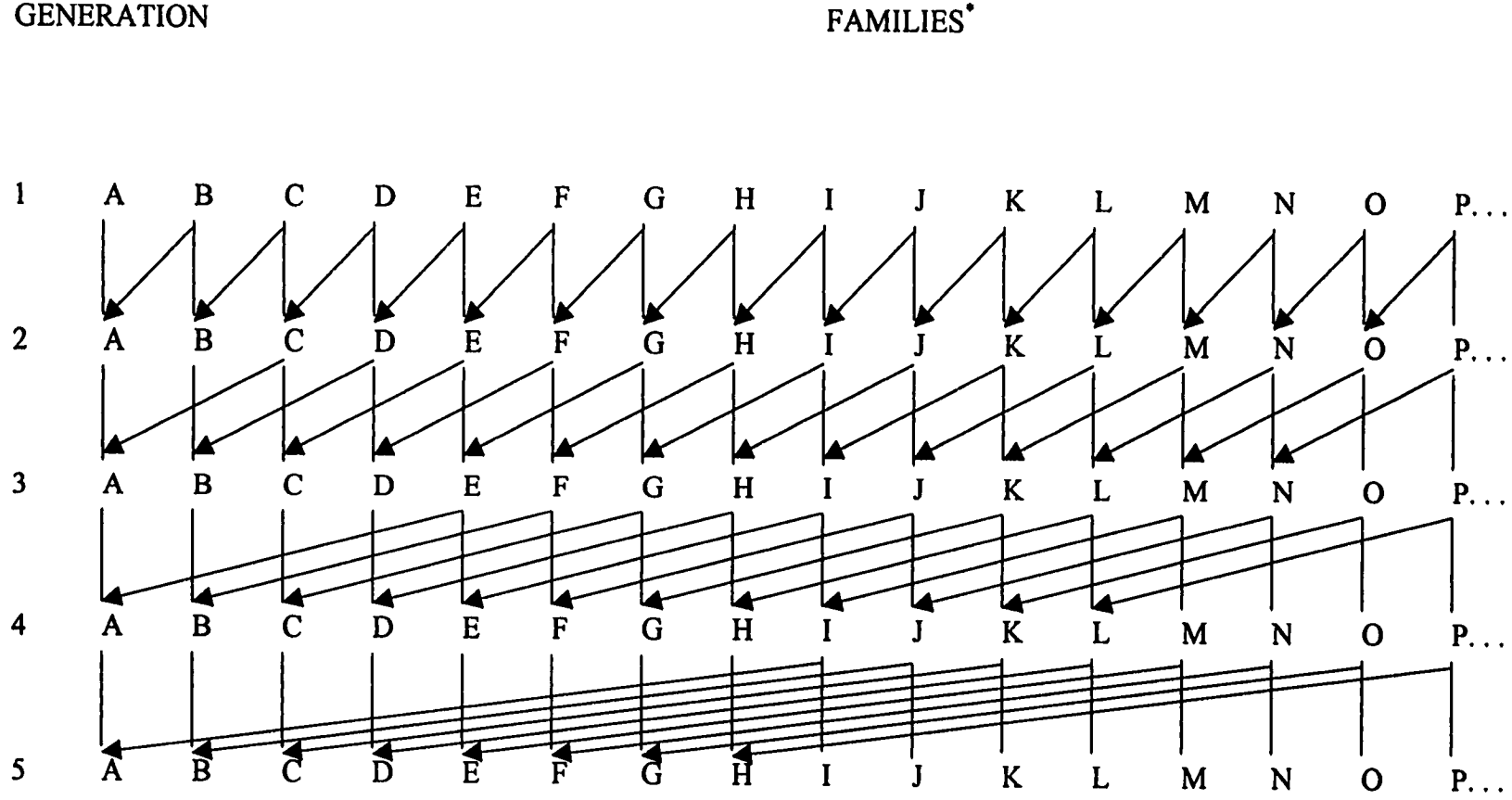
When it was decided that a random-bred control line was to be established, the oldest selected generation that was available was generation 2 (S_2). The RBC was established by sampling from selected parents from S_2 ($=S_{2sp}$). These became the parents of the first generation of RBC. Essentially, the RBC can be considered mostly S_3 genotypes contributing to the random bred control populations. Three lines were formed with each line of 20 males and 40 females pool-spawned in a breeding hapa. (A hapa is a term used for a net enclosure that is installed in a pond.). Four batches of fry were collected from each line within a period of one week. About 100 fry per batch were reared in separate hapas until they reached a body weight of about 3-5 g. Twenty-five (25) fish of mixed sex from each batch were taken at random and individually tagged. The identity of the batches in each line was maintained through the tags. All tagged fish from the same line were combined in one

hapa. At the time of propagation, 20 males and 40 females (5 males and 10 females from each batch) were stocked in a breeding hapa. The three lines were propagated using a rotation line crossing (e.g., Line 1 ♀♀ x Line 2 ♂♂, Line 2 ♀♀ x Line 3 ♂♂, Line 3 ♀♀ x Line 1 ♂♂).

Mean Selected Control Line (MSC)

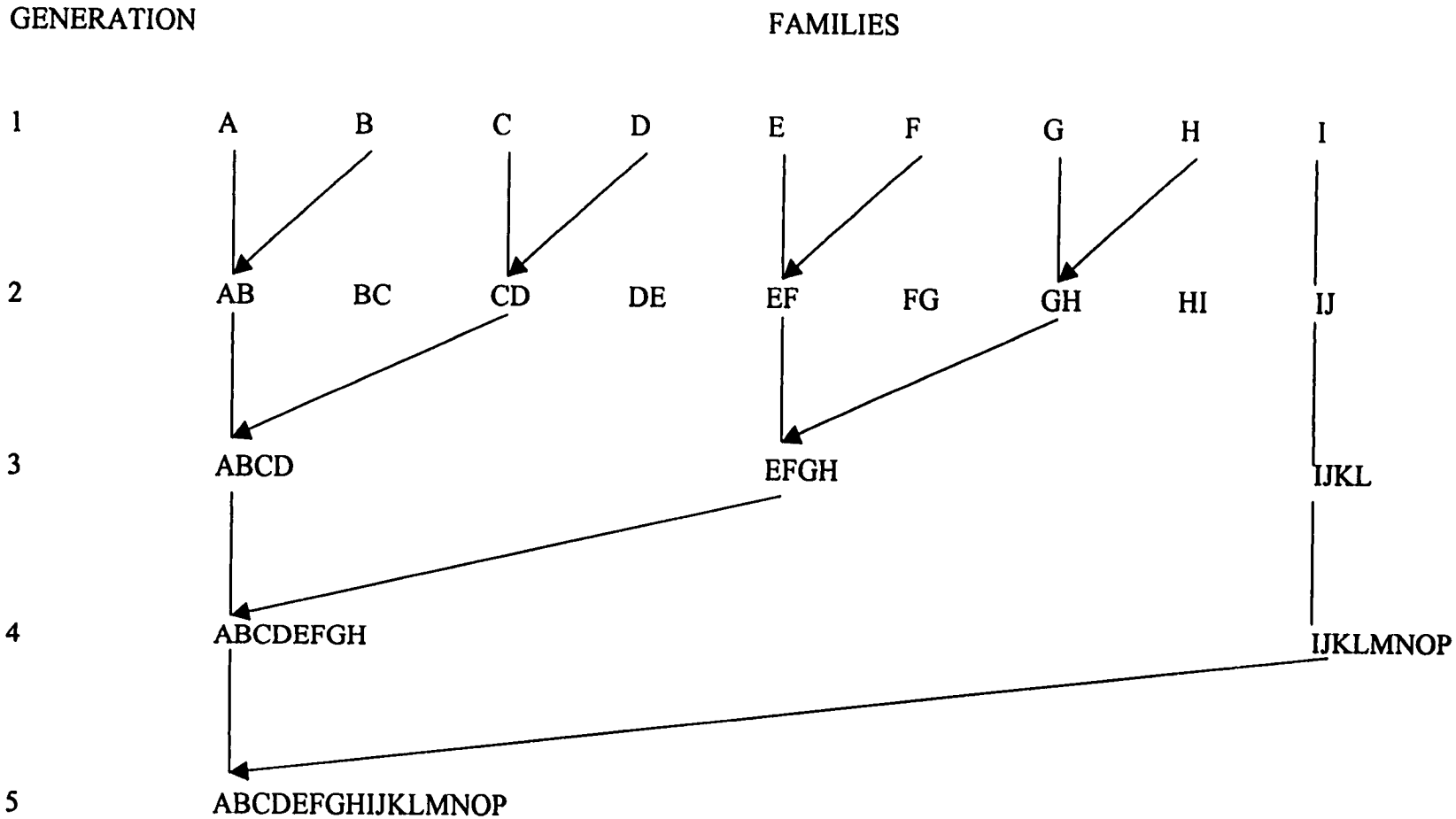
The rationale for the establishment of the MSC population was that natural selection favors those deviants closest to the population mean as opposed to artificial selection which favors the extreme deviants for a given trait. By selecting animals with mean phenotypic values, genetic change due to drift can be minimized in the control population making it a more stable control than random bred control population.

The MSC population was established from the first generation of the RBC population. Three lines were also formed, each line having 20 males and 40 females (5 males and 10 females derived from the mean of each of the four batches of RBC). The three lines were propagated by pool spawning, following a rotational line crossing. The maintenance of the lines was similar to RBC except that instead of obtaining random samples from each batch, the batch mean was determined. Twenty-five (25) fish with body weight closest to the mean were selected and tagged. The next generation was produced by pooled spawning 20 males and 40 females, again following the rotational line crossing described for the RBC population.



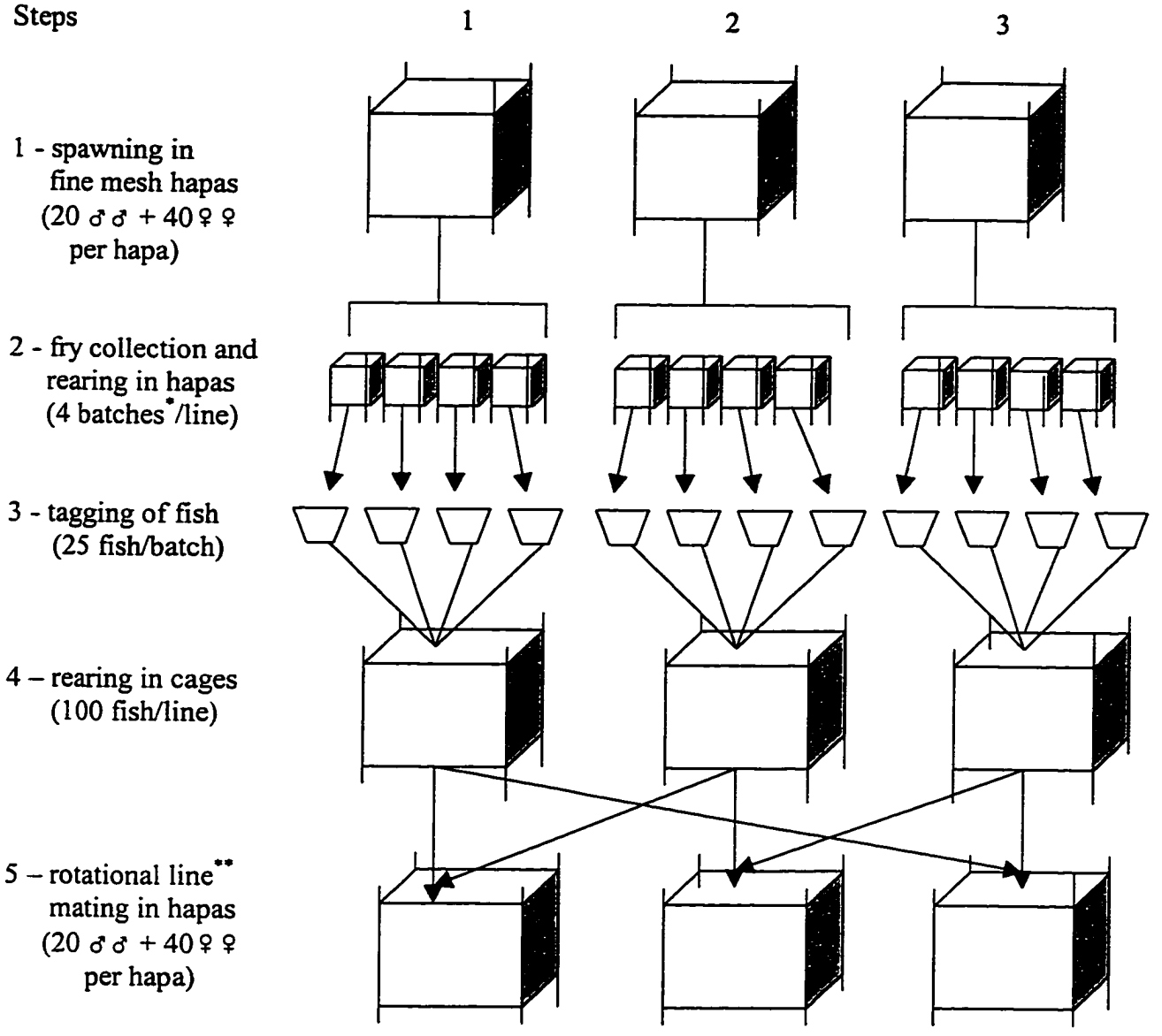
*One family per tank. The tanks are lettered. The female stays in the tank of birth while male is moved as indicated by the diagonal line (Brzeski *et al.*, 1989).

Figure 2.1 Family rotational mating scheme for 16 families.



The letters indicate the original families that have contributed to the genes in the family at any generation. There is equal contribution from all the lettered families (Brzeski *et al.*, 1989).

Figure 2.2 The pedigree of the A family is shown over 5 generations.



* batch is a collection of fry during short episodes of spawning (3-7 days)
** vertical line : source of females
diagonal line: source of males

Figure 2.3 Procedure in the establishment and maintenance of the control populations.

Chapter 3

RESPONSE TO SELECTION FOR BODY WEIGHT OF NILE TILAPIA (*Oreochromis niloticus*) IN DIFFERENT CULTURE ENVIRONMENTS

ABSTRACT

Within-family selection was practiced in Nile tilapia (*Oreochromis niloticus*) for 12 generations to increase body weight at 16 weeks. Response to selection was evaluated on the progenies from three selected generations (S_{10}, S_{11}, S_{13}) in tanks, hapas, and ponds. Two variants of control lines (random-bred control and mean selected control populations) were used to account for environmental changes during the course of the selection experiments. Two genetically improved strains (GIFT strain and genetically male tilapia strain) and a commercial strain (Israel strain) were included in the performance evaluation. The selected group consistently had the highest final body weights. The highest response was observed in the selection environment (tanks). A higher response occurred in the tank for S_{10} (68% as deviation from the RBC group) although response was still substantial at S_{13}. A significant interaction was observed in the 1996 GxE study but the interaction was not sufficiently large to produce changes in the ranking of the test groups. Overall, the result of this study showed that the selected group produced from within-family selection had improved growth performance. Selection response was similarly obtained in hapa and pond environments.

INTRODUCTION

Early selection experiments in fish did not have effective means of measuring genetic response. As Donaldson and Olson (1957: p. 95) wrote, "Many of the real advantages gained by selective breeding are difficult to measure. The improved quality of the fish is very obvious to those who have worked with the problem over a number of years. Other areas of improvement, such as increased growth rate and increased egg production, are simply matters of records." Fish breeding research was not alone in this dilemma. Gowe and Fairfull (1990) pointed out that early poultry breeding research did not recognize the need for genetic control procedures. The assumption must have been that any progress made had to be due solely to the selection program. Many experiments were limited to measuring phenotypic time trends, which could not be partitioned into respective genetic and environmental components owing to lack of controls or proper design.

Recent fish selection work has shown much improvement in terms of increased population size, low inbreeding levels by using planned mating schemes, and the maintenance of control populations to provide standard material for the evaluation of genetic trends. Kincaid (1979) reported the development and maintenance of standard reference lines of rainbow trout that are routinely used as control lines in their selection program. Hershberger *et al.* (1990) also maintained two distinct control lines, an internal control that was derived by sub-sampling all the families from the first generation of selection and a second control line acquired yearly from other hatcheries ("wild" controls).

These types of control lines were maintained for both the odd- and even-year selected lines of coho salmon.

The common methods for evaluating response to selection in fish selection experiments are the use of a random bred control population and divergent (high and low) selection experiments. Random bred control populations have been used by many investigators to provide a means for correcting for environmental trends or fluctuations that occur concomitantly with genetic changes brought about by artificial selection (Hill, 1972b; Fredeen, 1986). Theoretical aspects of the design and efficiency of such control populations have been discussed by Hill (1972a), who pointed out that several possible sources of error exist in the use of such controls for estimating genetic change. These include: random genetic drift in the control and selected populations because of restrictions in the size of the populations used; genetic trends in the control caused by natural selection; and the differential response of control and selected lines to environmental changes (e.g., genotype-environment interactions). Hill (1972b) also noted that with one or more control populations, both genetic drift and natural selection effects can be expressed as a trend in the mean genotype of the control populations over time. If the environment remains unchanged over a period of generations, and no trend develops in the control populations, there is evidence that the control has remained genetically stable.

In experiments with control populations, response is measured as a deviation of the selected line from the control while in divergent selection, the estimates of genetic change

can be achieved by contemporary comparison of such two divergent lines. The advantages and disadvantages of these and other types of control lines were mentioned in Chapter 1.

In this work, two variants of control lines - random bred control (RBC) and mean selected control (MSC) populations - were established to compare their consistency of performance in different culture conditions and over time. These control lines were used to determine the response to selection in a series of growth performance tests on the selected lines of Nile tilapia in various culture environments.

Genotype-environment interaction studies have become increasingly important to gain understanding about the consequences of such interactions on selection response. Significant genotype-environment interactions have been demonstrated in aquaculture species (Wohlfarth *et al*, 1983a, 1983b; 1986; Beachum, 1987; Hanke *et al.*, 1989; Dunham *et al.*, 1990; Romana-Eguia and Doyle, 1992; Sylven and Elvingson, 1992; Uraiwan *et al.*, 1995; Toro and Paredes, 1996). These studies provide an indication of the possibility to generate genetic diversity among breeds or strains through selective diversification programs (Doyle *et al.*, 1991). In some cases, genotype-environment interaction can be statistically significant but may account for a minute portion of the total phenotypic variance, thereby allowing researchers to ignore the interaction effect in planning a selection program (Gunnes and Gjedrem, 1978; 1981; Eknath *et al.*, 1993).

The selection experiment described in this thesis was undertaken entirely in tanks but the intended production units are cages and ponds. It is important to evaluate the selected line under the environmental and management conditions in which their progeny

are expected to perform (e.g., ponds) as well as the conditions under which the selection was made (tanks). In the present study, the pond experiments approximated the normal tilapia farming conditions in the Philippines.

This study was part of the general objective of evaluating the effectiveness of within-family selection for increased body weight in Nile tilapia. The specific objectives to be addressed in this chapter are: 1) to determine the response to selection on growth of the selected line of Nile tilapia in different culture environments, and 2) to determine the presence of genotype-environment interaction.

