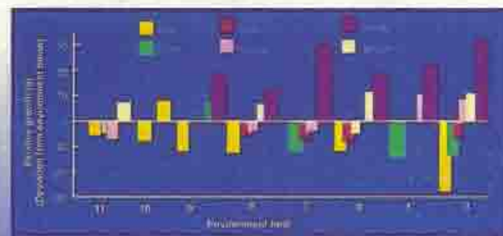
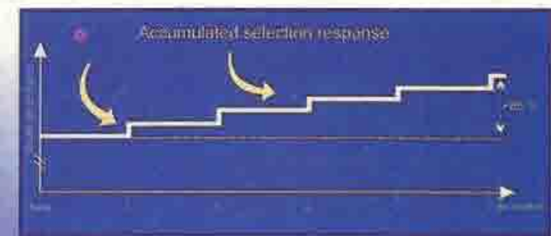
GENETIC IMPROVEMENT OF FARMED TILAPIAS (GIFT)

FINAL REPORT

(March 1988 to December 1997)



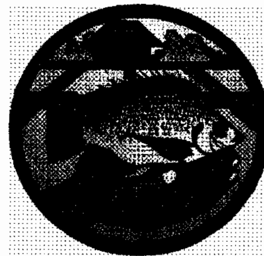


United Nations
Development Programme
Project No. GLO/90/016

International Center for Living Aquatic Resources Management

United Nations Development Programme
Sustainable Energy and Environment Division
Project No. GLO/90/016

GENETIC IMPROVEMENT OF FARMED TILAPIAS (GIFT) PROJECT



FINAL REPORT

March 1988 to December 1997

A. E. Eknath

Part 2

August 1998

ICLARM
International Center for Living Aquatic Resources Management
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LIST OF ATTACHMENTS IN GIFT PROJECT FINAL REPORT PART 2

1. Published manuscripts on standardization of the procedures.
2. Published manuscripts on the Project Activity "Genetic characterization of tilapia".
3. Protocol for maintenance of live genebank.
4. Published manuscripts on the Project Activity "Evaluation of culture performance of the eight strains in a wide range of farming systems under various agro-climatic conditions" (Generation 1 experiment).
5. Published manuscripts on the Project Activity "Estimation of the magnitude of non-additive genetic effects (heterosis) and thereby determine the breeding strategy: pure breeding and crossbreeding (Generation 2 experiment).
6. Estimated heritabilities within each environment, and across all environments, during each generation.
7. Manuscript (accepted for publication) on "Response to bi-directional selection for frequency of early maturing females in Nile tilapia".
8. Manuscript on: (a) Genetic variation in lysozyme and spontaneous haemolytic activities of blood serum from Nile tilapia; (b) Genetic associations of serum lysozyme and serum spontaneous haemolytic activities with survival and body weight in Nile tilapia.
9. Draft manuscript on "Combined effects of genetic improvement through selection and sex reversal on growth and survival of Nile tilapia (*Oreochromis niloticus*)".
10. Complementary studies published under FAC/CLSU research component of GIFT.
11. Studies and Reviews on "Genetics of Parasites and Diseases by T. Gjedrem.
12. Outline of guiding principles for conducting the "Research and development of salt tolerant Nile tilapia or for any aquaculture development in brackish/marine waters in general".
13. A compilation of Abstracts from Master of Science Theses.
14. Breeding Plans for Nile tilapia, Mrigal and Silver barb.
15. Record of visitors received by the GIFT Project.
16. Selected media coverage on GIFT.
17. List of meetings attended/papers presented by GIFT Staff.

Gift
GIFT Project
04 May 1997

ATTACHMENTS

Attachment 1.

Published manuscripts on standardization of the procedures

The Evolving Role of SQL in Genetics, Breeding and Selection Work*

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ABSTRACT

The Genetics Improvement of Farmed Tilapias (GIFT) project utilizes procedure SQL of the SAS System to manage, store and cross-correlate multiple sets of data generated from successive generations of selective breeding. Practical queries developed in SAS/SQL® will be presented and compared with traditional DATA and PROC steps. Implications of using Structured Query Language to the current selection and breeding work of the project will be discussed.

INTRODUCTION

The Genetic Improvement of Farmed Tilapias (GIFT) executed by the International Center for Living Aquatic Resources Management (ICLARM) is a collaborative research project co-financed by the Asian Development Bank (ADB) and the United Nations Development Programme/Division for Global and Interregional Programmes (UNDP/DGIP). The project began in April 1988 and will continue up to 1997 with support from UNDP/DGIP. Project collaborators are the National Freshwater Fisheries Technology Research Center of the Bureau of Fisheries and Aquatic Resources (NFFTRC/BFAR), the Freshwater Aquaculture Center of the Central Luzon State University (FAC/CLSU), the Marine Science Institute of the University of the Philippines (UP/MSI) and the Institute of Aquaculture Research (AKVAFORSK, Norway). The project is based in the Philippines with global research linkages.

The project focus is on Nile Tilapia (*Oreochromis niloticus*), an important fish species in small-scale aquaculture for many resource-poor farmers. The research thrust is to develop methodologies and policy guidelines that can be applied or adapted to a wide range of finfish species in national fish genetic improvement programs, notably carps and other indigenous species.