MATERIALS AND METHODS

Source of brood stock

Brood stock for the selected groups (S_{9sp}, S_{10sp}, and S_{12sp}) were derived from the 9th, 10th, and 12th selected generations of Nile tilapia produced by within-family selection. The selection experiment was conducted at the Freshwater Aquaculture Center of the Central Luzon State University (FAC-CLSU) with support from the International Development Research Centre. Details of the selection procedure are described in Chapter 2. The brood stock for the random bred control (RBC) line was obtained from the 2nd, 3rd, and 4th generations of RBC while the mean selected control (MSC) line was from the 3rd and 4th generations of MSC. The generation number used in this study refers to the parental stocks (e.g., using the labelling of selected generations described in Chapter 2, S_{9sp} were the

selected parents at the ninth selected generation, RBC_2 were randomly sampled brood stock from the 2nd generation of RBC, S_{12sp} were the selected parents at the twelfth generation, etc.). The materials used in this series of studies were offspring generations produced by pool spawning of a random sample of brood stock from the parental generations, as indicated. In effect, the test groups were equivalent to S_{10}, S_{11}, and S_{13} for the selected groups; RBC_3, RBC_4, and RBC_5 for the RBC groups and MSC_4 and MSC_5 for the MSC groups.

Israel strain was obtained from the Philippine Bureau of Fisheries and Aquatic Resources, the agency that distributes tilapia fingerlings throughout the country. This strain was most widely used for commercial production. The GIFT strain was taken from the 5th selected generation of GIFT strain produced through combined selection. This selection program was conducted by the Genetic Improvement of Farmed Tilapia (GIFT) project, a collaborative research project implemented by the International Center for Living Aquatic Resources Management (ICLARM) in collaboration with the Institute of Aquaculture Research in Norway (AKVAFORSK) and two national institutions: FAC-CLSU and the National Freshwater Fisheries Technology Research Center of the Philippine Bureau of Fisheries and Aquatic Resources (NFFTRC-BFAR). Genetically male tilapia (GMT) were derived from the offspring produced from the YY-male brood stock mated with normal females. The GMT was produced by the Genetic Manipulation for Improved Tilapia (GMIT) project at FAC-CLSU in collaboration with the University of Wales at Swansea.

Production of test groups and stocking

In experiment 1, the selected group was produced as part of the routine propagation of families of the selected lines in the tanks (described in Chapter 2). There were 21 full-sib families from Lines 1 and 2 that were included in this experiment. At the same time when the families were propagated in the tanks, the RBC line was produced from pool spawning of samples of brood stock drawn from the 2nd parental generation of RBC. Thirty (30) fry from the RBC group were added into each tank of full-sib family from the selected line. The two groups were size-matched to have the same initial weight as much as possible. The control fish were fin-clipped before stocking into the tank. The addition of RBC in the tank was done at the time the number of fish per family in the selected line was reduced to 100 per tank.

The production of test groups for experiments 2 and 3 consisted of pooled spawning of brood stock in ponds, each group in a different pond. The test groups were composed of SEL, RBC and ISR. Each pond was stocked with about 150 males and 450 females. When sufficient numbers of fingerling of the desired size (1-3 g) for the growth trial were observed, the ponds were drained and the fingerlings were collected. The stocking procedure consisted of size-matching among test groups and fin-clipping to identify the groups. The test groups were stocked communally in hapas and in ponds.

In experiment 4, the test groups were also produced in the pond by pooled spawning of the brood stock of each group in different ponds. The test groups were size-matched but

the comparison of the performance of the different test groups was done using separate stocking in replicated ponds.

The following is a description of the procedure for the production of the test groups for experiments 5, 6, 7, 8, and 9. For the selected group, spawning was done in 30 fine-meshed hapas. Each hapa measured 1-m^2 and was stocked with 1 male and 2 female brood stock. The RBC and MSC groups were produced from pool spawning of brood stock obtained from the various lines within each group (described in Chapter 2). Spawning was also done in 4-m^2 breeding hapas. The fry of other groups, e.g., GMT and GIFT, were obtained from the respective station or project that produced them. However, it was ascertained that the ages of the fingerlings that were obtained from these sources were similar or close to the ages of SEL, RBC, and MSC groups.

Batches of fry collected within a short period (1-3 days) were pooled for each group. Fry were reared for about six weeks in hapas to allow them to reach the size suitable for fin clipping. At stocking, the fingerlings were size-matched to obtain a uniform initial weight among the test groups as much as possible. The fish were then marked by fin clip and communally stocked in the different culture units.

Nine experiments were conducted for this study. The sequential numbering of the different experiments was based on the year the experiments were conducted. A chart indicating this numbering is shown in Table 3.1 while the full details of these experiments are presented in Table 3.2.

Feeding and fertilization

Fish were fed twice daily a commercial tilapia diet in the tank and hapa experiments except in hapa experiment 2. The pond in which the hapas were suspended was fertilized with chicken manure at the rate of 1 ton per hectare every two weeks. In pond experiments, the ponds were also fertilized with chicken manure at 1 ton per hectare every two weeks. Feeding of fish in the ponds with commercial tilapia diet was done only in Experiment 7.

Fish sampling and harvest

Fish sampling was carried out every 30 days to monitor growth performance in tanks, hapas, and ponds. In pond experiment 7, only the initial and final body weights were recorded. All experiments were conducted for a period of 120 days. At the end of each experiment, all stocked fish were collected, sexed, and individually weighed. Males and females were recognized by their genital papillae. In pond experiments 3 and 4, only a sample of fish (at least 50 fish per group) was individually weighed at harvest. The remaining fish were checked for fin clip markings, counted, and bulk-weighed by groups. There were some fingerlings collected from the pond experiments at harvest but these were minimal. Data were collected only for fish with known identity.

Data analyses

Analysis of group differences

The statistical analyses were done separately for each experiment within test environments. Body weights were analyzed using the General Linear Model (GLM) procedure (SAS Institute, 1989). The following statistical models were used:

$$Y_{ijk} = u + G_i + R_j + G_i*R_j + e_{ijk} \quad (1)$$

$$Y_{ijkl} = u + G_i + R_j + S_k + G_i*R_j + G_i*S_k + e_{ijkl} \quad (2)$$

where:

Y_{ijk} is the initial body weight,

Y_{ijkl} is the final body weight,

u is the overall mean,

G_i is the fixed effect of group,

R_j is the random effect of replicates,

S_k is the fixed effect of sex,

e_{ijkl} is the random error.

Model 1 was used for analyzing initial body weights and Model 2 was used for final body weights. First order interactions between group and replicates were included in Model

1. In Model 2, the first order interaction between group and replicates or sex effects were included. Multiple comparisons among pairs of means were done using Tukey's post hoc test at $P< 0.01$. Survival rate (%) was determined by dividing the total number of fish at harvest with the initial number of fish multiplied by 100. Survival data were transformed using the arcsin transformation and analyzed for statistical significance using the analysis of variance and Tukey's post hoc tests of pairwise differences in survival means (SAS Institute, 1989).

Genotype-environment interaction

Genotype-environment interactions were analyzed in the harvest data. Based on the year when the experiments were conducted, three genotype-environment studies were analyzed. These studies were comprised of the following experimments: 1993 GxE (Experiments 2 and 3), 1996 GxE (Experiments 5, 6, and 7), and 1997 GxE (Experiments 8 and 9). Varying combinations of the test environments were involved in these genotype-environment studies. These experiments were contemporaneous having the same source of test group materials and were conducted at almost the same time (although the experiments were started about 3-4 weeks apart but within a span of 6 months). The data from these experiments were analyzed to include a genotype-environment interaction in the model. GLM procedure (SAS Institute, 1989) was used on the following models:

$$Y_{ijklm} = u + E_i + G_j + R_k + S_l + E_i*G_j + E_i*R_k + E_i*S_l + G_j*R_k + G_j*S_l + R_k*S_l + E_i*G_j*R_k + E_i*G_j*S_l + E_i*R_k*S_l + G_j*R_k*S_l + E_i*G_j*R_k*S_l + e_{ijklm} \quad (3)$$

$$Y_{ijklm} = u + E_i + G_j + R_k + S_l + E_i*G_j + E_i*R_k + E_i*S_l + e_{ijklm} \quad (4)$$

where:

Y_{ijklm} is the final body weight,

u is the overall mean,

E_i is the fixed effect of the test environment

G_j is the fixed effect of group,

R_k is the random effect of replicates,

S_l is the fixed effect of sex,

e_{ijklm} is the random error.

Model 3 is the full model used in the analysis while model 4 was a reduced model showing the first-order interaction terms between the test environment and the other main effects. The interaction term between test environment and group was used to test for genotype-environment interactions. The GIFT strain was not included in the GxE analysis because it was used only in pond experiment 7.

The Random statement in GLM was used to specify the random effects in the model. To perform hypothesis test for each effect specified in the model using the appropriate error terms, the Test option was used (SAS Institute, 1989).

RESULTS

Growth in tanks

Mean values for initial and final body weights of the different test groups in tank experiments are shown in Table 3.3. Mean initial body weights did not differ significantly among the test groups in all tank experiments. There were also no significant differences in initial body weights among replicates. Significant differences in mean final weight were observed among groups with the selected group consistently the heaviest in all three experiments ($P<0.01$). In experiment 5, the two control groups did not significantly differ from one another in mean body weight. Significant differences in mean final body weights were observed among replicates in experiment 1 and 8.

The mean final body weights between sexes are shown in Table 3.4. The males were significantly heavier than the females within test group ($P<0.01$). Across groups, the mean final body weight of the males from the SEL group was significantly heavier in all three experiments. The mean final weights of the SEL females were also higher than the mean final weights of the females from the RBC and MSC groups. The mean final body weights between males of the RBC and MSC groups differed significantly but not between

females of RBC and MSC groups. The SEL females had comparable growth with the MSC males (experiments 5 and 8).

Sex ratio in tanks

The sex ratios within group are shown in Table 3.4. The Chi-square test (based on the difference from 1:1 ratio) for the sex ratios within groups differed significantly in the selected group in all three experiments ($P<0.01$). There was also a significant difference in the proportion of males and females of the MSC group in experiment 5 ($P<0.05$). The sex ratios of RBC group in experiments 1 and 5 and MSC in experiment 8 did not differ from a 1:1 ratio ($P>0.05$). In general, there was a higher proportion of females than males in the three experiments except in experiment 5 for the MSC group and experiment 8 for the selected group.

Survival in tanks

Mean survival rate of the test groups in the tanks are shown in Table 3.3. These values are mean survival over replicate tanks. The SEL group experienced significantly higher survival rate than the RBC group in experiment 1. In experiments 5 and 8, the SEL group had significantly lower survival than the RBC and MSC groups. The two control lines possessed similar survival rates. In experiment 8, some fish mortality occurred during the second month of the experiment (January-February 1997). This observation corresponded to a relatively low water temperature (about 22-24°C) during these months.

A disease occurrence was observed but the selected group was most affected in the tanks. This incidence may have accounted for the low survival of the selected group in this particular experiment.

Growth in hapas

Table 3.5 shows the initial and final mean weights and survival of the test groups in hapas. The test groups did not differ significantly in initial body weights except in experiment 9, where the selected group had significantly higher initial weight than the RBC and MSC groups ($P<0.05$). Final body weights in pooled sexes were significantly different among test groups with the selected group having a significantly higher mean final body weight in all three experiments ($P<0.01$). There was a significant replicate effect for initial body weight in experiments 2 and 6.

The mean final body weights of males and females in hapas are shown in Table 3.6. A significant sex effect was observed in all experiments with males being significantly heavier than females ($P<0.01$). The selected males were significantly larger than the males from the control groups. Similarly, among females, the SEL group experienced faster growth than females from the control groups except in experiment 2. The final mean body weight of the selected females did not differ from that of the males of the ISR strain. Also, the female SEL had comparable growth with the males of the MSC group in experiments 6 and 9. Significant differences in final body weights were observed among replicates in experiments 2 and 6. The overall improved growth performance of the test groups in

experiment 6, compared to experiments 2 and 9 may have been caused by improved feeding Also, in experiment 6, the net material that was used for the fabrication of hapas had bigger mesh size than the material used in experiments 2 and 9. The bigger mesh size provided better water exchange in the pond.

Sex ratio in hapas

The selected group and ISR strain exhibited a 1:1 sex ratio in experiment 2 (Table 3.6). The sex ratio of the RBC group showed a preponderance of females in experiment 2 and 9 (63% and 58%, respectively) but the sex ratio was not significantly different from 1:1 in experiment 6. The sex ratio of the MSC group was less consistent (greater proportion of males in experiment 6 but more females in experiment 9).

Survival in hapas

Mean survival rates in hapas are shown in Table 3.5. The percent survival among groups in experiment 2 was significantly different ($P<0.01$). ISR strain had the highest survival of 96% followed by RBC (90%), and SEL (77%). In experiment 6, the survival of RBC and MSC groups did not differ significantly while SEL had the lowest survival (68%). In experiment 9, there were no significant differences in mean survival rates among the test groups. The low survival of SEL in hapa experiment 6 was again attributed to a disease occurrence similarly observed in tank experiment 5. Note that hapa experiment 6 and tank

experiment 5 were conducted at almost the same time. This disease occurrence was also reported in other fish populations at the station and in nearby farms.

Growth in ponds

Mean initial and final body weights of the test groups in ponds are shown in Table 3.7. There were no significant differences in mean initial body weights among groups except in experiment 7, where the initial body weights of the SEL and GIFT strains differed significantly from RBC and MSC groups ($P<0.01$). Significant variation in initial weight was found only in experiment 3. For final body weight, there were significant differences among test groups with the SEL group consistently the heaviest among the groups except in experiment 7. There were no significant differences between the RBC and ISR groups in experiment 3. A significant difference between RBC and the GMT groups were observed in experiment 4. In experiment 7, the SEL group had similar final body weight with the GIFT strain while the two control lines had significantly lower mean body weights compared with SEL and the GIFT strain. Final body weights in experiment 7 were significantly different among replicates.

Table 3.8 shows the final mean body weight of males and females in ponds. A significant sex effect was observed in all pond experiments with males being significantly heavier than females ($P<0.01$). Among males, the selected group had significantly heavier mean body weight than the males of the other test groups except in experiment 7 where the selected group did not differ from the GIFT strain. The same pattern was

observed for the mean final body weights among females. In experiment 4, the GMT males did not differ significantly in mean final body weight from the selected females.

Sex ratio in ponds

The sex ratios of the different groups are shown in Table 3.8. There was no significant difference in the sex ratio of the selected group in all three experiments ($P > 0.05$). The sex ratios of the RBC group in experiments 3 and 7 did not differ from 1:1. Similarly, the sex ratio of the GIFT strain was not significant in experiment 7. A significant sex ratio indicated a higher proportion of males in the ISR strain in experiment 3 (59%), RBC group in experiment 4 (65%), and MSC group in experiment 7 (60%). The GMT had 97% males in experiment 4.

Survival in ponds

Survival data in experiments 3 and 4 were not available. In experiment 7, survival rate among groups ranged from 56% to 84%. RBC and GIFT strains had the lowest and the highest mean survival rate, respectively. There was no significant difference in the survival rate between the SEL and RBC groups in ponds (Table 3.7).

Response to selection

Table 3.9 shows the absolute difference in grams in mean body weight of the selected groups and mean body weight as percent of the control mean. The deviation of

mean body weights between the selected groups and the two variants of control lines (RBC and MSC) will be the focus of this section.

The growth difference between the selected group and the control groups was greatest in the tank environment in either pooled or separate sexes. In the tank experiments, the selected group was from 28.9 to 68% heavier than the RBC group and from 38.7% to 60% heavier than the MSC group. The growth difference between selected and MSC groups in tanks was slightly lower than with RBC group. In hapas, the response relative to the RBC group was less than what was observed in the tanks except in experiment 2. Relative to the MSC group, the response in hapas was close to the observed response in the tanks. In ponds, the response was moderate compared to those obtained in tanks and hapas, except in the second experiment where the SEL group was 41.8% heavier than the RBC group. The response for increased body weight in males and females followed the same pattern as when the sexes were pooled. The highest percentage difference in growth was observed in tank experiment 5 where males of the selected group differed by 82.5% relative to the RBC group while the least was between selected and the RBC groups in the hapa experiment 1 (6.2%).

Growth comparison with other tilapia strains

The mean differences of the selected groups from the ISR, GMT, and GIFT strains in absolute values and as percentages are shown in Table 3.9. These strains were included

in the various performance tests to compare the growth performance of the selected groups from a commercial strain and from other genetically improved strains.

From the results of the study, the selected group in the hapa experiment has shown superiority in growth performance over the ISR strain by 32.7% (pooled sexes) while the selected males and females were 25 and 44.5% heavier, respectively. A similar result was observed in the pond experiment although the magnitude of difference was less than what was observed in the hapas. With the predominantly male GMT, the selected group was 17.4% heavier than GMT in pooled sexes. The SEL males and females were 32.9% and 40.2% heavier than GMT males and females, respectively, but this comparison between the females involved only 10 GMT females.

In ponds, the selected group and the GIFT strain did not differ significantly in final mean body weights in either pooled or separate sexes. The final mean body weight of the selected group was heavier by 17.4% over that of the GMT in experiment 7. The SEL females and GMT males were comparable in mean final body weights.

Comparison of response between generations

The summary of response for the selected generation in the different culture environments is shown in Table 3.10. The average responses in S_{13} (1996 and 1997 experiments) were obtained and compared with the response obtained from S_{10} and S_{11}. Relative to using RBC line as control in the tanks, the observed response of 68% in S_{10} decreased to 45% in S_{12}. There was no testing made using MSC line as control in S_{10} but

the response in S_{13} was 41%. In the hapas, the response was 13.6% in S_{10} and 37.3% in S_{13} with reference to RBC line. The average response obtained in S_{13} using MSC as control was 36.2%. The response obtained in ponds was smaller than the response observed in the tanks and hapas. When RBC was used as control, there was an increase in response from 10% in S_{10} to 26.8% in S_{13} but the response in S_{10} was consistent with S_{13} when MSC was used as control line.

Selection response per generation

The selection responses per generation are shown in Table 3.11. When the percent improvement based on 12 generations of selection was assessed in each environment using the RBC as control, the mean responses were 4.49% in the tanks, 3.73 % in the hapas, and 2.68% in the ponds. On the other hand, when MSC was used as control, the response per generation were 4.1% in the tanks, 3.62% in the hapas, and 1.05% in the ponds. The highest response per generation was obtained after nine generations of selection in the tanks (9.7%). However, this high response could probably be due to the fact that the selected groups used in this test were from the full-sib families produced directly from the selection in the tanks. This means that these families were produced by the best mating pair (heaviest male and female from their respective families at selection). In contrast, the selected groups representing S_{13} in the series of testings were produced from pooled spawning of the selected parents including those that were not actually used to advanced the generation (e.g., including those from lower ranking fish at the time of selection).

Genotype-environment interaction

Tables 3.12, 3.13, and 3.14 show the summary of the mean final body weights of the test groups in the different culture environments for the 1993, 1996, and 1997 genotype-environment studies, respectively. These mean final body weights have been presented in earlier tables (Tables 3.3, 3.5, 3.7). For the 1993 GxE, the mean body weight across groups was 29.53 ± 11.84 g in hapas and 52.60 ± 12.84 g in ponds. This difference in the mean final body weight between hapa and pond was significant ($P < 0.01$). For the 1996 GxE, the mean body weights of the fish were 27.24 ± 12.78 g, 89.34 ± 31.79 g, and 106.71 ± 38.96 g in tanks, hapas, and ponds, respectively. Highly significant differences in mean body weights among environments were also observed ($P < 0.001$). For the 1997 GxE, the mean final body weight between tank and hapa environment was significantly different (40.11 ± 16.49 g in tank and 42.79 ± 16.19 g in hapa).

In general, the relative growth of the test groups was better in ponds than in tanks and hapas. This can be expected considering the more optimum conditions in the ponds (less fish per unit area) as opposed to the tanks and hapas. The phenotypic rankings were SEL>RBC>MSC in the 1993 and 1997 experiments and SEL>MSC>RBC in the 1996 experiment. These rankings were consistent in either sexes combined or separated.

The estimates of the mean squares for the main effects in the model and their interactions derived from the GLM procedure are given in Tables 3.15, 3.16, and 3.17 corresponding to the 1993, 1996, and 1997 GxE experiments. The reduced model for the analysis of variance was used. The tables of analysis of variance for the full model are

shown in the appendices. There was no significant genotype-environment interaction in the 1993 GxE study ($P < 0.05$). This interaction accounted for only 0.18% of the total variation. The environment and sex effects accounted for most of the variation (65.51% and 18.37%, respectively). For the 1996 GxE, except for the replicate effect, all the other main effects and their interactions were highly significant ($P<0.001$). The test environment and the sex effects accounted for 66.16% and 22.46% of the variation, respectively. The variation explained by the group x test environment effect was only 0.51%. The genotype-environment interaction was a magnitude interaction as the rankings of the test groups were not altered. In the 1997 GxE, there was also no significant genotype-environment interaction. The major factors contributing to the total variation were sex effect (63.76%) and group effect (19.97%). The group x test environment interaction was not significant. This component of variation was consistently the smallest (0.17%).

DISCUSSION

Overall, the results of these growth evaluations showed that the selected group produced from within-family selection had improved growth performance compared with the random-bred control and the mean selected control populations. This improvement in growth was observed consistently in tanks, hapas, and ponds. It was also apparent that the selected group experienced better growth than the Israel strain and the genetically male tilapia. As mentioned earlier, the Israel strain is the most commonly farmed strain in the Philippines. The results of this study indicate that Israel strain is a slow-growing strain. Eknath *et al.* (1993) obtained similar results in the growth evaluation of different strains of Nile tilapia.

An interesting result of the present is the comparable growth performance between the SEL and the GIFT strain. The GIFT strain was developed from a broad genetic base population composed of eight strains of Nile tilapia. Four of these strains were new genetic materials collected from tilapia populations in Africa. Such collections were made because the results of genetic characterization studies of existing farmed tilapias suggested their poor genetic status due to widespread introgression of genes from less desirable feral tilapia species, *O. mossambicus* (Macaranas et al., 1986) and possibly due to inbreeding (Eknath *et. al.*, 1991).

Aside from the base population, the major difference in the approach between the present selection experiment and that of the GIFT project is the method of selection that

was used. The GIFT project used a combined family selection approach in which individuals are selected on the basis of an index appropriately weighting the deviation of the full-sib family mean from the population mean and the deviation of individual performance from the mean of the individual's family. This methodology has been successful in terrestrial livestock development (mostly in developed countries) and in salmon breeding programs in Norway. It requires large facilities and the co-operation of a number of institutions and farms. The testing and the selection methods are likely only to be successful on a large scale where sufficient replication can be achieved.

The approach of the present study was to explore methods which are appropriate to smaller and less well-endowed facilities (Uraiwan and Doyle, 1986) and, as mentioned earlier, to utilize only locally adapted strains of tilapia as base populations for the selection program. Clearly, this work has shown that it was possible to improve the growth of locally adapted strains of Nile tilapia by using a within-family selection method.

Positive response to selection has been reported from the earlier selected generation of this stock. Abella *et al.* (1990) reported a higher growth rate of the selected *O. niloticus* than the random-bred control line after 2 generations of within-family selection. After 8 generations of within-family selection, Bolivar *et al.* (1994) found that the selected Nile tilapia were from 8 to 37% heavier than the random-bred control line. Roughly, this was equivalent to 3.6% improvement per generation. Beniga (1997) tested 'farmers' tilapia strain along with genetically improved tilapia strains utilizing GMT, GIFT and the selected line (S_{13} generation) in floating cages in Lake Sebu in southern Philippines. He found

significant growth difference between the 'farmers' strain and the genetically improved strains. Among the genetically improved strains, the selected line had significantly higher mean final weights than the GMT and GIFT strains ($P < 0.05$).

Growth of selected females

In 7 out of 9 experiments in the present study, the females of the selected group had similar or better growth than the males of RBC, MSC, ISR, and GMT groups. Another interesting result was the comparable final mean body weights of the selected females (73.58 ± 14.77 g) and the males GMT (72.84 ± 14.72 g). This result suggests that the within-family selection had effectively improved the growth of females.

The development and culture of the genetically male tilapia (GMT) was based on the premise that monosex male populations could enhance the yields of tilapia. On-station trials of GMT have shown that the yield of tilapia increased significantly by up to 58% compared with mixed-sex tilapia of the same strain (Mair *et al.*, 1995).

The uncontrolled reproduction of tilapia in a mixed-sex culture often results in stunting due to over-crowding, particularly in pond culture situations (Ofori , 1988; Mair *et al*, 1995). It is not surprising that many approaches are taken to control reproduction in tilapia (reviewed by Baroiller and Jalabert, 1989; Mair and Little, 1991). The most common approaches are manual sexing, hybridization, and hormonal sex reversal. A more recent technology is the sex manipulation to produce YY tilapia males (Mair *et al.*, 1997). All of these approaches are directed towards the production of all-male tilapia population.

The females are often discarded (e.g., manual sexing) in monosex culture because of the slow growth as the fish approach sexual maturity.

The present study has shown that mean growth performance of the selected females was comparable to an all-male tilapia population while the selected males were significantly heavier than the males from any of the test groups. This is an indication that mixed-sex culture of the selected Nile tilapia can result in a total yield more than what is to be expected from an all-male tilapia culture. The genetic basis for improvement of these two stocks was different and the resulting genotypes were also different, so it may not be relevant to make an absolute comparison of the growth performance of these two groups. Nevertheless, there is a common ground in the objectives of the GMT project and the present study and this is the production of faster growing fish for tilapia aquaculture in the Philippines. The results of this experiment provide the possibility of complementing the YY male technology by using the selected fish to produce YY brood stock. Another possibility is to use the selected females in the production of GMT fingerlings. The latter has been done on an experimental scale and preliminary results indicate the better performance of the GMT produced from using the selected females compared to other female sources (Pascual, pers. com.).

Reproductive traits such as age at spawning or fecundity in the selected Nile tilapia have not been investigated but a general observation from these experiments showed that reproduction in ponds was almost negligible. The correlated response to late maturation

may have occurred but it was not measured in the present study. This needs to be investigated in the selected lines of Nile tilapia.

Genotype-environment interaction

In general, the response was greater in tanks where selection was undertaken. In the presence of a strong genotype-environment interaction, part of the selection response might have been for adaptation to the specific set of environmental circumstances to which the population was subjected (in this study, the tank environment). It was apparent from the results that although the greatest response was achieved in the tank environment, improved growth was also observed in the hapa and pond environments. This shows that the response achieved in the tank by using within-family selection was carried over in hapa and pond environments.

The studies on genotype-environment interaction were limited to testing three kinds of culture environments (e.g., tank, hapas, ponds). There were three experiments with different combinations of the culture environments (details in the Materials and Methods). A significant interaction was observed in the 1996 GxE study but this interaction was not sufficiently large to produce a change in the rankings of the different test groups. In this study, the pond environment provided more optimal condition for growth than the tank and hapa environments. The results of the 1993 and 1997 GxE analyses did not reveal a significant test group x environment interaction.

Genotype-environment interactions have been reported in aquaculture species (see Tave, 1996, p. 250-253). Most of the studies indicated that a certain strain was best under a particular set of conditions. Eknath *et al.* (1993) found a significant but weak strain x test environment interaction in a growth performance test involving 8 strains of Nile tilapia. The breeding program proceeded by ignoring this interaction to develop a synthetic strain that could be used under diverse farming systems.

The genotype-environment interaction found under the conditions that were examined in this study was of minor importance. There was a consistent rankings of the groups across test environments. The testing of genotype by environment in these experiments was to look for indication that a breeding program in the tanks would have useful applications in production systems. Based on the lack of significance of genotype by environment interaction, the selection decision that was made in the tanks may be appropriate for other environments such as hapas or ponds.

Sex ratio

The sex ratio of the selected group was less predictable in the tank experiments. However, the significant proportion of females in experiment 1 was caused by skewed sex ratios in 7 out of 21 full-sib families. There is no plausible explanation for the skewed sex ratios in experiment 2 and 3 except for sampling error although there were 4 out of 10 replicates with skewed sex ratio in both experiments.

Based on the hapa and pond experiments, the pattern of sex ratios in the present study suggests that selection for body weight did not alter the expected 1:1 sex ratio common to Nile tilapia (Shelton *et al.* 1983). This finding does not correspond to the results of Behrends *et al.* (1988) where selection for body weight in a red tilapia strain has influenced sex ratios, indicating a correlated response to selection. A highly skewed sex ratio (84% males) was obtained. Likewise, Hulata *et al.* (1986) found that selection for increased body weight in the Ghana strain of Nile tilapia resulted in male dominated populations.

It was also probable that selecting independently in each sex has maintained this balanced sex ratio. In mass selection and where only a single cut-off point is created for the entire population, the tendency is that selected population will be composed of mostly the larger sex if the selection occurs before sexual dimorphism begins (Tave, 1995).

In summary, a significant response to within-family selection for increased body weight at 16 weeks over 12 generations of Nile tilapia was obtained relative to the control lines. The highest response was obtained in the selection environment (tanks) but improved body weight was similarly observed in hapas and ponds. The study also showed that the selected Nile tilapia from the within-family selection scheme had comparable growth with the GIFT strain that was developed from a combined selection method utilizing a broad genetic base. Improvement in tilapia production may be obtained if genetically improved strains will be used by farmers since the present

commercial strain (Israel strain) has inferior growth than the genetically improved strains as shown in this study.

Table 3.1 Numbering of the different experiments in tanks, hapas, and ponds[1]

Culture	Selected Generations			
	S_{10}	S_{11}	S_{13}	S_{13}[2]
Environments	1993	1994	1996	1997
Tanks	1	_	5	8
Hapas	2	_	6	9
Ponds	3	4	7	_

[1] Based on the year the experiments were conducted
[2] Repeat spawning of the S_{12} selected parents

Table 3.2 Details of the nine experiments carried out in tanks, hapas, and ponds.

Culture Environment	Year	Expt. No[1].	No. of units	Test groups[2]	No. of fish /unit
Tanks	1993	1	21	SEL_{10}-RBC_3	100 fish/SEL
					30 fish/RBC
	1996	5	10	SEL_{13}-RBC_4-MSC_4	50 fish/group
	1997	8	10	SEL_{13}-RBC_5-MSC_5	50 fish/group
Hapas	1993	2	4	SEL_{10}-RBC_3-ISR	35 fish/group
	1996	6	10	SEL_{13}-RBC_4-MSC_4	50 fish/group
	1997	9	8	SEL_{13}-RBC_5-MSC_5	22 fish/SEL[3]
					60 fish/RBC
					60 fish/MSC
Ponds	1993	3	3	SEL_{10}-RBC_3-ISR	100 fish/group
	1994	4	9	SEL_{11}-RBC_4-GMT	1000 fish/pond[4]
	1996	7	8	SEL_{13}-RBC_4-MSC_4-GIFT	50 fish/group

[1]Contemporary experiments (same time and same source of experimental materials): 1993 (Expts. 2,3); 1996 (Expts. 5, 6, 7) ; 1997 (Expts. 8, 9).
[2]Generation number indicated in the test groups refers to the offspring generation.
[3]Due to shortage of fingerlings from the selected group.
[4] Separate rearing; 3 replicated ponds per group

Table 3.3 Mean and number of fish (in parenthesis), standard deviation (SD) of initial and final body weights and mean survival of the different test groups of Nile tilapia in tanks (pooled sexes).

Expt. No.	Group	Initial		Final		Survival
		Mean weight, g	SD	Mean Weight, g	SD	%
1	SEL	1.33 (2100)[a]	0.21	35.33 (1977)[a]	9.94	94[a]
	RBC	1.34 (663)[a]	0.21	21.02 (548)[b]	8.06	87[b]
5	SEL	1.73 (363)[a]	0.34	35.85 (363)[a]	15.98	73[a]
	RBC	1.72 (441)[a]	0.37	22.38 (441)[b]	9.11	88[b]
	MSC	1.72 (437)[a]	0.39	24.99 (437)[b]	8.91	88[b]
8	SEL	3.18 (265)[a]	1.20	49.55 (265)[a]	20.79	53[a]
	RBC	3.51 (408)[a]	1.42	38.43 (408)[b]	14.42	82[b]
	MSC	3.40 (412)[a]	1.48	35.72 (412)[c]	12.42	83[b]

Within experiment, values superscripted with different letters are significantly different ($P<0.01$).

Table 3.4 Sex ratio, mean, and standard deviation (SD) of final weights of males and females of the different test groups of Nile tilapia in tanks.

Expt. No.	Group	Sex ratio (%) ♂♂	Sex ratio (%) ♀♀	Mean weight ♂ (g) Mean	Mean weight ♂ (g) SD	Mean weight ♀ (g) Mean	Mean weight ♀ (g) SD
1	SEL	42	58**	39.63[a]	10.15	32.21[c]	8.53
	RBC	48	52[ns]	23.70[b]	8.50	18.53[d]	6.76
5	SEL	33	67**	47.40[a]	16.38	30.29[c]	12.44
	RBC	46	54[ns]	25.97[b]	10.07	19.26[d]	6.81
	MSC	55	45*	29.07[c]	8.23	20.03[d]	6.99
8	SEL	61	39**	55.72[a]	21.01	40.00[c]	16.46
	RBC	41	59**	46.96[b]	15.26	32.45[d]	10.21
	MSC	48	52[ns]	39.76[c]	12.28	32.05[d]	11.39

Within experiment, values superscripted with different letters are significantly different ($P < 0.01$). For sex ratio within group, [ns] = not significant, * significant ($P < 0.05$), ** significant ($P < 0.01$)

Table 3.5 Mean and number of fish (in parenthesis), standard deviation (SD) of initial and final body weights and mean survival of the different test groups of Nile tilapia in hapas (pooled sexes).

Expt. No	Group	Initial		Final		Survival (%)
		Mean weight, g	SD	Mean weight, g	SD	
2	SEL	1.31 (140)[a]	0.24	33.96 (108)[a]	12.53	77[a]
	RBC	1.28 (140)[a]	0.23	29.89 (124)[b]	11.04	90[b]
	ISR	1.28 (140)[a]	0.21	25.59 (135)[c]	10.67	96[c]
6	SEL	1.67 (504)[a]	0.26	111.68 (344)[a]	35.32	68[a]
	RBC	1.68 (501)[a]	0.29	75.34 (411)[b]	23.84	82[b]
	MSC	1.68 (502)[a]	0.29	84.71 (416)[c]	25.05	83[b]
9	SEL[1]	2.33 (176)[a]	0.81	54.11 (172)[a]	20.09	93[a]
	RBC	2.18 (500)[b]	0.59	42.81 (467)[b]	15.55	95[a]
	MSC	2.10 (500)[b]	0.54	38.49 (454)[c]	12.79	94[a]

Within experiment, values superscripted with different letters are significantly different: initial weight ($P < 0.05$); final weight ($P < 0.01$). [1] Initial stocking density was only about 22 fish per hapa due to shortage of fingerlings

Table 3.6 Sex ratio, mean, and standard deviation (SD) of final weights of males and females in the different test groups of Nile tilapia in hapas.

Expt. No.	Group	Sex ratio (%) ♂♂	Sex ratio (%) ♀♀	Mean weight ♂ (g) Mean	Mean weight ♂ (g) SD	Mean weight ♀ (g) Mean	Mean weight ♀ (g) SD
2	SEL	50	50^{ns}	39.01^{a}	12.70	28.82^{cd}	10.10
	RBC	37	63**	34.70^{b}	13.15	27.13^{d}	8.56
	ISR	50	50^{ns}	31.24^{c}	10.55	19.95^{e}	7.30
6	SEL	45	55^{ns}	130.97^{a}	33.29	95.86^{c}	28.44
	RBC	48	52^{ns}	87.21^{b}	23.10	64.51^{d}	18.85
	MSC	60	40**	96.16^{c}	22.80	67.81^{d}	17.55
9	SEL	50	50^{ns}	62.29^{a}	18.07	45.93^{c}	18.72
	RBC	42	58**	52.56^{b}	14.64	35.75^{d}	12.00
	MSC	43	57**	45.94^{c}	13.19	32.98^{e}	9.20

Within experiment, values superscripted with different letters are significantly different ($P<0.01$). Sex ratio within group,^{ns} = not significant, * significant ($P < 0.05$), **significant ($P<0.01$).

Table 3.7 Mean and number of fish (in parenthesis), standard deviation (SD) of initial and final body weights and mean survival of the different test groups of Nile tilapia in ponds (pooled sexes).

Expt. No.	Group	Initial Mean weight, g	Initial SD	Final Mean weight, g	Final SD	Survival (%)
3	SEL	2.25 (150)[a]	0.62	55.70 (210)[a]	12.68	
	RBC	2.24 (150)[a]	0.96	50.62 (150)[b]	12.65	
	ISR	1.88 (150)[a]	0.78	50.45 (130)[b]	12.47	
4	SEL	0.43 (150)[a]	0.16	84.73 (150)[a]	21.31	
	RBC	0.42 (150)[a]	0.16	59.72 (150)[b]	17.13	
	GMT	0.42 (150)[a]	0.15	72.16 (150)[c]	15.05	
7	SEL	3.70 (398)[a]	0.79	118.42 (242)[a]	40.47	61[a]
	RBC	3.10 (399)[b]	1.09	93.41 (224)[b]	37.18	56[a]
	MSC	3.10 (398)[b]	1.03	107.18 (303)[c]	35.93	76[b]
	GIFT	3.55 (392)[a]	0.77	117.36 (330)[a]	39.40	84[c]

Within experiment, values superscripted with different letters are significantly different ($P<0.01$).

Table 3.8 Sex ratio, mean, and standard deviation (SD) of final weights of males and females in the different test groups of Nile tilapia in ponds.

Expt. No.	Group	Sex ratio, %		Mean weight ♂ (g)		Mean weight ♀ (g)	
		♂♂	♀♀	Mean	SD	Mean	SD
3	SEL	50	50^{ns}	62.90^{a}	11.74	48.65^{c}	9.12
	RBC	47	53^{ns}	57.73^{b}	12.14	44.25^{d}	9.28
	ISR	59	41^{*}	55.91^{b}	11.78	42.51^{d}	8.63
4	SEL	48	52^{ns}	96.86^{a}	20.73	73.58^{c}	14.77
	RBC	65	35^{**}	63.86^{b}	17.56	51.84^{d}	13.10
	GMT	97	3^{**}	72.84^{c}	14.72	52.48^{d}	10.93
7	SEL	46	54^{ns}	142.07^{a}	40.9	98.05^{d}	26.64
	RBC	54	46^{ns}	113.95^{b}	35.14	68.84^{e}	21.31
	MSC	60	40^{**}	123.57^{c}	32.28	82.19^{f}	25.32
	GIFT	48	52^{ns}	137.09^{a}	38.96	98.79^{d}	26.64

Within experiment, values superscripted with different letters are significantly different ($P<0.01$). Sex ratio within group, ^{ns} = not significant , * significant ($P < 0.05$), ** significant ($P < 0.01$).