At the start of the project, breeders from 8 strains of Nile tilapia were collected, and about 10,000 tagged, purebred offspring were stocked for individual performance recording. The pure strains were then crossed to produce second generation of about 20,000 tagged and performance recorded crossbred offspring of all strain combinations. From the third generation onwards, the best performing breeders from the previous generation have been used as parents to produce about 20,000 individually tagged and recorded offspring of mixed origin in each generation.

A major accomplishment of the GIFT project is an improved synthetic strain of *O. niloticus*. Developed within four years of extensive experimentation, this strain had outperformed the most widely cultured strain in the Philippines by 60% in terms of growth and survival. A large, unique database on tropical aquaculture genetics has also evolved. This database records ancestry, survival or mortality, reproduction, hatching and growth performances of *O. niloticus*.

Objectives

The genetic information that this large database provides also creates new constraints on information access and management (e.g., storage and personnel requirements), hence the project developed SAS/SQL® applications as a provision to cross-correlate information generated across generations. This paper aims to present some of these practical queries and demonstrate solutions to database management problems.

The GIFT Database and Problems

The GIFT database is a compilation of different genetic data gathered from a series of carefully designed experiments conducted by the project since 1988. It includes data generated from systematic evaluation of different strains in diverse farming systems through the various stages of strategic decisions reached (such as selection or crossbreeding) to the development of a continuous genetic improvement program. It also consists of data gathered from on-farm trials in cooperation with small-scale resource poor farmers - the beneficiaries of the project.

In order for the GIFT data to be useful, the following provisions were set up :

a) mechanisms for organizing and storing the data;
b) ways of finding and retrieving the data; and
c) mechanisms for cross-correlating the information to be retrieved and used later.

However, in setting up the above provisions, problems on software, hardware and application level likewise existed.

Alternatives to Problems

The first attempt to the problem was choosing a database management software. Some issues considered were: compatibility issues underlying a platform operating system; and "multiple system architecture" - supporting older and "other" operating system releases.

The second attempt was selecting an appropriate database structure, appropriate to the overall application needs of the project. Selecting a database structure involved not only design decisions, but also management considerations as to the relationship between flexibility, sophistication and response time of retrieval and the cost of building an appropriate database that meets stated retrieval requirements.

The best and most suitable database structure to the GIFT database is relational architecture.

A relation is a mathematical concept that is similar to that of a set. Relations are represented physically as two-dimensional tables arranged in rows and columns. The relational theory was originally developed by E. F. Codd (SAS/SQL® User's Guide), an IBM researcher. This architecture allowed the use of relationships defined in database tables without requiring the user to understand how data are physically stored and managed.

ICLARM Contribution No. 916. Paper presented at the 18th SAS User Group International Conference (SUGI 18), New York, New York, USA, 9-12 May 1993.

The relational theory of E. F. Codd gave rise to ANSI's Standard Structured Query Language or SQL (SAS/SQL® User's Guide). This is a universal language that was developed to access, extract or update data stored in relational databases or tables coming from multiple sources (SAS 1989).

The recent Release 6.06 of the SAS System on OS/2 was chosen as the project team's standard software to handle database management and analysis. The SAS System provided powerful tools in database management, implementing SQL.

Procedure SQL of the SAS System can give control on data on three levels:

a) extract value;
b) create or delete tables and views; and
c) modify or delete parts of the database.

The SQL procedure can be used interactively during a SAS session or within batch programs. The above features and relevance of SQL will be examined in performing queries.

There are three possible ways of performing a query in the SAS System: a) using traditional DATA and PROC steps; b) using the SQL procedure; and c) a combination of traditional steps and SQL.

Examples of Queries

Example 1:

Tracing the ancestors of selected breeders is one of the interesting queries performed by the project. This process enables detection of inbreeding and gene frequency count of the current stock. If breeders had evolved after four cycles, these have descended from 16 purebred ancestors in the first generation, i.e., this fish has parents, grandparents, great grandparents and great great grandparents which can be extracted from GENE4, GENE3, GENE2 and GENE1 files respectively. Figure 1 illustrates the hierarchical structure of the ancestry query.

The query extracts parameters from three different files. The major pivot or reference is the variable *tagno* of SELECT.SSD. This reference variable is the breeder which the ancestry is being traced. This is related to *sire_c* and *dam_c* of GENE4 - parent file. The parent crosses of *sire_c* and *dam_c* can be found on the next file GENE3 - grandparent file known as *mcross* and *fcross*. To distinguish between parent of *sire_c* and *dam_c*, aliases are used for *mcross* and *fcross*. The *grandf_s* and *grandm_s* are aliases of parents of *sire_c* while *grandf_d* and *grandm_d* are aliases of parents of *dam_c*. Note that the link between different files is reflected by the WHERE statement. The ORDER statement sorts output to screen in ascending order by *tagno* of SELECT.SSD. Figure 2 shows the linked file.

Using SQL Procedure to perform the query:

```
proc sql;
    select a.tagno, b.sire_c, b.dam_c,
           c.mcross as grandf_s, c.fcross as
           grandm_s, d.mcross as grandf_d,
           d.fcross as grandm_d
    from in.select as a, in.gene4 as b,
           in.gene3 as c, in.gene3 as d
    where (a.tagno = b.tagno) &
           (b.sire_c = c.tagno) &
           (b.dam_c = d.tagno)
    order by 1;
```

The query output (Fig. 2) shows that of the eight breeders, six share common male cross or sire, implying these fishes (F001 & F002; F003 & F004; F005 & F006) are half-sibs. Traced parents of sires further show they had emerged from a single set of parents (A1A4-108 x A3A3-341), hence the sires are full-sibs at the third cycle experiment. The female cross of breeders or dam on the other hand show, six of them are full-sibs (F002 & F003; F006 & F007; F001 & F004) at the third cycle experiment.