Table 3.9 Growth difference of the selected Nile tilapia from the control lines (response to selection), and growth comparison with Israel, GMT, and GIFT strains.

Culture Environment	Expt. No.	Group Comparison	Difference from control mean					
			Pooled sexes		Males		Females	
			g	%	g	%	g	%
Tank	1	SEL/RBC	14.31	68.0	15.93	67.2	13.68	73.8
	5	SEL/RBC	13.47	60.9	21.43	82.5	11.03	57.2
		SEL/MSC	10.86	43.4	18.33	63.0	10.26	51.2
	8	SEL/RBC	11.12	28.9	8.76	18.6	7.55	23.2
		SEL/MSC	13.84	38.7	16.51	40.1	7.95	24.8
Hapa	2	SEL/RBC	4.07	13.6	4.31	12.4	1.39	6.2
		SEL/ISR	8.37	32.7	7.77	24.9	8.87	44.5
	6	SEL/RBC	36.34	48.2	43.76	50.2	31.35	48.6
		SEL/MSC	26.97	31.8	34.81	36.2	28.05	41.4
	9	SEL/RBC	11.3	26.4	9.73	18.5	10.18	28.5
		SEL/MSC	15.62	40.6	16.35	35.6	12.95	39.3
Pond	3	SEL/RBC	5.08	10.0	5.17	8.9	4.40	9.9
		SEL/ISR	5.25	10.4	6.99	12.5	6.14	14.4
	4	SEL/RBC	25.01	41.8	33.00	51.7	21.74	41.9
		SEL/GMT	12.57	17.4	24.02	32.9	21.10	40.2
	7	SEL/RBC	25.01	26.8	28.12	24.6	29.21	42.4
		SEL/MSC	11.24	10.5	18.50	14.9	15.86	19.3
		SEL/GIFT	1.06	0.9	4.98	3.6	-0.74	0.0

Table 3.10 Summary of response in each of the tested generation (as percent of the control group).

Culture Environments	Control group	Average response, %		
		S_{10}	S_{11}	S_{13}
Tanks	RBC	68.0	-	45.0
	MSC	-	-	41.0
Hapas	RBC	13.6	-	37.3
	MSC	-	-	36.2
Ponds	RBC	10.0	41.8	26.8
	MSC			10.5

[1]Average response for S_{13} from the 1996 and 1997 experiments.

Table 3.11 Selection response per generation.

Environment	Selected generation	Control Group	Response per generation, %	Mean response per generation, %
Tank	S_{10}	RBC_3	9.70	9.70
	S_{13}	RBC_4	6.09	4.49
		RBC_5	2.89	
	S_{13}	MSC_4	4.34	4.10
		MSC_5	3.87	
	Mean			6.09
Hapa	S_{10}	RBC_3	1.94	1.94
	S_{13}	RBC_4	4.82	3.73
		RBC_5	2.64	
	S_{13}	MSC_4	3.18	3.62
		MSC_5	4.06	
	Mean			3.09
Pond	S_{10}	RBC_3	1.43	1.43
	S_{11}	RBC_4	5.22	5.22
	S_{13}	RBC_5	2.68	2.68
		MSC_4	1.05	1.05
	Mean			2.59

Table 3.12 Mean body weight and standard deviation (SD) of SEL, RBC, and MSC groups in tanks, hapas, and ponds (1993 GxE).

Test groups	Test environments			
	Hapas		Ponds	
	Mean	SD	Mean	SD
SEL	33.96[a]	12.53	55.70[d]	12.68
RBC	29.89[b]	11.04	50.62[e]	12.65
MSC	25.59[c]	10.67	50.45[e]	12.47
Mean across environment	29.53	11.84	52.60	12.84
Total no. of observations	369		527	

Values with different superscript letter are significantly different ($P < 0.01$).

Table 3.13 Mean body weight and standard deviation (SD) of SEL, RBC, and MSC groups in tanks, hapas, and ponds (1996 GxE).

Test groups	Test environments					
	Tanks		Hapas		Ponds	
	Mean	SD	Mean	SD	Mean	SD
SEL	35.85[a]	15.98	111.68[c]	35.32	118.42[f]	40.47
RBC	22.38[b]	9.11	75.34[d]	23.84	93.41[g]	37.18
MSC	24.99[b]	8.91	84.71[e]	25.05	107.18[h]	35.93
Mean across environment	27.24	12.78	89.34	31.79	106.71	38.96
Total no. of observations	1241		1171		769	

Values with different superscript letter are significantly different ($P < 0.01$).

Table 3.14 Mean body weight and standard deviation (SD) of SEL, RBC, and MSC groups in tanks, hapas, and ponds (1997 GxE).

Test groups	Test environments			
	Tanks		Hapas	
	Mean	SD	Mean	SD
SEL	49.55[a]	20.79	54.11[d]	20.09
RBC	38.43[b]	14.42	42.81[e]	15.55
MSC	35.72[c]	12.42	38.49[b]	12.79
Mean across environment	40.11	16.49	42.79	16.19
Total no. of observations	1093		1085	

Values with different superscript letter are significantly different ($P < 0.01$).

Table 3.15 Analysis of variance of final body weight from the GLM procedure (1993 GxE).

Sources of variation	DF	MS	Contribution to total variation, %
Environment	1	79415.73*	65.51
Group	2	4481.03**	3.70
Replicate	3	11574.65ns	9.54
Sex	1	22267.58**	18.37
Environment x Group	2	217.99*	0.18
Environment x Replicates	2	2089.98**	1.72
Environment x Sex	2	1107.86**	0.90
Error	826	66.94	0.06

** - highly significant ($P < 0.001$), * - significant ($P < 0.01$), ns - not significant ($P > 0.05$).

Table 3.16 Analysis of variance of final body weight from the GLM procedure (1996 GxE).

Sources of variation	DF	MS	Contribution to total variation, %
Environment	2	1532449.86**	66.16
Group	2	174107.38**	7.50
Replicate	9	7619.54ns	0.33
Sex	1	520379.93**	22.46
Environment x Group	4	11864.74**	0.51
Environment x Replicates	16	10968.77**	0.47
Environment x Sex	2	58192.32**	2.51
Error	3180	402.16	0.02

** - highly significant ($P < 0.001$), * - significant ($P < 0.01$), ns - not significant ($P > 0.05$).

Table 3.17 Analysis of variance of final body weight from the GLM procedure (1997 GxE).

Sources of variation	DF	MS	Contribution to total variation, %
Environment	1	9986.16^{ns}	7.90
Group	2	25213.41**	19.97
Replicate	9	2875.95^{ns}	2.27
Sex	1	80510.71**	63.76
Environment x Group	2	214.65^{ns}	0.17
Environment x Replicates	7	1928.15**	1.52
Environment x Sex	1	2682.38*	2.12
Error	2177	166.44	0.13

** - highly significant ($P < 0.001$), * - significant ($P < 0.01$), ^{ns} - not significant ($P > 0.05$).

Chapter 4

GROWTH PERFORMANCE OF NILE TILAPIA (*Oreochromis niloticus*) UNDER SEPARATE AND COMMUNAL TESTING

ABSTRACT

Communal and separate testings were used to evaluate the growth performance of the progenies from the 9th generation selected parents (SEL), random-bred control population (RBC), and Israel strain (ISR) of Nile tilapia (*Oreochromis niloticus*). The testings were made simultaneously in hapas and ponds. The initial size differences among the test groups were minimized by size-grading. There was no significant genotype x type-of-rearing interactions both in hapa and pond conditions. Genotype by environment interaction was not significant under communal rearing. For testing purposes, communal rearing gives the same results as would be obtained under separate rearing. This will make testing programs less expensive as they require fewer resources. The magnitude of growth differences among groups varied between separate and communal rearing. The absence of a significant genotype and rearing method interaction suggests that communal rearing could provide an important impact for commercial production when alternative stocks are compared by the farmers.

INTRODUCTION

A basic foundation in developing fish breeding programs is the choice of genetic species or strains with desirable performance for the trait or traits of interest. Usually the initial stage will involve performance evaluation of genetic groups for the desired attributes. Similarly, the later stage requires an evaluation of the genetic stock produced from the breeding program before the product can be recommended or made commercially available for farm conditions. Therefore, an optimal testing method to evaluate performance of groups of fish is an important aspect of genetic stock improvement programs. Performance testing allows for the assessment of productivity of genetic groups (species, hybrids, or strains) to provide a basis for future management or research plans. However, the lack of adequate testing facilities, the large environmental variation between testing units, and the bias of the initial size variation of the fish are major impediments in performance testing of different groups of fish (Buck *et al.*, 1970; Wohlfarth and Moav, 1985, 1993; Dunham *et al.*, 1982; Doyle and Talbot, 1986a).

A testing method termed as 'communal testing' was developed for common carp (*Cyprinus carpio*). This method consists of stocking different genetic groups together in the same culture unit (Wohlfarth and Moav, 1972, 1985). This method of testing was found appealing because it eliminates environmental variation between groups, increases the number of test groups, and reduces the number of units required for testing (Dunham *et al.*, 1982; Wohlfarth and Moav, 1985; McGinty, 1987). However, in common carp, because of the positive correlation of weight gain on initial weight, the observed growth data from

communal testing require correction for the bias caused by variation in initial weight among test groups. The correction technique involves estimating the regression of weight gain on initial weight by the so-called 'multiple nursing' technique and using the coefficient of regression as a correction factor (Wohlfarth and Moav, 1972, 1985).

Basiao (1994) found no significant difference between the growth of a commercial strain and a Thai strain of Nile tilapia under the size-graded experiment but a significant difference was observed under a mixed-sized experiment when the initial size was used as a covariate. The real genetic difference between the two groups was evident (in this case, the two strains were not different) and the difference observed under the mixed-sized experiment was caused possibly by strong competition between groups with variable initial sizes.

Wohlfarth and Moav (1985) found that the relative growth rankings of different genetic groups of common carp (*Cyprinus carpio*) were identical when each group was stocked separately in into a series of replicated ponds and when the groups were stocked together into communal ponds. Thus, growth estimates from communal testing were reliable predictors of expected genetic differences in separate ponds. Dunham *et al.* (1982) also obtained similar rankings of the different test groups of channel catfish in communal and separate rearing. In tilapia studies, Circa *et al.* (1995) reported that the relative growth performance of the different strains of Nile tilapia was consistent under communal and separate rearing in rice-fish culture environment and in hapa environment (Danting *et al.*, 1995). In contrast, McGinty (1987) found opposite relative rankings of different tilapia species in separate and communal rearing indicating possible different genetic mechanisms

under the two rearing conditions.

Genotype-environment interaction may arise as a result of differences in response of the genotypes to changes in the environments (Falconer, 1989). It is clear from the literature that genotype-environment interaction may be encountered under a wide variety of situations. In general, it would seem important to take whatever precautions are necessary to detect its presence in selection studies.

In the present study, the growth of a selected group (SEL), a commercial strain (Israel strain), and a random-bred control group were evaluated simultaneously under communal and separate testing in hapas and ponds. The initial size differences among the test groups were minimized by obtaining samples of similar sized fish from each group. This size-matching procedure minimizes environmentally-induced variation (possibly due to the quality of the eggs or age of the fry) that can otherwise affect the final weight of the fish (Doyle and Talbot, 1986a). The objectives of this study were: 1) to evaluate the response to selection under communal and separate testing of Nile tilapia; 2) to compare communal and separate testing; and 3) to determine genotype-environment interaction in the two rearing methods.

MATERIALS AND METHODS

Test groups

The test groups involved a selected line of Nile tilapia (SEL) that was generated through within-family selection, a random-bred control group (RBC), and a commercial Israel strain of Nile tilapia (ISR). The SEL group was produced from the spawning of randomly sampled brood stock derived from the ninth selected generation (S_{9sp}) while the RBC was produced from pooled spawning of brood stock from the 2nd generation of RBC. Brood stocks of ISR strain were obtained from the stock maintained by the Philippine Bureau of Fisheries. The ISR strain was used because of its widespread use as a commercially farmed strain in the Philippines. The spawning of each group was done in separate ponds, where each pond was stocked with 150 males and 450 females. When sufficient numbers of fingerlings of the desired size for the growth trial (1-3 g) were observed, the ponds were drained and the fingerlings were collected. Growth evaluation studies were conducted simultaneously in hapas and ponds using communal and separate rearing. The details of the experiments are presented in Table 4.1.

Hapa experiment

For separate rearing of the test groups, four replicated hapas were each stocked with 105 fish of mixed-sex of one of the three test groups. Four additional hapas were stocked communally with 35 fish from each group. The fish were size-matched to obtain a sample with as similar initial weight as possible for all groups. The size-matching was initially

done by weighing a sample of fish to obtain the mean. The fish that were close to the mean weight were selected while those from the extremes of the distribution curve were discarded. Sample size was not a problem in this study because of the large number of fingerlings that were produced for each group.

The fish were marked by fin clipping for group identification prior to stocking in communal hapas. Since there were three groups, two groups were marked by left and right pelvic fin-clipping and the third group by pectoral fin-clipping. All fish were individually weighed at stocking into the hapas. Fish sampling was done every 4 weeks to monitor growth. The hapas (1.5 x 1.5 x 1.5 m) were installed in a 1000-m^2 earthen pond. There was no supplemental feeding provided so the fish depended on the natural food present in the pond. The pond where the hapas were suspended was fertilized with chicken manure at the rate of 1 ton per hectare every two weeks. The study was terminated after a grow-out period of 16 weeks. All harvested fish were checked for their fin clip markings, sexed, and individually weighed. The males were distinguished from females by examining genital papillae. Final data were obtained only for fish of known identity.

Pond experiment

Three replicated 500-m^2 ponds were stocked with 1000 mixed-sex fish of one of the three test groups (SEL, RBC, and ISR). An additional three ponds were stocked communally with the three test groups at stocking density of 333 fish per group. Similar to the hapa experiment, the fish were size-matched to obtain similar initial weights at stocking. The fish were marked by fin clipping to identify each group in the communal ponds.

The ponds were fertilized with chicken manure at the rate of 1 ton per hectare every two weeks. The fish were not given supplemental feeds. Fish sampling was done every four weeks. Ponds were harvested after 16 weeks. All harvested fish were checked for their fin clip markings, counted, sexed, and bulk-weighed. Because of the number of fish in the pond tests, a random sample of 50 fish was weighed individually per group in each replicate in the communal rearing and 50 fish per replicate in the separate rearing. Final data were collected only for fish with known identity.

Data Analysis

The data used for communal rearing in this chapter were previously presented in Chapter 3 but were being repeated here for comparison to the separate rearing.

Final body weights were analyzed separately for each test environment (hapa and pond) using the generalized linear model (GLM) procedure (SAS Institute 1989) with the following model:

$$Y_{ijklm} = u + T_i + G_j + R_k + S_l + T_i*G_j + T_i*R_k + T_i*S_l + G_j*R_k + G_j*S_l + R_k*S_l + T_i*G_j*R_k + T_i*G_j*S_l + T_i*R_k*S_l + G_j*R_k*S_l + T_i*G_j*R_k*S_l + e_{ijklm} \quad (1)$$

$$Y_{ijklm} = u + T_i + G_j + R_k + S_l + T_i*G_j + T_i*R_k + T_i*S_l + e_{ijklm} \quad (2)$$

where:

Y_{ijklm} is the final body weight,

u is the overall mean,

T_i is the fixed effect of testing method,

G_j is the fixed effect of group,

R_k is the random effect of replicates,

S_l is the fixed effect of sex,

e_{ijklm} is the residual effect.

Model 1 comprised of the full model and Model 2 was a reduced model involving only the first-order interactions between the testing methods and the other main effects. Multiple comparisons among pairs of group means were done using Tukey's post hoc test at $P< 0.01$.

To analyze the genotype-interaction effect, the data from the pond were appended to the data on hapa experiment and a new variable (environment) was added to the appended data set. The data were analyzed separately by testing method. The model used was:

$$Y_{ijklm} = u + E_i + G_j + R_k + S_l + E_i*G_j + E_i*R_k + E_i*S_l + G_j*R_k + G_j*S_l + R_k*S_l + E_i*G_j*R_k + E_i*G_j*S_l + E_i*R_k*S_l + G_j*R_k*S_l + E_i*G_j*R_k*S_l + e_{ijklm} \quad (3)$$

$$Y_{ijklm} = u + E_i + G_j + R_k + S_l + E_i*G_j + E_i*R_k + E_i*S_l + e_{ijklm} \quad (4)$$

where:

Y_{ijklm} is the final body weight,

u is the overall mean,

E_i is the fixed effect of the environment,

G_j is the fixed effect of group,

R_k is the random effect of replicates,

S_l is the fixed effect of sex,

e_{ijklm} is the residual effect.

Model 3 comprised of the full model and Model 4 was a reduced model involving only the first-order interactions between the testing methods and the other main effects.

The difference in sex ratio (based on 1:1 ratio) within groups was analyzed using a Chi-square test. Mean survival among test groups in hapas was analyzed using one-way analysis of variance.

RESULTS

Hapa experiment

Mean initial and final body weights of the test groups are shown in Table 4.2. There were no significant differences in initial body weight among communally-reared groups but significant differences were observed in the separately-reared groups. The ISR strain had a slightly higher initial weight than the SEL and RBC groups ($P<0.05$). The overall mean

initial weight in the communally-reared groups (1.29 ± 0.22 g) was significantly lower than the initial weight in the separately-reared groups (1.39 ± 0.24 g). Significant differences in initial weights were observed among replicates. Mean final body weights among test groups were significantly different ($P<0.01$). The SEL group had significantly higher mean final weight than the RBC and ISR groups in communal and separate rearing.

Table 4.3 shows the mean squares from the GLM procedure. Overall means between communal (29.53 ± 11.84 g) and separate rearing (43.99 ± 12.54 g) in hapas were significantly different at $P < 0.05$. Group and sex effects were highly significant, contributing 22.62% and 25.38 %, respectively to the variation observed on final body weights. The replicate effect and all the interaction effects were not significant. The growth curves of the test groups in hapas are presented in Figures 4.1 and 4.2. The growth divergence among groups in hapas became distinct after 4 and 8 weeks of culture under separate and communal rearing, respectively.

Table 4.4 shows the mean final body weight of males and females of the three groups in hapas. Males were significantly heavier than females ($P<0.01$). Across groups, the SEL males were significantly heavier than the males from the other two groups. For the females, the SEL group was significantly heavier that the RBC and ISR groups in separate rearing but in communal rearing, the SEL and RBC females did not differ significantly in mean final body weights ($P>0.05$). The SEL females and ISR males did not differ in final body weight under communal rearing.

The sex ratios of the different groups were similar between communal and separate rearing (Table 4.4). There was no significant difference in sex ratio between the SEL and

ISR groups ($P > 0.05$) but a significantly higher proportion of females was observed in the RBC group ($P < 0.01$).

The survival rates among groups in communal rearing were 78%, 90%, and 96% for SEL, RBC, and ISR groups, respectively. The SEL group had significantly lower survival in communal rearing. The survival rates in separate rearing were 89%, 93%, and 84% for SEL, RBC, and ISR groups, respectively, with the ISR strain having the lowest survival rate. There were no significant differences in survival rates between communal and separate rearing in hapas.