Requirement in linking through procedure SQL:

The method of joining data from multiple sources to produce a final data set requires a common variable or an "identifier" between data sets. The "merge" performed in SQL can be performed using the SELECT statement to reference two or more tables. The controlling factor is one or more key variables performed by the WHERE statement. This "identifier" variable for each file need not be named similarly.

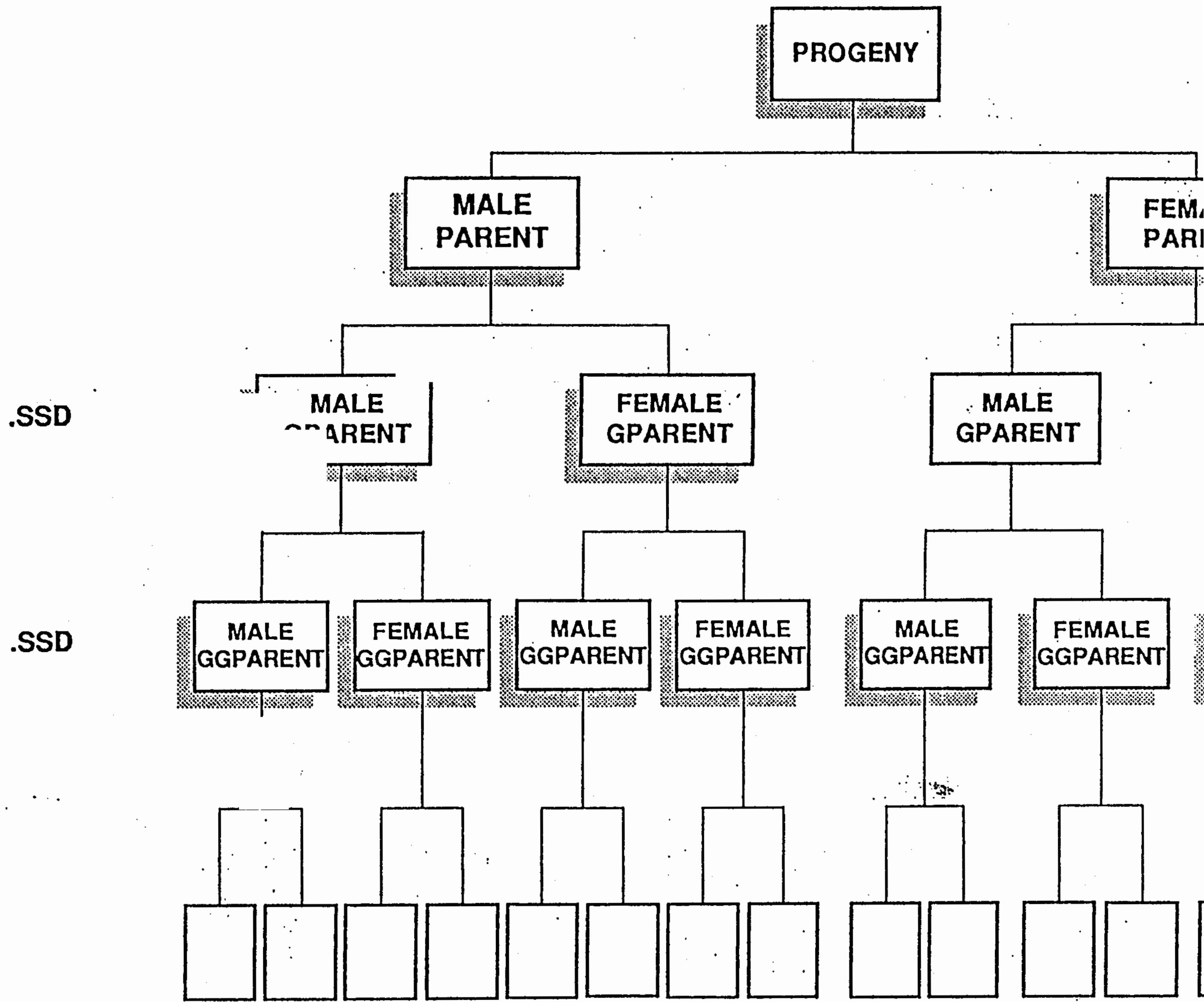
Correspondingly, the traditional step is capable of performing the above query.

```
proc sort data=in.breed out=sel; by tagno;
proc sort data=in.gene4(keep=tagno sire_c
   dam_c) out=g4; by tagno;
data all; merge sel g4; by tagno;
  if bv=.then delete; rename
   tagno=famno;
data all1; set all; tagno=sire_c;
proc sort; by tagno;
proc sort data=in.gene3(keep=tagno mcross
   fcross) out=g3; by tagno;
data all2; merge g3 all1; by tagno;
   if bv=.then delete;
data all3; set all2(drop=tagno);
   tagno=dam_c;
proc sort data=all3; by tagno;
data g3_dam; set g3;
   rename mcross=mcr_dam fcross=fcr_dam;
data all4; merge g3_dam all3; by tagno;
  if bv=.then delete;
data all6; set all4(drop=tagno);
   rename famno=tagno;
proc sort;by tagno;
```

BREED and GENE4 files were sorted using *tagno* as the identifier. Only required variables were kept in GENE4 (step 1). The two files (SEL.SSD and G4.SSD) were merged to create a new file (ALL.SSD) and observations with missing *bv* values were deleted (step 2). Deletion often happens when there is no one-to-one match between two or more files being merged. The identifier *tagno* was renamed in this file as *famno*. The next file created (ALL1.SSD) was a duplication of ALL.SSD except that a new column, *tagno*, was created based from *sire_c*. This file was sorted by *tagno* (step 3). The purpose is to search for the parents of *sire_c* from GENE3 file. Step 4 involves sorting GENE3.SSD by *tagno*, keeping only the crosses (parents) of each *tagno* and creating a temporary output data set called G3.SSD. Step 5 onwards is a repetition from step 2 to 4 to get the parents of *dam_c*. Note that a series of file creation was done to rename the parameter to label the parents properly.

Requirements in linking using traditional DATA and PROC steps:

There are many ways to link or join data coming from multiple sources using the traditional programming steps. Like SQL, this procedure also requires a key "identifier", and data must



(B1. SSD) from G4 file (parents) to G1 file (great-great grandparents).

Figure 2. Output of query 1 - tracing ancestry of selected breeders.

① TAG NUMBER INDICATING FAMILY NUMBER	② TAG CODE FOR SIRE	③ TAG CODE FOR DAM	④ GRANDF_S	⑤ GRANDM_S	⑥ GRANDF_D	⑦ GRANDM_D
F001-001	AA1-102	H3-091	A1A4-108	A3A3-341	A1A3-146	A4P4-231
F002-002	AA1-102	Q1-052	A1A4-108	A3A3-341	A1P4-095	A3A3-110
F003-003	AA1-113	Q1-024	A1A4-108	A3A3-341	A1P4-095	A3A3-110
F004-004	AA1-113	H3-096	A1A4-108	A3A3-341	A1A3-146	A4P4-231
F005-005	AA1-110	H2-081	A1A4-108	A3A3-341	A1A3-146	A4P2-132
F006-006	AA1-110	MM3-116	A1A4-108	A3A3-341	A3P1-084	A1A4-081
F007-007	AA1-103	MM3-087	A1A4-108	A3A3-341	A3P1-084	A1A4-081
F009-009	AA1-035	N3-030	A1A4-108	A3A3-341	A3P4-095	A1A4-136

① G5 fish = Extracted from select data
② Male parent of G5 fish
③ Female parent of G5 fish } Extracted from GENE4 data
④ Male parent of G4 male fish
⑤ Female parent of G4 male fish } Extracted from GENE3 data
⑥ Male parent of G4 female fish
⑦ Female parent of G4 female fish } Extracted from GENE3 data

be sorted or indexed by this key. Thus, if the column name (to act as "identifier") did not match the column name of the other file, renaming column names is a pre-requisite before sorting. If the files involved were not standardized, manipulations will be time consuming and extra effort is needed to understand and re-structure the data.

Example 2:

Queries are also performed during multivariate analysis that require parameters coming from different sources, such as extracting growth performance parameter from each generation for further genetic analysis, e.g., estimation of heritability by offspring-parent regression. The common practice is to query using the traditional DATA and PROC steps and store permanently the resulting data set.

Instead of using multiple DATA and PROC steps to link data sets by common variables each time a similar analysis is required, CREATE VIEW or TABLE can be performed. The former technique saves disk storage because instead of storing the merged or linked file, a VIEW definition is saved instead of actual data. The later keeps the file as a SAS file either permanently or temporarily. The following query extracts body weights (day 0 and day final) of breeders' and body weights of parents and grandparents on 90 day rearing.

The above query is similar to query #1 except that this time growth parameters are being extracted for analysis. The initial and final weights of breeders were extracted together with the final body weights of its parents. There is the option to store the resulting table permanently or simply store temporarily or permanently the query definition. Later procedures such as PROC REG can access the VIEW definition.

Using the procedure SQL:

```
proc sql;
   /* if you want to create permanent SAS
        table */
   create tables libref.multivar
   /* or if you simply want to store the
        definition and access later in
        subsequent SAS procedures
   create VIEW libref.multview as */
   select a.tagno, a.bdwt90 as fin_wt,
        a.bdwt0 as init_wt,
        b.bdwt90 as fin_sire,
        c.bdwt90 as fin_dam
   from in.select as a, in.gene4 as b,
        in.gene3 as c
   where (a.tagno = b.tagno) &
        (b.sire_c = c.tagno) &
        (b.dam_c=d.tagno)
   order by 1;
```

DISCUSSION

The attempt by the project team to implement relational structures for its databases facilitates better computing. It eliminates redundancy in storing similar information, standardizes data structures and increases efficiency in almost all aspects of computing work (storage, processing and access).

Using the traditional step, multiple joins can still be performed, but in a less sophisticated way. Codes developed in this method are hard to read and maintain. Researchers accessing the file need to understand history of file creation and its structures

and location. A great disadvantage of traditional step is felt during multivariate analysis. There is a tendency to store queries as permament files - this practice competes with the current disk space problems of the project and finally becomes an entry to the projects list of databases for maintenance.