Pond experiment

The growth curves of the different test groups in communal and separate rearing during the 16-week culture period are shown in Figures 4.3 and 4.4. The mean initial and final body weights of the different test groups in ponds are shown in Table 4.5. The mean initial body weights among groups were not significantly different under separate rearing ($P > 0.05$). However, under communal rearing, the ISR strain had a significantly lower mean initial weight than the RBC and SEL groups. The overall mean initial weight in communally-reared groups (2.13 ± 0.81 g) was significantly different from those in the separately-reared groups (1.79 ± 1.11 g).

Mean final weights were significantly different among groups ($P < 0.01$) in both rearing methods. The SEL group has significantly higher mean final body weight than RBC and ISR groups in communal rearing while the RBC and ISR groups did not differ from each other in mean final weight. The RBC group performed similarly under the two

rearing methods. The overall mean was 52.6 ± 12.84 g in communal rearing and 56.37 ± 19.02 g in separate rearing. There was no significant difference between the two rearing methods. Similar to the hapa experiment, most of the variation was due to group and sex effects. The replicate effect and interaction effects were not significant (Table 4.6).

The mean final weights of males and females in communal and separate rearing are shown in Table 4.7. Significant differences in mean final weight were observed between males and females ($P < 0.001$). In communal rearing, the SEL males differed significantly from RBC and ISR males but RBC and ISR males did not differ from each other in mean final weight. The pattern was similar for the female groups. For separate rearing groups, the mean final weights among males and females across groups were significantly different. The SEL groups had the heaviest mean final body weights both in male and female groups. Comparable mean body weights were observed between the females of the SEL group and the males of the RBC groups.

The sex ratios within groups were similar in communal and separate rearing. The sex ratio of the SEL and RBC groups did not differ significantly from 1:1. The ISR group had significantly more males in both testing methods.

Genotype-environment interaction in communal and separate rearing

Tables 4.8 and 4.9 present the marginal mean squares for communal and separate rearing, respectively. No significant genotype-environment interaction was observed when comparing the final body weight of the test groups in communal rearing. The environment contributed considerably to the total variation (63.5%) followed by the sex effect (17%)

while group effects accounted for only 3.2% of the variation. In separate rearing, there was a significant genotype-environment for body weight ($P<0.001$) but this accounted for only 1% of the total variation. The major variations were almost equally contributed by group and sex effects (35% and 33.7%, respectively).

Response to selection in communal and separate rearing

Higher selection response (as a percent of the RBC group) was observed in separate rearing both in hapas and ponds (Table 4.10). In communal rearing, the selected group was 13.6% and 10% heavier than the RBC group in hapas and ponds, respectively, while in separate rearing, the selected groups were heavier by 25.5% in hapas and 35.3% in ponds.

Comparison between SEL group and the ISR strain showed a higher percent difference than with RBC group except in communal rearing in ponds where the magnitude of difference of the selected group with RBC and ISR groups was the same. The differences in mean body weight between the SEL and the ISR strain were 36.7% and 49.2% in hapas and ponds, respectively.

The correlation between final weight in separate and communal rearing were 0.95 and 0.98 for hapa and pond environments, respectively. These correlation coefficients, although high and positive, were not significant because only 3 paired observations (3 test groups) were involved in the correlation analysis.

DISCUSSION

This is the first time, under the present study, that the response to selection was evaluated both in communal and separate testing. In these experiments, the growth performance of the different groups obtained in separate and communal rearing was the same in terms of ranking of the groups. For testing purposes, communal rearing gives the same results as would be obtained under separate rearing. This would make testing programs less expensive as they require fewer resources.

The magnitude of difference in growth performance among groups was different in separate and communal rearing. However, the absence of a significant genotype and rearing method interaction suggest that communal rearing could provide an important impact for commercial production when alternative stocks are compared by the farmers.

Comparison of response in separate and communal rearing

The results of communal experiments must be highly correlated with separate experiments for communal rearing to yield useful information (Moav and Wohlfarth, 1973, 1991). The results of the present study have shown that the group that had faster growth in communal rearing also experienced faster growth in the separate rearing. This result was consistent in both the hapa and pond environments. The high but not statistically significant correlation between the final weights in communal and separate rearing points that communal rearing can be used as an indication of the possible ranking in separate rearing. This was consistent with the findings of Wohlfarth and Moav (1985), Dunham *et*

al. (1983), Circa *et al.* (1995) and Danting *et al.* (1995).

Competition between different genetic groups may be a problem in the communal stocking method. Such behaviour can be influenced by differences in initial size of the individuals or groups of fish (Gunnes, 1976; Dunham *et al.*, 1982). In tilapia, communal rearing of mixed-sized groups has been known to depressed the growth of the small-sized group due to competition with the medium- and large-sized groups or both (McGinty, 1985). In the present study, the initial sizes for all groups in each replicate were made similar by size-matching. If competition was present among groups, it was not due to the initial size differences. But it may be possible that in between the initial and final growth phase, when each fish begins to express individual growth potential, such competition may have eventually become important (Doyle and Talbot, 1986b).

Moav and Wohlfarth (1974, p. 193) found that "the genetic group which had faster growth rate in separate ponds was more competitive in communal ponds and that its relative growth rate was even higher in the presence of inter-group competition". McGinty (1987) also found more magnification of differences in weight gain between species of tilapia in communal rather than in separate rearing. In contrast, the present study showed consistently that growth differences among groups were larger in separate rearing both in hapa and pond environments. This result can be seen as a distinct advantage particularly to the fish farmer since improved stocks such as those produced from fish breeding programs are not likely to be stocked communally under commercial production system. The integrity of the more productive stocks is usually maintained through monospecific culture.

Genotype-environment interactions

Under communal rearing, there was no significant genotype-environment interaction but a significant genotype-environment interaction was observed under separate rearing. However, the interaction was mainly a magnitude interaction. Even if the test groups had improved response to the better environment in ponds, the rank ordering remained the same in hapa as well as in pond environment.

Macaranas *et al.* (1997) observed significant strain differences in growth performance of four tilapia stocks raised in two culture environments. Eknath *et al.* (1993) documented a significant genotype-environment interaction for growth, mainly a magnitude interaction in strain evaluation involving 7 strains of Nile tilapia tested in 11 diverse culture environments. The interactions were relatively minor and the conclusion made was that development of specialized strains for each of the farming systems was not necessary. Iwamoto *et al.* (1986) and Dunham *et al.* (1990) also obtained significant genotype-environment interactions for growth in rainbow trout and catfish, respectively, and suggested that selection of strains adapted for specific aquaculture application may eventually lead to increased productivity.

Initial size variation

The problem of initial weight differences seemed to have been satisfactorily resolved with common carps with the use of the multiple-nursing technique (Wohlfarth and Moav 1985). This correction technique was also found valid for genetic experiments with

channel catfish (Dunham *et al.*, 1982). With tilapias however, the method of multiple nursing to generate an environmentally induced correction factor for initial size differences was found not appropriate (Wohlfarth *et al.*, 1994; Kulikovsky *et al.*, 1994). In both studies, the mean difference in weight gains between the large and small multiple nursed samples were either zero or negative. This meant that the small multiple-nursed samples had higher final weight than the large multiple-nursed samples resulting in a zero or negative correction factor. Because of these inconsistent results, the comparison of growth differences among genetic groups in tilapia was, therefore, based on the observed weight gains (Wohlfarth *et al.*, 1994, Kulikovsky *et al.*, 1994).

A method which has been used in reducing the phenotypic variance in size in tilapia is the 'collimation' technique. This technique, suggested by Doyle and Talbot (1986a) consisted of grading the population according to size and selecting individuals near the mean, while discarding those from the tails of the size distribution curve. Jarimopas (1986) applied the collimation technique in tilapia using weight-specific selection where she obtained a heritability of 0.20. Uraiwan (1990) and Bolivar *et al.* (1994) used within-family selection with collimation to remove the non-genetic environmental variance in growth associated with asynchronous spawning or maternal effects in tilapia. Substantial response to selection has been obtained from these experiments.

In the present study, size-grading was done to obtain a sample of fish of the same size before initiating the growth trials. The size-grading was done at an early phase of the life cycle when the fish were about 1-3 g in weight. Examination of the growth curves showed the similarity of growth rate extended up to week 4 in most cases, even until week 8

in communal hapas. It was only in separate pond testing that the selected group diverged abruptly from stocking to week 4. This shows that the genetic difference in growth among these groups was expressed later on in the growth phase. Doyle and Talbot (1988: p. 455) indicated that "fish can go through a major disruption in the grow-out environment (such as the size-grading that was done in the present study) and then re-emerge with relative growth rates similar to what they had before." If this is the case, the growth differences among groups that were observed in this study are an inherent indication of the genetic growth potential of the groups and the size-grading procedure done early in the growth phase could not have affected the manifestation of these differences in later growth phase.

In summary, the selected group consistently had the highest mean final weight in hapa and pond environments. Higher selection response (relative to the random-bred control line) was observed under separate rearing method. There was no significant genotype-environment interaction under communal rearing while the interaction observed under separate rearing was not sufficiently large to produce "line crossing" or a change in the rank of the test groups. Finally, the growth ranking of the test groups was similar in communal and separate rearing methods. This suggests that communal rearing method can be used in routine performance testings.

Table 4.1 Details of the communal and separate rearing experiment

Rearing Method	Culture unit	No. of units	No. of fish/unit	No. of fish /group
Communal	Hapa	4	105	35
	Pond	3	1000	333
Separate	Hapa	12	105	_
	Pond	9	1000	_

Table 4.2 Mean and number of fish (in parenthesis), standard deviation (SD) of initial and final body weights of Nile tilapia under separate and communal rearing in hapas.

		Initial		Final	
Rearing Method	Group	Mean weight (g)	SD	Mean weight (g)	SD
Communal	SEL	1.31 (140)[a]	0.24	33.96 (109)[a]	12.53
	RBC	1.28 (140)[a]	0.23	29.89 (126)[b]	11.04
	ISR	1.28 (140)[a]	0.21	25.59 (134)[c]	10.67
Separate	SEL	1.36 (420)[b]	0.25	52.11 (375)[d]	13.07
	RBC	1.38 (420)[b]	0.25	41.51 (389)[e]	9.82
	ISR	1.43 (420)[c]	0.22	38.12 (353)[f]	9.97

Columns with values superscripted with different letters are significant different ($P < 0.001$).

Table 4.3 Analysis of variance of final body weight from the GLM procedure (Hapa experiment).

Sources of variation	DF	MS	Contribution to total variation, %
Type	1	55688.75*	51.95
Group	2	10999.52**	10.26
Replicate	3	6888.26ns	6.42
Sex	1	27206.32**	25.38
Type x Group	2	988.04ns	0.92
Type x Replicate	3	5013.50*	4.67
Type x Sex	1	352.89*	0.33
Error	1460	68.81	0.06

** - highly significant ($P < 0.001$), * - significant ($P < 0.01$), ns - not significant ($P > 0.05$).

Table 4.4 Mean final body weights of males and females of the three test groups of Nile tilapia under communal and separate rearing in hapas.

Rearing Method	Group	Sex ratio (%)		Mean weight ♂ (g)		Mean weight ♀ (g)	
		♂♂	♀♀	Mean	SD	Mean	SD
Communal	SEL	50	50^{ns}	39.01^{a}	12.71	28.82^{gh}	10.10
	RBC	37	63**	34.70^{b}	13.15	27.13^{h}	8.56
	ISR	50	50^{ns}	31.24^{cg}	10.55	19.95^{i}	7.30
Separate	SEL	50	50^{ns}	58.91^{d}	13.02	45.43^{j}	9.08
	RBC	35	65**	47.55^{e}	10.88	38.30^{k}	7.44
	ISR	51	49^{ns}	43.35^{f}	9.77	32.73^{l}	6.81

Sex ratio within group, ns – not significant, ** significant ($P < 0.01$)
Columns with values superscripted with different letters are significantly different ($P<0.001$). Male and female columns, means with different superscipt letter are significantly different.

Table 4.5 Mean and number of fish (in parenthesis), standard deviation (SD) of initial and final body weights of Nile tilapia under separate and communal rearing in ponds.

Rearing Method	Group	Initial		Final weight (g)	
		Mean weight (g)	SD	Mean weight (g)	SD
Communal	SEL	2.25 (150)[a]	0.62	55.70 (210)[a]	12.68
	RBC	2.24 (150)[a]	0.96	50.62 (182)[bd]	12.65
	ISR	1.88 (130)[b]	0.78	50.64 (135)[b]	12.47
Separate	SEL	1.82 (150)[c]	0.97	71.01 (163)[c]	21.81
	RBC	1.83 (150)[c]	1.23	52.48 (156)[d]	12.72
	ISR	1.73 (150)[c]	1.11	47.59 (165)[e]	12.33

Columns with values superscripted with different letters are significantly different ($P<0.001$). [1] Sample size only.

Table 4.6 Analysis of variance of final body weight from the GLM procedure (Pond experiment).

Sources of variation	DF	MS	Contribution to total variation, %
Type	1	4030.48*	4.68
Group	2	19455.24**	22.62
Replicate	2	5436.50ns	6.32
Sex	1	44992.48**	52.31
Type x Group	2	6685.28ns	7.77
Type x Replicate	2	5145.77ns	5.98
Type x Sex	2	168.11ns	0.19
Error	879	93.52	0.11

** - highly significant ($P < 0.001$), * - significant ($P < 0.01$), ns - not significant ($P > 0.05$).

Table 4.7 Mean final body weight of males and females of the three test groups of Nile tilapia in communal and separate rearing in ponds.

Rearing Method	Group	Sex ratio (%) ♂♂	Sex ratio (%) ♀♀	Mean weight ♂ (g) Mean	Mean weight ♂ (g) SD	Mean weight ♀ (g) Mean	Mean weight ♀ (g) SD
Communal	SEL	50	50^{ns}	62.90^{a}	11.74	47.13^{f}	8.76
	RBC	57	43^{ns}	57.73^{b}	12.14	43.92^{g}	8.75
	ISR	59	41^{**}	55.91^{b}	11.78	42.22^{g}	8.63
Separate	SEL	54	46^{ns}	77.00^{c}	22.27	64.00^{h}	19.13
	RBC	48	52^{ns}	60.68^{dh}	12.07	44.92^{i}	7.50
	ISR	67	33^{**}	51.41^{e}	12.11	39.70^{j}	8.31

Sex ratio within group, ^{ns} – not significant, ** significant ($P < 0.01$)
Columns with values superscripted with different letters are significantly different ($P<0.001$).

Table 4.8 Analysis of variance of final body weight from the GLM procedure (Communal rearing).

Sources of variation	DF	MS	Contribution to total variation, %
Environment	1	78485.61*	63.50
Group	2	3931.61**	3.20
Replicate	2	17001.55ns	13.70
Sex	1	21065.58**	17.0
Environment x Group	2	150.94ns	0.12
Environment x Replicate	2	1902.90**	1.50
Environment x Sex	1	1111.79**	0.90
Error	826	73.28	0.06

** - highly significant ($P < 0.001$), * - significant ($P < 0.01$), ns - not significant ($P > 0.05$).

Table 4.9 Analysis of variance of final body weight from the GLM procedure (Separate rearing).

Sources of variation	DF	MS	Contribution to total variation, %
Environment	1	36180.70*	25.3
Group	2	50023.74**	35.0
Replicate	2	4704.04ns	3.3
Sex	1	48175.38**	33.7
Environment x Group	2	1422.86**	1.0
Environment x Replicate	2	1971.61**	1.4
Environment X Sex	1	211.97ns	0.15
Error	1343	120.98	0.08

** - highly significant ($P < 0.001$), * - significant ($P < 0.01$), ns - not significant ($P > 0.05$).

Table 4.10 Difference (%) in mean body weight of selected Nile tilapia from RBC and Israel strain in communal and separate rearing in hapas and ponds.

Rearing Method	Culture Environment	Group	Pooled sexes		Males		Females	
			g	%	g	%	g	%
Communal	Hapa	SEL/RBC	4.1	13.6	4.3	12.4	1.7	6.2
		SEL/ISR	8.4	32.7	7.8	24.9	8.7	44.5
	Pond	SEL/RBC	5.1	10.0	5.2	8.9	4.4	10.0
		SEL/ISR	5.2	10.0	7.0	12.5	6.14	14.4
Separate	Hapa	SEL/RBC	10.6	25.5	11.6	23.9	7.1	18.6
		SEL/ISR	14.0	36.7	15.5	35.9	12.7	38.8
	Pond	SEL-RBC	18.5	35.3	16.3	27.0	19.1	42.5
		SEL-ISR	23.4	49.2	25.6	49.8	24.3	61.2

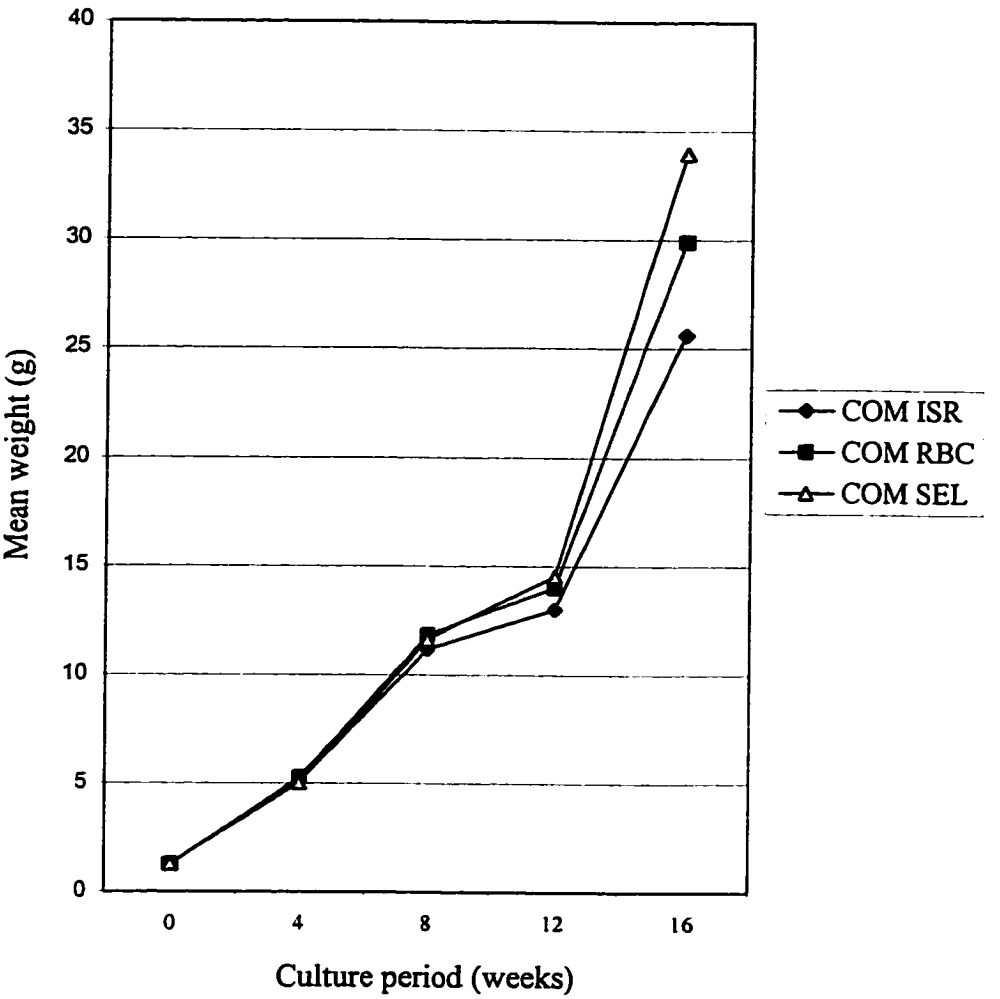


Figure 4.1 Growth curves of the test groups in communal rearing in hapas.