SQL on the other hand can define, manipulate and control relational databases or tables. There is no need to specify the structure of the data, its location and/or its data type. One instead concentrates on what data to extract, rather on how to select them. Creating TABLES or VIEWS is a major advantage. Frequently used queries can be stored as a VIEW definition without the need of additional disk storage and maintenance. Since codes in SQL is straightforward, it is easier to maintain and even debug.

The main advantage of traditional step over SQL lies on hardware requirements. Traditional procedures do not require very powerful computer processors. No normalization technique during database design is needed. Using procedure SQL means increasing requirements of computer processing to provide better flexibility. Investment on hardware level is high to accommodate sophistication of the SQL procedure.

Although both methods can link the files, the CPU time for each approach did not vary much, but the traditional step in most cases will have a lower CPU requirement but will need more personnel development time. For neophytes of procedure SQL, the learning curve for SQL is not really steep, especially if one has an adequate background in SAS programming.

For research on genetic selection and establishment of breeding programs, performing queries through SQL will be cost-effective. It can yield significant savings in staff development time, data storage and maintainability of both database and source codes. It also facilitates rapid decision making.

The project hopes to implement SQL procedure in its SAS/AF® applications and to utilize fully SAS/SQL® combined with the flexibility of traditional steps in database management. A major on-going activity is the documentation of all available files and the marking of identifier variables to facilitate linking and joining datasets anytime by any interested user. VIEWS will be implemented throughout to allow easy access to a current information without increasing storage requirements. The project also hopes to utilize SAS/SQL® for its training activities.

AKNOWLEDGEMENTS

The authors would like to thank the GIFT project team staff for the motivation to develop this application. The comments of Dr. Roger S. V. Pullin, Mr. Jay Maclean and Hans Bentsen and to SAS Philippines Institute for supporting this application paper.

REFERENCES

SAS Institute Inc. (1989), *SAS Guide to the SQL Procedure.* SAS Institute, Cary, North Carolina.

TRADEMARKS

SAS, SAS/AF and SAS/SQL are trademarks of SAS Institute, Cary, North Carolina.

Asian Fisheries Science 7(1994):129-133.
Asian Fisheries Society, Manila, Philippines

Effect of Sampling Frequency on the Growth and Survival of Nile Tilapia *Oreochromis niloticus* in Hapas*

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Abstract

Five sampling frequencies: weekly (S1), every two weeks (S2), every three weeks (S3), monthly (S4) and initial and final sampling only (S0) were used to investigate the effect on growth and survival of Nile tilapia *Oreochromis niloticus*. The study was conducted in ten 1-m^3 hapas suspended in a 500-m^2 fertilized pond following routine sampling procedure over a 140-day period without supplementary feeding.

The highest mean body weight was observed in S0 (76.74 g) while the lowest in S1 (38.82 g). The observed differences on the mean final body weights were significant among treatments ($P<0.01$). A 25-50% growth depression of Nile tilapia was observed in this study. The stress-induced effects of frequent sampling resulted in slower growth of fish in hapas.

There were no significant differences on the number of fish surviving among treatments. The highest survival was 97% observed in S2, while the lowest, 65%, was obtained in S3. Significant variation in sex ratios was observed only in S1 and S3 ($P<0.05$). There were more females than males in these treatments, however, no differential effects of sampling frequency on the sexes were noted.

Introduction

Sampling of fish at periodic time intervals is useful in looking at trends on growth, maturity, reproduction or conditions of health under natural or culture environments. Sampling methods in experimental units like hapas or cages often require lifting the net, scooping out a certain number of fish and holding the fish out of the normal culture environment for a period of time until the fish are

*ICLARM Contribution No. 1063.

weighed and measured. In ponds, repeated seining is done to get the number of fish samples required from a population; while tanks or aquaria may need draining. While it is essential to obtain data for certain purposes, the stress-related effects of these procedures may affect growth rate and other traits in fish (Mazeaud et al. 1977) which may confound results of experiments.

There is lack of standardized methods for fish sampling in aquaculture experiments and no literature available indicating a standard time interval for monitoring growth of fish. In nutrition research, for instance, the sampling intervals used are variable.

This experiment was part of the pre-project studies to standardize methods under the collaborative research project on Genetic Improvement of Farmed Tilapias (GIFT). The objective of this study was to investigate the effect of sampling frequencies on growth and survival of *Oreochromis niloticus* in hapas.

Materials and Methods

Ten 1-m^3 net hapas were each stocked with a random sample of 30 fish from a full-sib family averaging 0.88 g in weight. The hapas were arranged in two columns (at five hapas per column) and installed in the middle of a 500-m^2 earthen pond. The pond was fertilized every two weeks using inorganic (16-20-0) and organic (chicken manure) fertilizers at the rate of 100 $kg \cdot ha^{-1}$ and 1 $t \cdot ha^{-1}$, respectively. No supplementary feeds were given. Five sampling frequencies were used: weekly (S1), every two weeks (S2), every three weeks (S3), monthly (S4) and initial and final sampling only (S0). Each treatment was replicated twice.