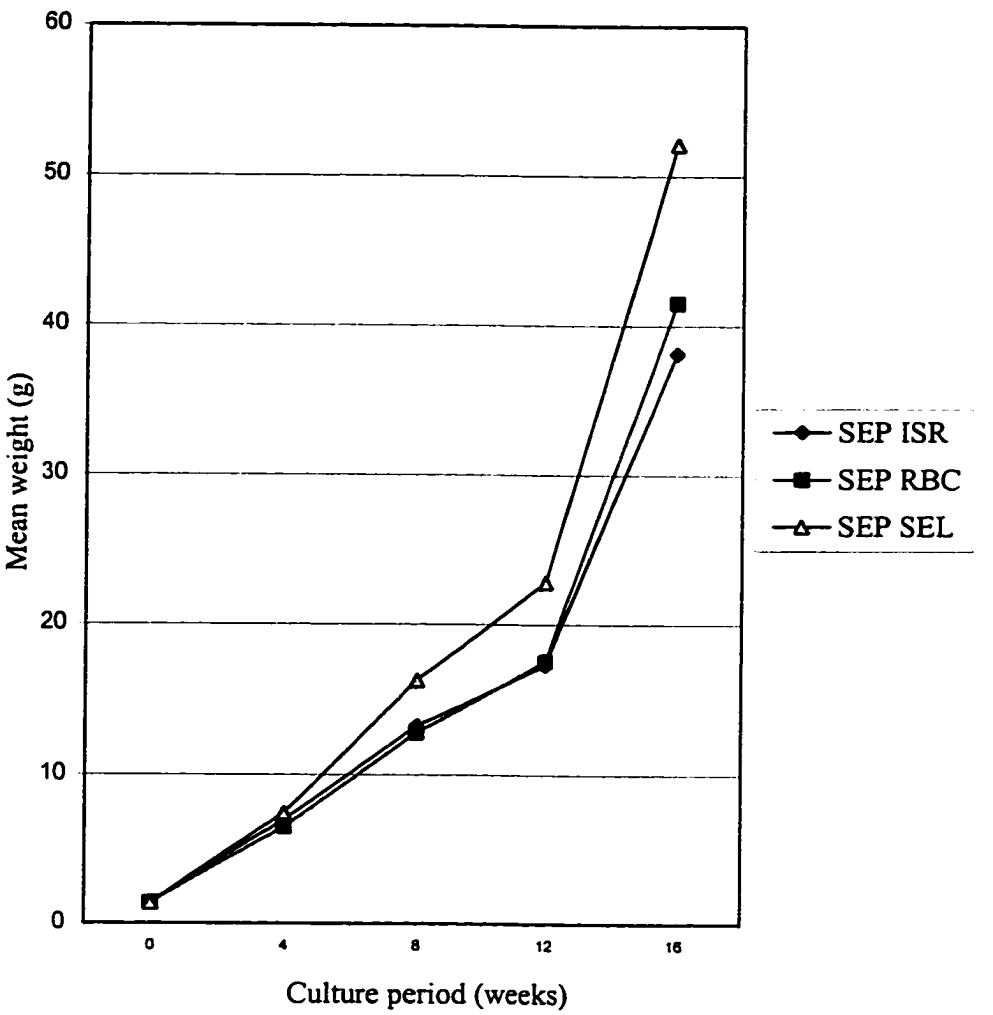


Figure 4.2 Growth curves of the test groups in separate rearing in hapas.

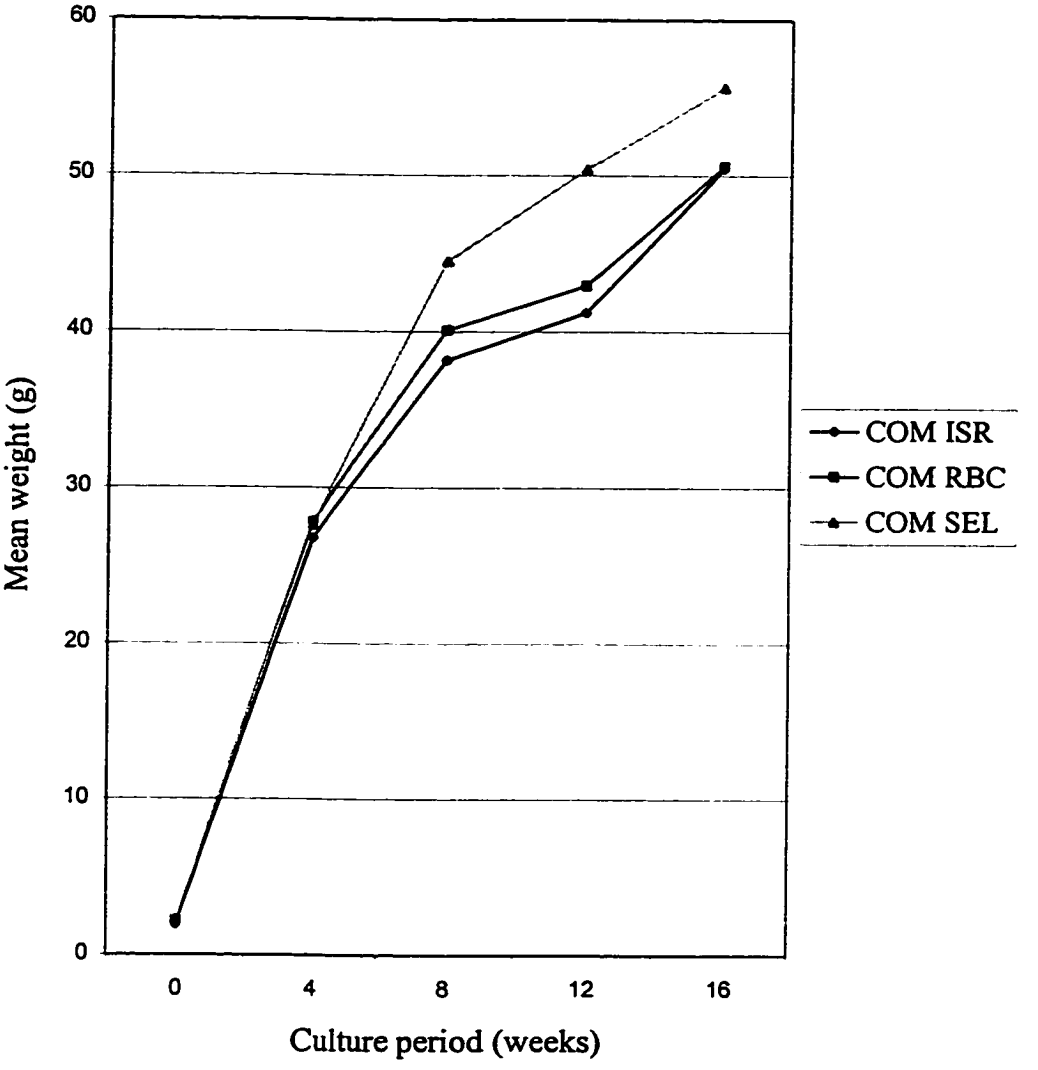


Figure 4.3 Growth curves of the test groups in communal rearing in ponds.

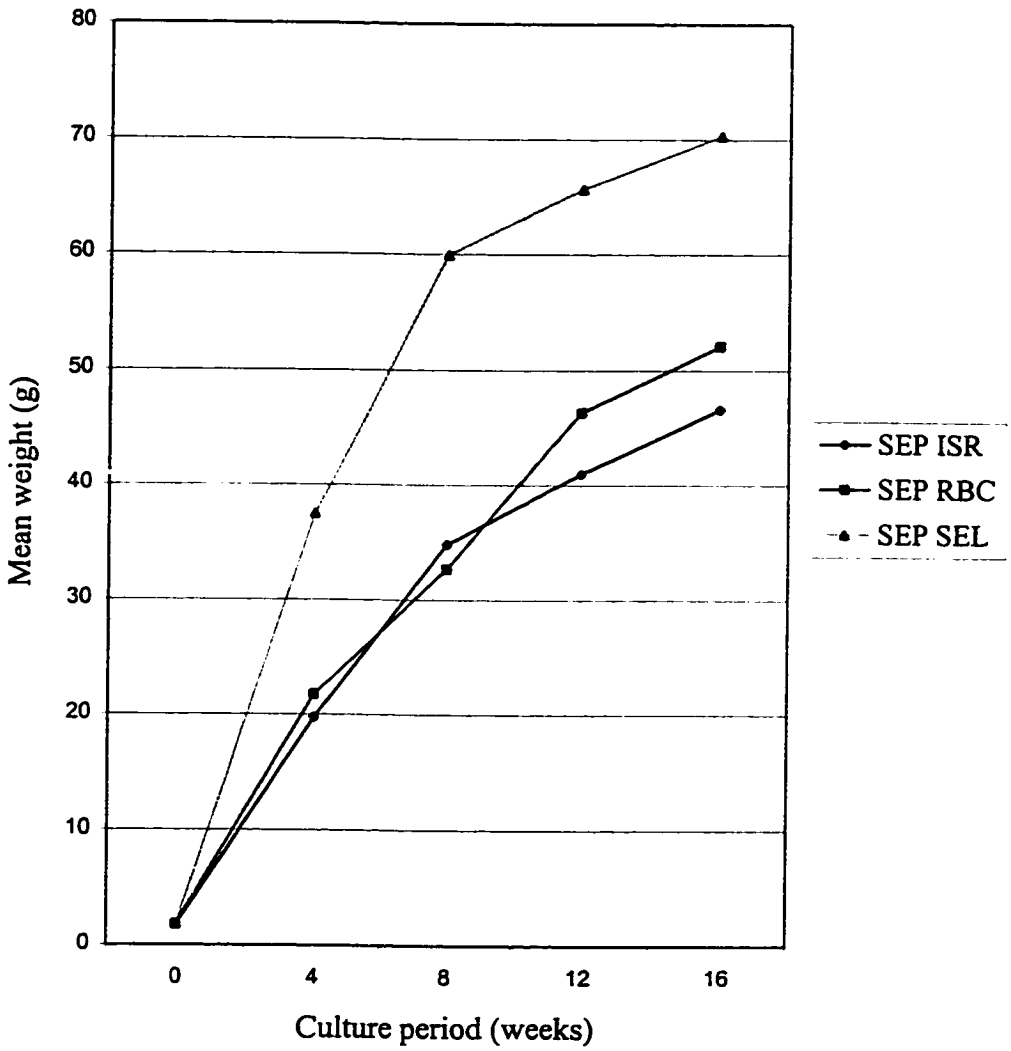


Figure 4.4 Growth curves of the test groups in separate rearing in ponds.

Chapter 5

RESPONSE TO SELECTION FOR BODY WEIGHT IN NILE TILAPIA (*Oreochromis niloticus*) USING A SINGLE-TRAIT ANIMAL MODEL

ABSTRACT

Within-family selection for improved growth at 16-weeks was undertaken on Nile tilapia (*Oreochromis niloticus*) from 1986 to 1996. Data from 12 generations of selection were analyzed by a single trait Restricted Maximum Likelihood fitting an animal model. The heritability of body weight at 16 weeks in the base population was estimated as 0.385. A continuous genetic response for body weight was found with an expected mean increase in body weight of 2.19 ± 0.27 g or 12% per generation in Line 1 and 2.21 ± 0.25 g or 12.9% per generation in Line 2. A realized heritability estimate of 0.14 was obtained based on the regression of mean breeding values on cumulative selection differentials after 12 generations. The inbreeding coefficient was 6.3% after 12 generations with an average inbreeding rate of 1.4% per generation. The family rotational mating used to propagate the families was effective in keeping the inbreeding level to a minimum even at a high selection intensity. Overall, the low inbreeding levels, high selection intensities and the relatively high heritability for body weight at 16-weeks in the base population that was used in this selection experiment resulted in substantial response using the within-family selection method.

INTRODUCTION

Data from selection experiments are often used to obtain estimates of realized heritability, genetic correlations, and related parameters, such as genetic means and responses to selection, using the standard least-square procedures (Becker, 1984). For such procedures, the environmental effects are accounted for by using an unselected control or by using divergent selection (Falconer, 1989). However, in large-animal experiments, a major problem is that environmental fluctuations are difficult if not impossible to control which makes the estimates of the genetic trend biased by environmental effects (Hill, 1972a, b).

Henderson's (1973) pioneering work provided a technique that enables separation of genetic and environmental effects when predicting bull and cow breeding values. The technique has now dominated the analysis of data from livestock improvement schemes, both in the prediction of breeding values and the estimation of genetic parameters. Sorensen and Kennedy (1984, 1986) showed that a mixed-model procedure could be used to analyze data from selection experiments to provide estimates of genetic parameters and of selection response.

Sorensen and Kennedy (1984) compared least-squares and mixed-model estimates of selection response and concluded that a least-square estimator is unbiased provided that the records have been properly adjusted for fixed effects, that the selection is within generation (no overlapping generations) and there is only one record per candidate for selection. On the other hand, the mixed-model estimator is unbiased and individual breeding values have minimum variance of prediction error provided that selection is within levels of fixed effects, the variances of the random effects before selection are known, and

that the relationship matrix is complete. This means that all animals involved in the selection decisions, regardless of whether they contributed offspring, must be included in the analysis. This model of analysis, known as the 'animal model', defines additive genetic effects for all animals individually and accounts for all variances and covariances among them (Meyer and Hill, 1991). A major strength of the animal model approach is that performance and genetic relationship for all individuals in all generations are utilized simultaneously (Gall *et al.*, 1993).

The use of the animal model to estimate response is under an assumption of additive genetic inheritance and an infinitesimal model; that is, the trait is considered to be determined by infinitely many unlinked genes each of small effect, and gene frequencies are assumed not to change due to selection. Simulation work suggests that animal models provide good approximations of breeding values and selection responses for traits controlled by small numbers of genes if the genetic model is additive even if selection has been practiced (Maki-Tanila and Kennedy, 1986).

Most early published estimates of genetic parameters in tilapia breeding have been calculated by methods of least squares or by regression of response on selection differentials. Recently, the fish breeding literature shows that the animal models are beginning to be used to estimate variance components in species like rainbow trout (Su *et al.*, 1996; Elvingson and Johansson, 1993). Animal models based on best linear unbiased prediction procedures have been rarely used to evaluate the response from selection in fish populations that have undergone several generation of artificial selection. Gall *et al.* (1993) have shown an example of its application in estimating genetic change based on a subset of data from a rainbow trout selection program.

Sorensen and Kennedy (1986) have discussed the conditions under which the mixed-model approach adequately partitions phenotypic trend into its genetic and environmental components and to estimate response to selection. They stressed, however, that this does not imply that selection experiments should be designed without the use of contemporaneous controls. They added that selection experiments should be designed not only with unselected controls but should also be replicated if facilities are available.

This chapter presents the analysis of the accumulated data from a 10-year selection experiment for increased body weight in Nile tilapia by the use of an animal model. There was no unselected control line in the early part of selection experiment. Instead, a repeat mating of selected parents from the older generation was used as control for testing the response to selection. The progeny from older parents were compared with the progeny from the most recent selected generation. Although some positive estimates of response were obtained, this procedure proved to be unreliable, in part, because of the difficulty of spawning the older animals and because of the small number of repeat mating that could be done. During the middle part of the selection experiment, control populations were established and were used in growth performance testings reported in Chapters 3 and 4.

The study was based on data obtained from a selection experiment conducted at the Freshwater Aquaculture Center (FAC) of the Central Luzon State University, Philippines from 1986 to 1996. The selection work focused on the Nile tilapia (*O. niloticus*), a species of great importance to Asian aquaculture. Twelve generations of within-family selection were performed for increased body weight. The objectives of this study were: 1) to investigate the response to selection for 16-week body weight in Nile tilapia using an animal

model; and 2) to estimate genetic parameters of 16-week body weight in the base population using REML methodology with an animal model.

MATERIAL AND METHODS

Base population

The base population in this selection experiment was taken from the second generation of high growth line of Nile tilapia developed from a separate selection experiment (Abella *et al.*, 1986). Four strains of Nile tilapia, namely Israel, Singapore, Taiwan, and 'FAC' strain were combined to create a founder population for that previous selection experiment. The 'FAC' strain was collected from the breeding ponds of the Freshwater Aquaculture Center and believed to have a record of ancestry from an earlier introduction of the Singapore strains (Lester *et al.*, 1988). Random samples of brood fish from the high growth line were obtained to establish a base population used for the present study (Abella *et al.*, 1990).

The experiment

The selection experiment started with 19 full-sib families. Each family was reared in a single concrete tank. Size-grading was carried out when the mean weight of the fry reached about 0.5 g. The concept of size-grading in tilapia selection procedure was developed to remove the possible bias on selection that was attributable to excessive variation in initial weight caused by maternal effects (Doyle and Talbot, 1986a). The size-grading was done by measuring 30 random samples of fish to determine the mean initial

weight in each family. The largest and the smallest fish in each family were culled and those fish with weight closest to the family mean were saved. The family size was reduced to 100 fish during this procedure. This reduction along with the size grading was done to keep the family size within the limits of the rearing capacity of the tanks, thereby reducing the possible effect of competition due to high stocking density or initial size differences (Doyle and Talbot, 1986b).

The basis of selection was the body weight at 16 weeks. All individuals were weighed in grams. Ten heaviest females and 8 heaviest males were selected within each family. The selected fish were tagged with modified Floy tags and were held separately by sex in the tank. When the next generation was to be produced, the two heaviest females from a family were mated with the heaviest male from another family. The mating scheme followed that of the rotational mating scheme (Kincaid, 1977) described in Chapter 2. The priority was the spawning of the heaviest female. If this was delayed, then the full-sib family from the second heaviest female was considered for further rearing and selection. All the procedures involved in this selection experiment (from spawning to selection) were done entirely in outdoor concrete tanks.

The selection started with a single line from the base population up to generation 5. At generation 6, each family from the selected line was split into two, forming a second line. The two lines were managed similarly. There were losses of some families caused by failure or delay in reproduction, but these were minimal. Also, beginning at generation 6, the initial number of fry that was reared per family was standardized to 200 as opposed to rearing all the fry produced by one female, a practice that was done from generation 1 to generation 5.

Data

The data were obtained for 16-week body weight for a total of 19,581 fish in Line 1 and 19,943 fish in Line 2. For the analysis, and because the second line originated from the first line, the two lines had the same records starting from generation 0 to generation 5. The two lines were analyzed separately.

Pedigree information was available and it traced back to the fish in generation 0 where the parents were not known and the fish were assumed not be related. The pedigree information included all fish whether they contributed progeny or not.