The routine sampling procedure included lifting the hapas, collecting all the fish, and putting them in aerated water. Anesthetic (Quinaldine) was used to minimize fish movement during measurement. Fish were blot-dried to remove excess water on the body before individual weights were recorded at every sampling period.

Final body weights and survival were analyzed according to the following generalized linear model (GLM):

$$Y_{ijkl} = a + F_i + R_j + S_k + eijkl \qquad \text{...model 1}$$

where:

- Y_{ijk} is the final body weight/survival
- a is a constant
- F_i is the effect of the ith sampling frequency
- R_j is the effect of the jth replication
- S_k is the effect of kth sex
- eijkl is the random error.

For analysis within sexes, S_k was deleted from the above model (model 2). Differences in sex ratio were analyzed using the chi-square test.

Results

Survival and Sex Ratios

There were no significant differences in the number of fish surviving among treatments. The highest survival was 97% observed in S2; the lowest, 65%, was obtained in S3, while the rest gave 95% survival (Table 1). The low survival in S3 was due to the accidental escape of some fish in one of the replicate hapas towards the end of the experiment. The male to female ratio differed significantly from 1:1 only in S1 and S3 (P<0.05). The sex ratio in S2, S4 and S0 were not significantly different from 1:1.

Table 1. Percentage overall survival, males and females and sex ratio of Nile tilapia *Oreochromis niloticus* in five sampling frequencies.

Treatments	Survival (%)			Sex ratio	
	Overall	Males	Females	Male	: Female
S1	95	35	65	1.00	: 1.85*
S2	97	55	45	1.23	: 1.00^{ns}
S3	65	31	69	1.00	: 2.25*
S4	95	53	47	1.11	: 1.00^{ns}
S0	95	51	49	1.03	: 1.00^{ns}

*Significantly different from 1:1 (P<0.05)
^{ns} Not significant

Growth

The least square means (LSM) of final body weights and body weights of males and females, according to models 1 and 2, are presented in Table 2. The marginal mean squares (MS) from the GLM model for various fixed effects are presented in Table 3. Except for the treatment x sex interaction effect, the MS for all other effects in the model were significant (P<0.01). This indicated no differential effects of sampling frequency on the sexes. The highest mean final body weight of fish was obtained in S0, and the lowest in S1. Males were significantly heavier than females in all the treatments. The trend in final body weights of males and females was similar to the overall mean body weights.

Table 2. Least square means and standard errors (in parentheses) of final body weight of Nile tilapia *Oreochromis niloticus* according to models 1 and 2 in five sampling frequencies. (Means in columns with the same superscript are not significantly different at P>0.05).

Treatments	Mean weight (g)		
	Overall	Males	Females
S1	38.82^{a} (1.45)	40.66^{a} (2.34)	36.99^{a} (1.72)
S2	50.46^{b} (1.38)	55.82^{bc} (1.86)	45.11^{b} (1.86)
S3	57.04^{c} (1.84)	56.99^{c} (3.03)	57.09^{cd} (2.05)
S4	56.87^{c} (1.39)	60.60^{cd} (1.92)	53.15^{d} (2.02)
S0	76.74^{d} (1.39)	81.72^{e} (1.98)	71.71^{e} (1.94)

Table 3. Degrees of freedom (df), marginal mean squares (MS) and F values for the fixed effects in the model.

Effects	df	MS	F Value
Treatments	4	10,411.3	94.5*
Replicates	1	1,513.6	13.7*
Sex	1	2,456.9	22.3*
Treatment * sex	4	223.6	2.0^{ns}

*Significant at P<0.01.
^{ns}Not significant.

Discussion

About 25-50% growth depression on Nile tilapia caused by frequent sampling was observed in this study. The greatest difference on final body weight was obtained between S1 and S0, and the least between S3 and S0. These marked differences on growth were probably caused by stress of handling which have physiological effects on fish (Miles et al. 1974; Mazeaud et al. 1977; Adedire and Oduleye 1983; Matty 1985; Kutty 1986). Another influence of stress is on tilapia nutrition. Fish (1960) as cited by Balarin (1979) reported that handling *Sarotherodon mossambicus* tended to reduce the acid concentration of the digestive juices. This would imply that stressed fish digest food less efficiently (Balarin 1979). Mabaye (1971), again as cited by Balarin (1979), did not note any significant difference between growth rates of 'handled' and 'non-handled' fish, although the former generally tended to have lower growth rates.

Fish sampling is essential in growth and nutrition studies which may require data on fish biomass at certain periods to determine growth curves or to adjust the amount of experimental feeds. A glance at 15 nutrition studies on tilapia in aquaria, tanks or cages in the literature showed weekly and every two weeks as the most common fish sampling intervals which means, based on the results of this study, a possible confounding effect between stress and the experimental diet on the growth rates of fish.

One limitation of this work was that the experiment was done in hapas. Fish sampling in ponds by seining to capture fish can be more stressful and complex because it tends to disturb the whole pond ecosystem. The sampling practice in ponds needs similar investigation.

Sampling every three weeks was adopted in the early stages of the GIFT project because more data points were needed to describe the growth performance of the different strains of tilapia. After this study, fish sampling was done at stocking and at harvest in subsequent growth testing.