Statistical Analyses

Breeding values and genetic parameters

Predicted breeding values were calculated by an animal model using the PEST (Prediction and Estimation) program developed by Groeneveld *et al.* (1990). The use of an animal model for the prediction of breeding values requires prior knowledge of the additive genetic and phenotypic variances of the trait concerned or at least their ratio or the heritability in the base population (Kennedy, 1981). In the present study, the variances were not known. Consequently, the variances were obtained by estimating variance components first from the data set. This procedure has been shown to give unbiased estimates of the variances if an animal model is used with a complete relationship matrix and the estimates were obtained using maximum likelihood procedure (Kennedy, 1981; Sorensen and Kennedy, 1986). The estimates of additive genetic and residual variances were then used in the mixed-model equations to compute breeding values from the same data set.

The estimation of variance components was carried out using Restricted Maximum Likelihood (REML) fitting an animal model. Fixed effects were generations (0-12), tanks (1-19), and sex (1-2). A software package called REML-VCE (Groeneveld, 1996) was used to estimate variance components. In matrix notation, the model used both for estimating breeding values and variance components was:

$$y = Xb + Za + e$$

wwhere:

y is the vector of observations,

b is the vector of fixed effects,

a is the vector of random additive genetic values with Var $(a) = A\sigma_a^2$

e is the vector of residual effects with Var $(e) = I\sigma_e^2$

X and Z are known incidence matrices relating the elements of b and a to the observations. A is the additive genetic relationship matrix (Henderson 1976) with diagonal elements of $1 + F_i$, where F_i is the inbreeding coefficient of animal i, and off-diagonal element equal to the numerator of Wright's (1922) coefficient of relationship between two animals, and I is an identity matrix. σ_a^2 and σ_e^2 are the additive genetic and residual variances, respectively.

The mixed model equations corresponding to the model used were:

$$\begin{bmatrix} X'X & X'Z \\ Z'X & Z'Z+A-1\lambda \end{bmatrix} \begin{bmatrix} b \\ a \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \end{bmatrix}$$

where $\lambda = \sigma^2_e / \sigma^2_a = (1-h^2)/h^2$, h^2=heritability in the base population.

The predicted breeding values (selection response) were obtained by averaging the elements of â each generation. The genetic response for body weight was estimated from the regression of the average breeding values on generation number.

Within-family heritability from REML analysis for 16-week body weight was calculated as:

$$h^2 = (\sigma^2_a /2)/((\sigma^2_a /2) + \sigma^2_e)$$

Selection differentials, realized heritability and intensity of selection

Selection differentials were calculated within each family as the average difference of the selected fish from their respective family means. Cumulative selection differentials were obtained by adding the selection differential for the first and second generation to obtain a value for generation 2, adding the differentials from the first, second, and third generations to obtain a value for generation 3, and so on. The standardized selection differential (actual intensity of selection) was calculated by dividing the selection differential by the corresponding phenotypic standard deviation of 16-week body weight.

An estimate of realized h^2 for 16-week body weight was obtained by regressing the breeding values (from the animal model) on cumulative selection differentials.

Calculation of inbreeding

The coefficient of inbreeding for each individual was calculated from the pedigree data using the algorithm by Meuwissen and Luo (1992). The inbreeding coefficient for a specific generation was calculated as the mean of the individual inbreeding coefficients. The rate of inbreeding in each generation was calculated following the formula by Falconer (1989):

$$(F_t - F_{t-1})/(1-F_{t-1})$$

where F_t is the inbreeding coefficient in generation t and F_{t-1} is the inbreeding coefficient in generation t-1.

RESULTS

Number of fish and phenotypic means

The number of fish for each generation, the observed means, and standard deviation for 16-week body weight in each generation are given in Table 5.1. The observed means were variable in both lines but indicated some phenotypic increase during the course of the experiment. Line 2 had slightly higher observed means than Line 1. The comparison of observed means between the two lines did not show any significant difference ($P = 0.70$). Splitting the selected line at generation 5 resulted in two sub-population of Nile tilapia with very similar trends with respect to the fluctuations in phenotypic means (Figure 5.1).

Genetic parameters in the base population

Estimated heritabilities for body weight from REML analyses were 0.38 and 0.39 for Lines 1 and 2, respectively. The estimates of additive genetic variances were 30.85 in Line 1 and 35.94 in Line 2. The corresponding residual variances were 25.40 and 28.32.

Predicted genetic response

Average predicted breeding values per generation are presented in Table 5.2 and Figure 5.2. A continuous genetic response for body weight was found in both lines. As with their phenotypic means, the two lines did not differ significantly in the predicted breeding values (P=0.98). The regression of the average predicted breeding values on generation number showed that the expected increase in body weight was 2.19 ± 0.27 g or 12% per generation in Line 1 and 2.21 ± 0.25 g or 12.9% per generation in Line 2. Over 12 generations, the average predicted breeding values increased by an estimated of 28.73 g and 29.63 g for Line 1 and Line 2, respectively. The genetic response to selection was very similar for the two lines.

Selection intensity and realized heritability

The actual mean intensities of selection per family are presented in Table 5.3. The selection intensities were high corresponding to 2% of the population being selected. The realized h^2 was estimated to be 0.14.

Inbreeding

The estimated inbreeding coefficients are presented for Line 1 (Figure 5.3). The inbreeding coefficients of the two lines were almost similar. The fish used to establish the base population were assumed to be neither inbred nor related. The average inbreeding coefficients for the first three generations were zero. The inbreeding coefficient was 6.3% after 12 generations of selection while the average rate of inbreeding was calculated to be 1.4% per generation.

DISCUSSION

Genetic parameters

Directional selection reduces additive genetic variance for selected traits, which introduces bias when estimating genetic parameters. By using an appropriate animal model with complete additive genetic relationship matrix, changes in the selection mean and variance due to drift and selection can be accounted for (Kennedy *et al.*, 1988). The relationship matrix must be complete to tie all selected individuals back to the base population prior to selection (Sorensen and Kennedy, 1984).

The aim of using the animal model methodology from the data derived from within-family selection was to verify the response to selection and to estimate the genetic parameters in the base population. The heritability estimated from this method indicated a substantial genetic variation prior to selection. The genetic trend shows a steady progress of the selection response up to generation 12. There is no comparable study that indicates a long-running selection experiment in tilapia such as the present study where a selection

response has been reported.

Eknath *et al.* (1993) estimated the heritability for growth at 90 days in Nile tilapia to be 0.24. The mixed base population from combining genotypes from 4 wild populations of tilapia and 4 locally adapted strains was formed from this selection program. The average genetic gain across five generations of selection carried out using a combined selection has been about 12 to17% per generation (Dey and Eknath, 1997).

In the present study, the genetic response that has been achieved on selection for body weight at 16 weeks was about 12% per generation using within-family selection. This finding was supported by the results of the growth performance testings in various culture environments reported in Chapter 3. It is interesting that the response was greater in later generations (Figure5.2). Some factors involved certainly could have influenced this change in the genetic trend such as improved management of the stocks, improved environment and therefore improved growth rate of the fish.

Rotational mating and inbreeding

The rotational mating scheme has been effective in minimizing inbreeding. This mating scheme has also proven to be manageable even with limited facilities and it integrated well with the within-family selection method where complete pedigree was maintained. A slow accumulation of inbreeding was observed in the selected population. This was expected with the structured mating system that was used to advance the generations where full-sib matings were avoided (Kincaid, 1983).

With the initial 19 families used in the rotational mating, inbreeding was expected to occur only after 5 generations but because of losses of some families, a certain level of

inbreeding had occurred beginning generation 4. After generation 5, the cycle of mating was repeated which explained the subsequent increases in inbreeding. In some generations, certain families did not have either males or females. A decision was made to obtain a 'filler' for the sex that was lacking from the families either from within the line or from the corresponding family in the other line. This situation happened rarely so it did not seem to affect the level of inbreeding.

Following a rotational mating scheme, Uraiwan (1990) had calculated individual inbreeding coefficient of 0.8% after 5 generations of within-family selection. This is about the same estimate as what was observed in this study (Figure 5.3). Kincaid (1977) have used a rotational line crossing to reduce the rate of inbreeding accumulation in trout brood stocks.

There are relatively few inbreeding studies in fish. Kincaid (1983) reviewed the effects of inbreeding in fish populations. Generally, inbreeding depression becomes apparent based on levels of inbreeding in the range of 25-60%. Kincaid (1976) observed that an inbreeding coefficient of 12.5% resulted to depression on body weight of rainbow trout.

Within-family selection

The success of managing the within-family selection program has confirmed that this method would be useful in a breeding program with limited facilities (Uraiwan and Doyle, 1986). This study is probably the longest-running selection experiment that had used within-family selection method with rotational mating in Nile tilapia. In the longer term, the expected selection response would likely be reduced as a possible result of a proportionate

decline in additive genetic variance of the trait due to inbreeding eventually, but using within-family selection, the selection would partially act on those individuals with families not exhibiting inbreeding depression.

Overall results document substantial selection response after 12 generations of within-family selection. The mixed-model methodology was used to verify this response from the data that was generated from the selection experiment. It was not intended to change the selection protocol. The maintenance and use of control population had adequately satisfied the need for estimating genetic response.

Table 5.1 Number of fish (N), mean body weight and standard deviations (SD) in each generation of selected lines of Nile tilapia.

Generation	N	Mean	SD
0	1541	13.60	6.75
1	1676	29.92	7.46
2	1720	31.40	9.58
3	1631	16.91	6.29
4	1618	31.26	11.20
5	1623	11.06	5.83

	Line 1			Line 2		
	N	Mean	SD	N	Mean	SD
6	1249	17.69	6.84	1345	17.42	6.32
7	1471	25.25	8.62	1427	26.40	13.03
8	1406	18.47	8.63	1485	26.09	8.67
9	1481	34.91	10.63	1533	38.11	10.75
10	1363	30.70	10.00	1536	35.35	10.50
11	1440	44.80	9.47	1408	47.41	12.41
12	1362	35.49	8.14	1400	40.38	11.53

Table 5.2 Predicted mean breeding values (BV) and standard deviation (SD) for body weight in each generation of selected lines of Nile tilapia

Generation	BV	SD
0	-0.265	3.34
1	0.871	4.88
2	1.238	7.03
3	2.023	4.06
4	2.008	8.23
5	5.527	4.24

	Line 1		Line 2	
	BV	SD	BV	SD
6	5.361	3.78	5.540	4.63
7	6.465	5.78	4.382	9.92
8	10.286	6.60	11.546	5.85
9	12.546	4.94	11.971	5.82
10	16.382	6.76	17.385	7.06
11	23.829	5.10	22.112	8.08
12	28.734	4.06	29.635	6.14
Regression[1]				
Observed	1.34	0.66	1.83	0.65
Genetic	2.18	0.27	2.21	0.25

[1] Regression of generation means (observed) and mean breeding values (genetic) on generation number

Table 5.3 Mean selection differentials (S) and selection intensities (*i*) in each generation of selected lines of Nile tilapia

Generation	S (g)	*i*
0	15.18	2.50
1	13.19	2.58
2	14.76	2.35
3	14.22	2.94
4	16.19	2.37
5	9.93	2.94

	Line 1		Line 2	
	S (g)	*I*	S (g)	*i*
6	13.94	2.58	13.49	2.71
7	18.75	2.86	24.69	2.64
8	13.87	2.90	15.18	2.72
9	27.4	2.81	24.50	2.64
10	15.62	2.60	22.61	2.78
11	19.28	2.43	26.49	2.89
12	19.03	2.55	24.60	2.66

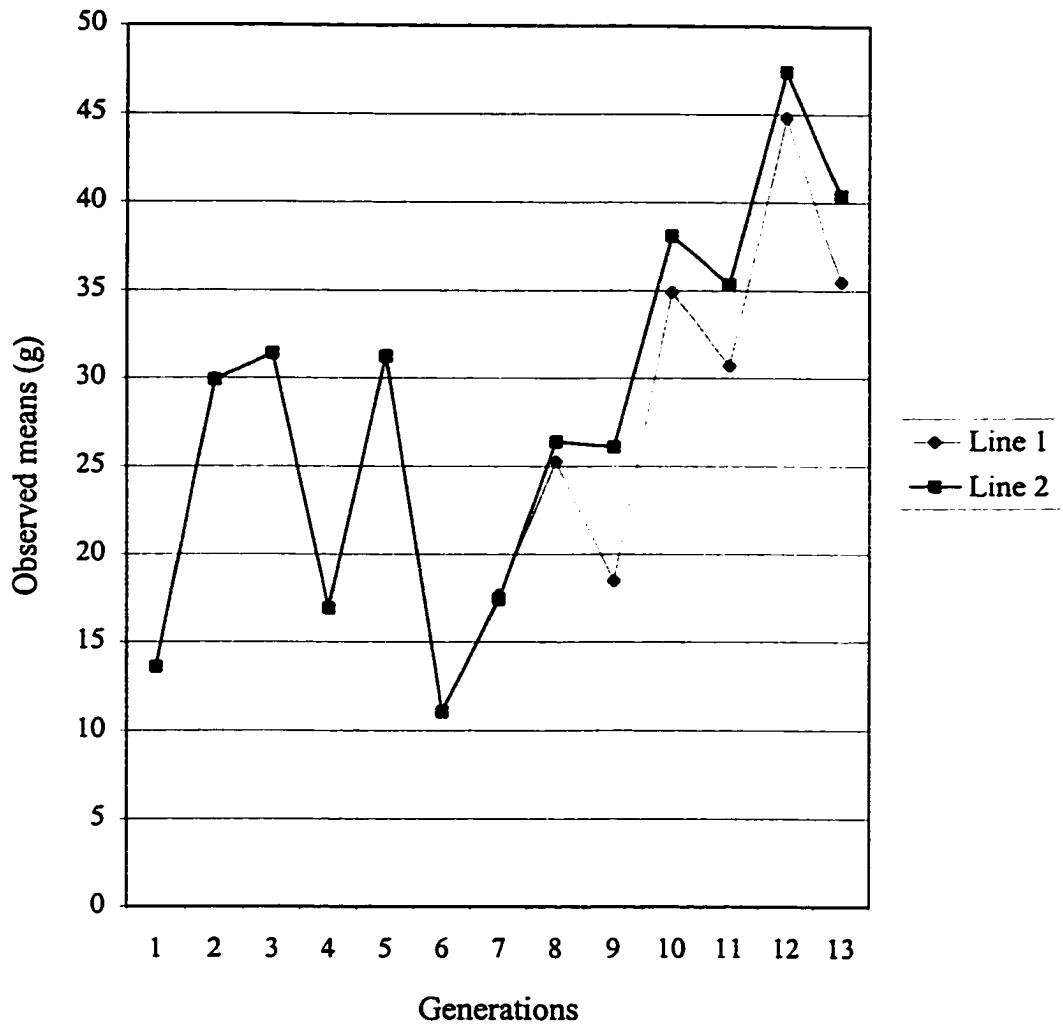


Figure 5.1 Observed means in the two selected lines of Nile tilapia.

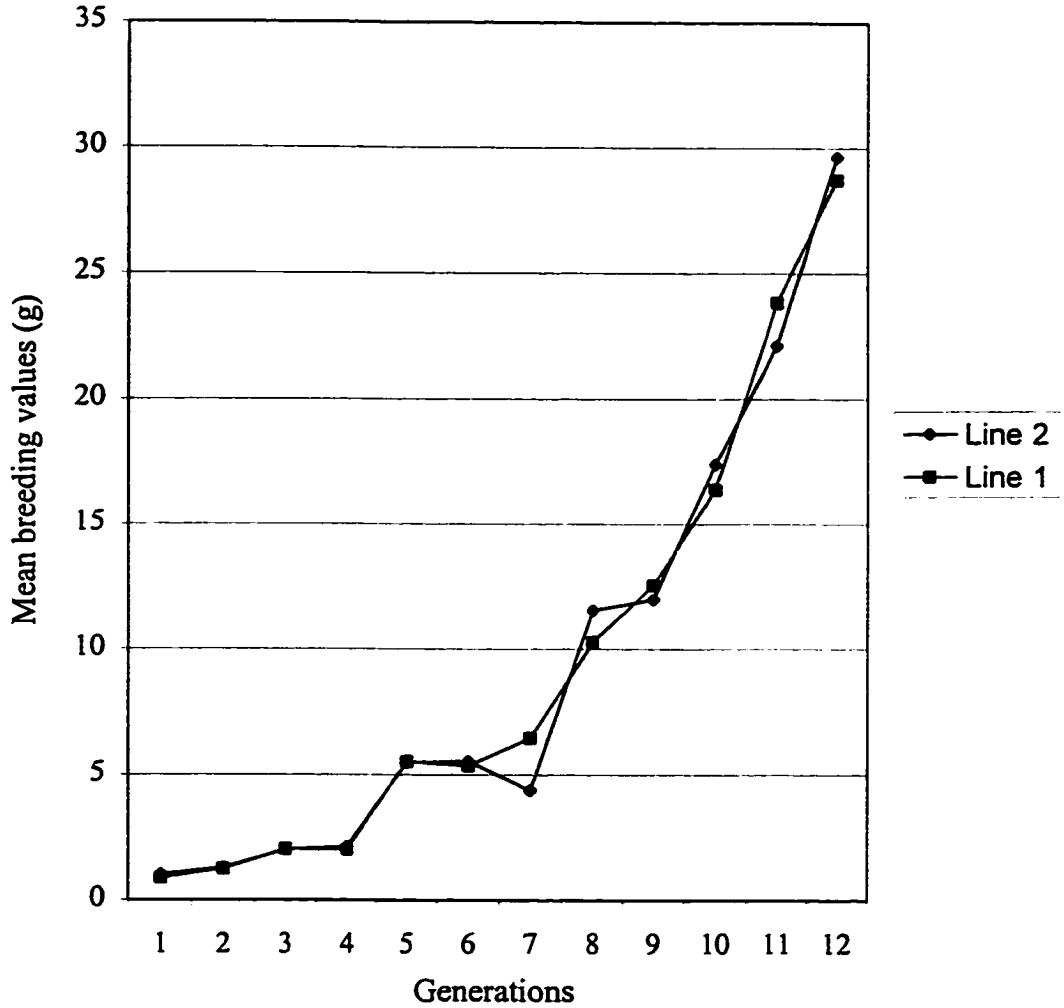


Figure 5.2 Mean breeding values in the two selected lines of Nile tilapia

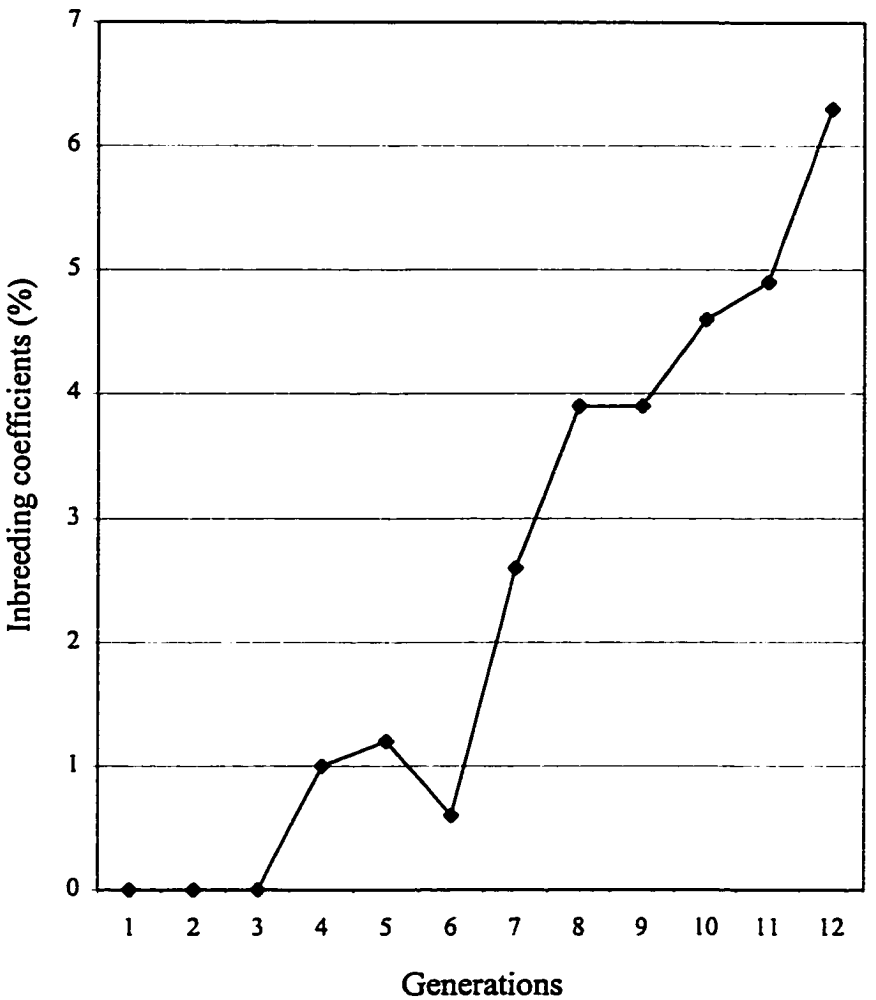


Figure 5.3 Inbreeding coefficients in 12 generations of selection in Nile tilapia.