Acknowledgments

This study forms part of the collaborative research project on Genetic Improvement of Farmed Tilapias (GIFT), co-financed by the Asian Development Bank (RETA 5279) and the United Nations Development Program/Division for

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References

Adedire, C.O. and S.O. Oduleye. 1983. Stress-induced water permeability changes in *Oreochromis niloticus*. Proceedings of the International Symposium on Tilapia in Aquaculture: 123-133.

Balarin, J.D. 1979. Tilapia: a guide to their biology and culture in Africa. University of Stirling. 38 pp.

Fish, G.R. 1960. The comparative activity of some digestive enzymes in the alimentary canal of tilapia and perch. Hydrobiologia 15:151-178.

Kutty, M.N. 1986. An analysis of factors affecting individual fish growth and pond fish production. Aquaculture research in the Africa region. Proceedings of the African Seminar on Aquaculture. International Foundation for Science, Pudoc Wageningen, Netherlands: 129-142.

Mabaye, A.B.E. 1971. Observation on the growth of *Tilapia mossambica* fed on artificial diets. Fisheries Research Bulletin Zambia 5:379-396.

Matty, A.J. 1985. Fish endocrinology. Billing & Sons Limited, Worcester. 267 pp.

Mazeaud, M.M., F. Mazeaud and E.M. Donaldson. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Transactions of the American Fisheries Society 106(3):201-212.

Miles, H.M., S.M. Loehner, D.T. Michaud and S.L. Salivar. 1974. Physiological responses of hatchery reared muskellunge (*Esox masquinongy*) to handling. Transactions of the American Fisheries Society 103:336-342.

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EVALUATION OF GROWTH PERFORMANCE TESTING METHODS FOR STRAIN COMPARISONS OF NILE TILAPIA (*Oreochromis niloticus*)*

Danting Ma. Jodecel, Ambekar E, Eknath
and Hans B, Bentsen

ABSTRACT

Different methods to compare groups of fish in aquaculture environments had been tested. Providing adequate replicates to minimize environmental variation is often a technical problem. The paper reports on strain comparison using communal testing, separate testing in replicates and testing with internal reference. Communal testing and separate testing are methods used for comparing groups in a common or in different unit respectively, while testing with internal reference involves conclusion of reference fish in a culture unit to provide internal statistical control.

The 7 strains of Nile tilapia (*Oreochromis niloticus*) used in the sutdy includes 3 African strains from Egypt, Ghana, Sénégal, and 4 cultured Asian farmed strains known in the Philippines as Israel, Singapore, Taiwan and Thailand. A pair-wise mating of breeding pairs from each strain was stocked. Swim-up fry were collected, separately for each strain until they reached taggable size of 3-5g. Red tilapia used as internal control was of the same age as the seven strains. Growth performance testing was carried out in 2m x 2m x 2m cages.

The results showed significant differences ($P<0.01$) in the growth performance of the seven strains when reared separately, communally in replicates and with internal control. There was no significant difference between replicates of separately or communally stocked test cages and in communally stocked test cage with internal reference fish. Including the internal reference affected the mean growth performance of the test fish when stocked separately. The ranking of the seven strains was relatively consistent in the different testing methods (Egypt and Sénégal strain as the best performing strain and Ghana/Singapore/Israel strain, poorest performing strains).

The implications of the results for resolving statistical problems created by replication are also discussed.

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* **Poster paper presented at the 5th International Symposium in Genetics in Aquaculture, June 19-25, 1994. Dalhousie University, Halifax Canada.**

Attachment 2.

Published manuscripts on the Project Activity

"Genetic Characterization of tilapia"

Use of RNA:DNA ratios as an index of nutritional status Of six Nile tilapia (*Oreochromis niloticus*) strains under different environments*

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Six strains of Nile tilapia reared under different culture conditions were assessed r their nutritional status using muscle RNA:DNA ratios. The six strains included four rains collected from Africa (Egypt, Ghana, Kenya and Senegal) and recently imported the Philippines and two Philippine farm strains known locally as the "Singapore" ıd "Taiwan" strains.

Individually tagged fish were raised in three test (farm) environments. Previous udies revealed significant differences in growth performance of these local strains in ›nds in three Philippine agroclimatic regions. Two test environments, with similar ›nd management practices, were considered as low growth (LG) and high growth IG) environments. The third test environment was a "stressful" low temperature (LT; 7-22°C) environment. A laboratory feeding experiments with fed and starved fish ·ovided controls for comparisons.

The mean RNA:DNA ratios for the groups were: control-fed fish 4.34, control-arved fish 3.32, LG fish 3.13, HG fish 3.45, and LT fish 1.48. The two Philippine rm strains did not survive in the LT environment. Although the RNA:DNA ratio for G was higher than for the other two environments, it was still lower than the control-d fish which were fed to satiation. The RNA:DNA ratio in LT was very much lower ıan the control-starved fish. A strong positive correlation was observed between ›ecific growth rate and RNA:DNA ratio ($r^2=0.9$, $P<0.001$). The results indicated ıat RNA:DNA ratio could be used to predict genetic potential for growth performance ;pecially in poor environments where gravimetric techniques failed to discriminate the :lative growth performance of different strains.

Source: Poster Session Abstract. Aquaculture 111 (1993) p. 295.