Chapter 6

GENERAL DISCUSSION AND CONCLUSIONS

The general objective of this thesis was to evaluate a selection method suitable for small-scale genetic improvement programs. Within-family selection was applied to improve the growth of locally adapted strains of Nile tilapia (*Oreochromis niloticus*). The specific objectives were: 1) to quantify the response to selection for growth rate of Nile tilapia in tanks, hapas, and ponds; 2) to evaluate the effect of communal and separate rearing in hapas and ponds on the response to selection; 3) to determine genotype-environment interactions, and 4) to determine response to selection through the analysis of the accumulated data from within-family selection experiments using a single-trait animal model.

Within-family selection

In search for a proper selection method that can be used for tilapia genetic improvement, especially under condition of limited facilities, Uraiwan and Doyle (1986) have found that within-family selection method would be suitable to improve the performance of Nile tilapia. The rationale behind the application of this selection approach is that it removes the environmental variance due to maternal effects and other environmental causes (e.g., climate, water quality, nutrition), permits high selection intensities, minimizes inbreeding, eliminates the extensive need for individual tagging, and reduces the demand for facilities (Uraiwan and Doyle, 1986).

In the present study, the within-family selection method was used to increase body weight at 16 weeks. The selection was done in tanks to provide more control of the matings and easier monitoring of fish growth. In the propagation of the selected families, the rotational mating scheme avoided significant inbreeding for 5 generations of the 19 families. This mating scheme has proven to be extremely easy to manage, especially in association with the within-family selection scheme where the complete pedigree was maintained.

Selection response

Twelve selected generations have been produced and results of the different performance testing in tanks, hapas, and ponds indicated that the within-family selection method was effective in improving the growth of Nile tilapia. The study also showed that the current commercial strain of Nile tilapia (Israel strain) was an inferior strain compared with the genetically improved strains (SEL, GMT, and GIFT).

The aim of using the animal model methodology from the data derived from within-family selection was to verify the response to selection and estimate the genetic parameters in the base population. The heritability estimated from this method indicated substantial genetic variation prior to selection. The genetic trend shows a steady progress of the selection response up to generation 12. There is no comparable study that indicates a long-term selection experiment in tilapia which used within-family selection and where substantial response have been reported such as in the present study.

Based on genetic theory, the additive variance is expected to reduce to a steady state due to gametic phase disequilibrium. Increased in inbreeding will cause further the reduction in the genetic variance. In the tilapia population used in this selection experiment, the genetic variance appeared to have been maintained and inbreeding remained consistently low after 12 generations of selection. Only properly designed breeding programs can produce responses over a large number of generations without serious loss of additive genetic variation. This has been verified in long-term selection experiments and in several breeding programs in farm animals (Robertson, 1980).

Choice of selection methods

There are several breeding schemes that can be used to improve a fish population by genetic means. Selective breeding and hybridization are the two traditional approaches that have been used, and they have been used to improve all major crops and livestock grown to date. More recent techniques include chromosomal manipulation, production of sex-reversed brood stock, and genetic engineering. The decision to choose the most appropriate breeding program should carefully consider the biology of the species. Studies with tilapia have shown that individual selection to improve growth rate has been ineffective for a number of reasons. One of these is the inability to spawn tilapia synchronously. Research has also suggested that because tilapia spawns over a several-month period, within-family selection is the selective breeding program that is suitable to improve growth rate (Uraiwan and Doyle, 1986).

The choice of a selection procedure, particularly for tilapia aquaculture is a matter to be decided not only on genetic but also on economic grounds given the present scale of

the highly diverse and small-scale tilapia industry in Asia. On-farm selective breeding, using a simple, low-cost within-family selection method can be practiced by small-scale farmers to manage and improve their fish stocks (Brzeski and Doyle, 1995). This will empower farmers to use strains of their choice and to not be continually dependent on commercial hatcheries.

Communal versus separate rearing

Communal rearing method gave the same results as would be obtained under separate rearing although the magnitude of difference in growth performance among groups was different in separate and communal rearing. Contrary to the general idea that growth differences would be magnified under a competitive environment such as in communal rearing of different test groups, this study has shown that growth between groups was more magnified under separate rearing.

Communal rearing is now commonly used in tilapia performance testing. This would make testing programs less expensive as they require fewer resources. The non-significant test group by rearing method interaction suggest that communal rearing could provide an important impact for commercial production when farmers wants to compare alternative stocks.

Genotype-environment interactions

The genotype by environment interactions reported in the present study were mainly magnitude interactions. The test groups had improved response to the better environment in ponds, but the rank ordering remained the same in hapa as well as in pond environments.

This suggests that the selection decision that was made in the tank environment can yield selection response in other environments whether in a hapa or pond environment. It also implies that there is need to develop more than one strain under the range of environments that were used in this study.

Recommendation for future studies

The selected fish in the present study were more sensitive to environmental stress than the control populations. The genetic correlation between growth and survival rate needs investigation in the selected population. Although several studies have shown positive genetic correlation between growth and survival rate in salmonids (reviewed by Fjalested *et al.*, (1993), no comparable investigations can be found in tilapias. This is probably because in general, the tilapias are known for their tolerance to environmental stressors, including the presence of pathogens, hence survival rate has not been given special attention in tilapia genetic studies. However, with the intensification of culture for this species, high survival will become an important breeding goal in the future. It is not possible to record this trait with the selection method used in the present study. Other approaches need to be applied, e.g., family selection.

Another trait that would be worthwhile investigating is sexual maturation. The absence or very small number of reproductions observed in the different test environments indicates that reproductive traits such as age at spawning or fecundity in the selected Nile tilapia may have been affected. A correlated response to late maturation may be possible but it was not measured in the present study. This needs to be investigated in the selected lines of Nile tilapia

Practical implications

The outputs of this study were twofold: improved methodology for programs of fish selection under operating constraints and improved stocks of tilapia for use in small-scale aquaculture in the Philippines. The methodological work can have an impact on the design of selection experiments for tilapia in limited facilities while the adoption of the genetically improved stocks will increase the production of Nile tilapia without necessarily changing the level of management or farm input.

The present study has shown that mean growth performance of the selected females was comparable to an all-male tilapia population while the selected males were significantly heavier than the males from any of the test groups. The results of this study provide the possibility of complementing the YY male technology by using the selected fish to produce YY brood stocks. Another possibility is to use the selected females in the production of GMT fingerlings. The use of the selected fish in the application of YY-male technology or in sex reversal technology, can raise further the yield potential of Nile tilapia.

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APPENDICES

Appendix 1. Analysis of variance of final weight using the full model (1993 GxE).

Sources of variation	DF	MS	F-value	P > F
Env	1	77961.55	22.8	0.01
Group	2	3577.85	9.16	0.04
Env x Group	2	224.01	6.83	0.42
Rep	3	11137.86	4.13	0.12
Env x Rep	2	2217.27	63.32	0.001
Group x Rep	6	215.87	3.24	0.22
Env x Group x Rep	4	34.95	0.70	0.62
Sex	1	24192.30	14.55	0.07
Env x Sex	1	1050.33	22.10	0.04
Group x Sex	2	31.99	0.40	0.71
Env x Group x Sex	2	47.51	0.95	0.46
Rep x Sex	3	558.70	6.97	0.02
Env x Rep x Sex	2	196.61	3.95	0.11
Group x Rep x Sex	6	80.10	1.56	0.32
Env x Group x Rep x Sex	4	49.73	0.75	0.55

Env = Environment; Rep = Replicate

Appendix 2. Analysis of variance of final weight using the full model (1996 GxE).

Sources of variation	DF	MS	F-value	P > F
Env	2	1532449.86	19.88	0.01
Group	2	172165.40	12.39	0.01
Env x Group	4	11798.54	14.63	0.001
Rep	9	7619.54	0.73	0.67
Env x Rep	9	10968.77	12.66	0.001
Group x Rep	18	493.39	0.69	0.75
Env x Group x Rep	32	815.76	1.14	0.35
Sex	1	511004.81	8.84	0.08
Env x Sex	2	56134.50	74.17	0.001
Group x Sex	2	3171.89	5.31	0.01
Env x Group x Sex	4	719.66	1.02	0.41
Rep x Sex	9	498.43	0.76	0.65
Env x Rep x Sex	16	763.22	1.07	0.41
Group x Rep x Sex	18	602.49	0.85	0.63
Env x Group x Rep x Sex	32	711.60	1.76	0.01

Env = Environment; Rep = Replicate

Appendix 3. Analysis of variance of final weight using the full model (1997 GxE).

Sources of variation	DF	MS	F-value	P > F
Env	1	9986.16	1.80	0.30
Group	2	26360.20	10.28	0.04
Env x Group	2	580.65	3.19	0.71
Rep	9	2875.95	1.20	0.39
Env x Rep	7	1928.15	7.50	0.23
Group x Rep	18	463.12	106.64	0.90
Env x Group x Rep	14	181.77	0.38	0.96
Sex	1	81006.96	14.37	0.07
Env x Sex	1	3763.94	7.13	0.03
Group x Sex	2	19.84.26	6.70	0.01
Env x Group x Sex	2	183.73	0.38	0.68
Rep x Sex	9	583.74	1.62	0.41
Env x Rep x Sex	7	529.20	1.17	0.37
Group x Rep x Sex	18	292.55	0.62	0.82
Env x Group x Rep x Sex	14	480.18	2.88	0.001

Env = Environment; Rep = Replicate

Appendix 4 Analysis of variance of final weight in communal and separate rearing using the full model (Hapa Experiment).

Sources of variation	DF	MS	F-value	P > F
Type	1	53297.60	10.27	0.05
Group	2	10830.12	17.34	0.01
Type x Group	2	1013.26	1.43	0.30
Rep	3	7019.96	1.23	0.40
Type x Rep	3	5194.49	7.36	0.02
Group x Rep	6	625.63	0.88	0.55
Type x Group x Rep	6	707.50	9.32	0.01
Sex	1	26718.89	40.80	0.01
Type x Sex	1	304.48	4.01	0.09
Group x Sex	2	308.48	4.06	0.07
Type x Group x Sex	2	55.36	0.73	0.52
Rep x Sex	3	655.39	8.63	0.01
Type x Rep x Sex	3	249.97	3.29	0.10
Group x Rep x Sex	6	41.21	0.54	0.76
Type x Group x Rep x Sex	6	75.90	1.15	0.33

Rep = Replicate

Appendix 5 Analysis of variance of final weight in communal and separate rearing using the full model (Pond Experiment).

Sources of variation	DF	MS	F-value	P > F
Type	1	4591.70	1.00	0.42
Group	2	15797.83	3.40	0.14
Type x Group	2	5577.15	1.22	0.38
Rep	2	4499.04	1.00	0.58
Type x Rep	2	4568.94	1.00	0.44
Group x Rep	4	4654.05	1.02	0.49
Type x Group x Rep	4	4562.86	15.20	0.01
Sex	1	42924.52	320.65	0.01
Type x Sex	1	94.48	0.31	0.60
Group x Sex	2	88.70	0.29	0.75
Type x Group x Sex	2	21.17	0.07	0.93
Rep x Sex	2	133.88	0.44	0.66
Type x Rep x Sex	2	322.12	1.07	0.42
Group x Rep x Sex	4	276.54	0.92	0.53
Type x Group x Rep x Sex	4	300.04	3.30	0.01

Rep = Replicate

Appendix 6 Analysis of variance of final weight using the full model according to type of rearing (Communal Rearing).

Sources of variation	DF	MS	F-value	Prob > F
Env	1	69431.60	20.11	0.02
Group	2	2893.77	7.81	0.05
Env x Group	2	208.51	3.12	0.22
Rep	3	10289.28	4.67	0.15
Env x Rep	2	2190.58	33.45	0.01
Group x Rep	6	202.89	1.76	0.26
Env x Group x Rep	4	65.47	1.90	0.27
Sex	1	22954.80	17.17	0.13
Env x Sex	1	1156.20	32.25	0.03
Group x Sex	2	89.10	1.00	0.46
Env x Group x Sex	2	35.81	1.03	0.43
Rep x Sex	3	542.37	6.31	0.03
Env x Rep x Sex	2	146.42	4.24	0.10
Group x Rep x Sex	6	85.96	2.38	0.18
Env x Group x Rep x Sex	4	34.40	0.53	0.70

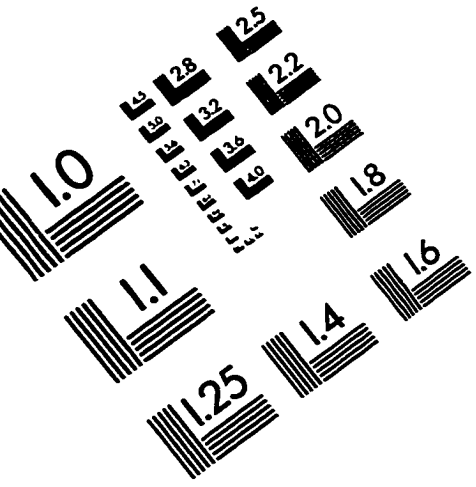
Env = Environment; Rep = Replicate

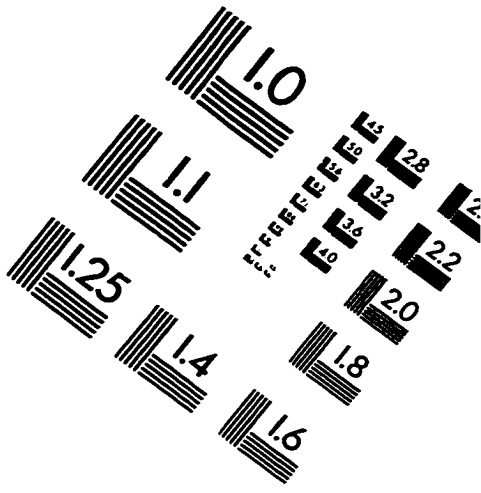
Appendix 7 Analysis of variance of final weight using the full model according to type of rearing (Separate Rearing).

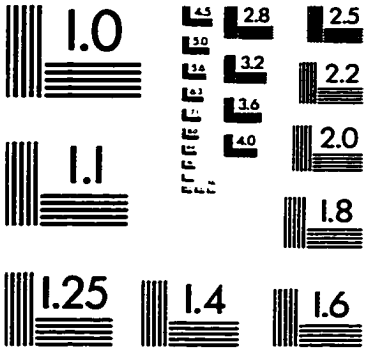
Sources of variation	DF	MS	F-value	Prob > F
Env	1	33973.51	12.05	0.00
Group	2	33115.35	67.70	0.00
Env x Group	2	1104.95	0.16	0.85
Rep	3	3610.89	3.64	0.90
Env x Rep	2	1947.36	0.28	0.76
Group x Rep	6	6416.37	0.84	0.59
Env x Group x Rep	4	6826.44	20.67	0.01
Sex	1	50855.41	40.20	0.07
Env x Sex	1	1053.56	8.10	0.10
Group x Sex	2	266.40	3.80	0.83
Env x Group x Sex	2	130.06	0.39	0.69
Rep x Sex	3	146.26	0.46	0.72
Env x Rep x Sex	2	582.93	1.77	0.28
Group x Rep x Sex	6	320.58	0.89	0.57
Env x Group x Rep x Sex	4	330.16	4.09	0.01

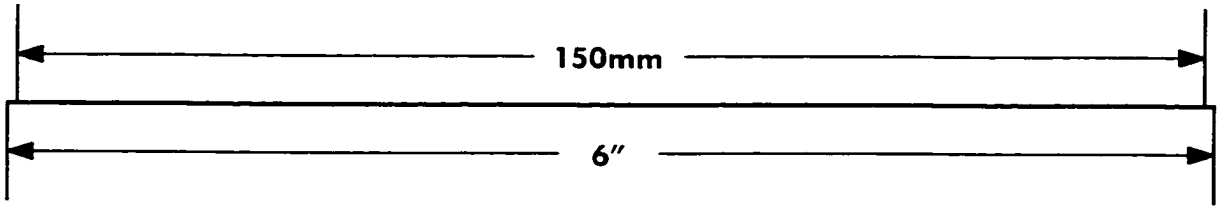
Env = Environment; Rep = Replicate

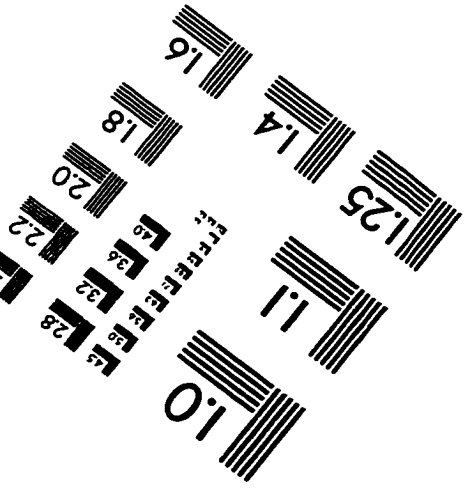
IMAGE EVALUATION TEST TARGET (QA-3)

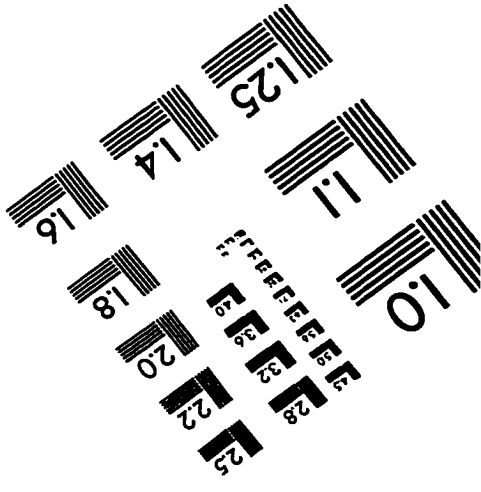












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