Genetic improvement of farmed tilapia: documentation and genetic characterization of strains*

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The genetic diversity of cultured Nile tilapia (*Oreochromis niloticus*) stocks in the Philippines is low and, consequently, may form a poor base for the development of a selective breeding program. A new population with a wider genetic base is being established in the Philippines by combining germplasm recently brought from Africa with local Philippine farm stocks. The four African stocks were from Egypt, Ghana, Kenya and Senegal; the four Philippine stocks are known locally as the "Israel", "Singapore", "Taiwan", and "Thailand" strains.

The stocks were characterized using morphometric and isozyme markers. All stocks shared alleles at 14 monomorphic and 16 variable loci. Observed heterozygosity ranged from 0.026 to 0.071. Among African stocks, characteristic allele frequency differences were observed at Ast-1, Adh, G3pdh-2, Mdh-1 and Sod loci. A dendogram constructed from Nei's Genetic Distrance (D) values revealed: (1) a cluster of the Philippine stocks with the Egypt and Ghana stocks; (2) a distinct separation of the Senegal stock; and (3) a larger separation of these two groups from the Kenya stock. The latter supports the recognition of the Kenya tilapias by Dr. E. Trewavas as a different subspecies (*O. n. vulvani*) from all others tested here (*O. n. niloticus*).

Principal component analysis was done on data from a truss network of 21 landmark points on the body outline. The first two principal components accounted for 91.5% of the variation (PCI, 89.5%; PC2, 2%). A plot of PCI against PC2 residuals separated out the Kenya strain, which has a relatively shorter and more streamlined midbody region than the other strains. Overall, however, the analysis indicated very few morphological differences among the eight strains.

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* **ICLARM Contribution No. 714.**
Source: Poster Session Abstract. Aquaculture 111 (1993) p. 296.

DOCUMENTATION OF GENETIC RESOURCES FOR AQUACULTURE - THE ROLE OF Fishbase*

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* ICLARM Contribution No 849.

ABSTRACT

Thorough documentation of the genetic resources of aquatic organisms is vital for their evaluation, conservation and utilization in aquaculture. The sustainability and future evolvability of aquaculture will depend upon wise management of such resources. Their documentation has been inadequate so far because of many problems: lack of accessible information on the origins and histories of farmed breeds and the status of wild populations; lack of standardized approaches and nomenclature; and lack of resources for the task, especially in developing countries. Fishbase, a large relational database on the biology of finfish, is now being developed at ICLARM in cooperation with the Food and Agriculture Organization of the United Nations (FAO) and with the support of the Commission of the European Communities (CEC). For aquaculture, Fishbase is beginning to document the differences among wild populations and farmed breeds and hybrids, including their growth, morphology and other performance traits in various culture systems and genetic characteristics such as biochemical differences and karyotypes. Fishbase provides data, descriptive text and color pictures of fish and maps showing their distribution. The paper discusses future directions for the documentation of genetic resources for aquaculture with reference to Fishbase and other activities.

INTRODUCTION

The need to maintain biodiversity for ecological, economical, and ethical reasons is now widely accepted. Biodiversity is a keyterm in IUCN's strategic document Global Biodiversity Strategy (Anon. 1992) and was, after "global warming", the second main topic on the agenda of the United Nations Conference on Environment and Development (UNCED) in Rio de Janerio. IUCN points out that "the key to conserving genes, species, and ecosystems is by increasing our knowledge of biodiversity".

The conservation of fish genetic resources is essential to provide genetic material for sustainable food production. There is an increasing awareness of the need to understand the genetic variability of wild and cultured fish populations for the future management of the genetic resources. For the maintenance, utilization and evaluation of these genetic resources one such priority is thorough documentation (Pullin 1988).

Presently, much of the data remains scattered in unpublished field notes and reports and in languages that constrain its accessibility and use. Moreover, relevant information in textbooks and scientific papers is often inconsistent in it usage of units and terminology. Collating and proper categorization of such information is the main task of Fishbase, a large relational database under development at the International Center for Living Aquatic Resources Management (ICLARM), Manila in cooperation with the Food and Agriculture Organization of the United Nations (FAO) and with the support of the Commission of the European Communities (CEC) (Froese 1990; Pauly and Froese 1991; Palomares *et al*, 1991). Fishbase provides global information on fish in a standardized form, to be made available to institutions worldwide, particularly those in developing countries.

This paper describes the role of Fishbase in documenting information on fish genetic resources.

GENERAL CONSIDERATIONS

Documenting key information on the biological diversity of the 24,000 known species of fish is a major challenge. Fishbase is beginning with the objective of documenting those species of

Source: p.63-68. In D. Penman, N. Roongratri and B. McAndrew (eds.) International Workshop on Genetics in Aquaculture and Fisheries Management. ASEAN-EEC ADCP, Bangkok, Thailand.

major importance in fisheries and aquaculture as part of its first phase of development. Fishbase compiles key information on nomenclature, morphology, physiology, genetics, ecology, population dynamics, reproduction, diseases, and conservation status. This information is linked with the occurrence of the species in the wild or in captivity.

The best way to protect wild fish germplasm is to maintain the original habitats (Pullin, 1990). ICLARM and its collaborators intend to collect and maintain (as live fish and cryopreserved spermatozoa) representatives of some of the wild stocks of finfish that are important for aquaculture, as an insurance against their extinction or genetic change from human interventions such as escapes from aquaculture or releases for fisheries enhancement. However, *ex situ* preservation cannot completely replace protection of original gene pools in natural habitats. Captive fish populations will also undergo genetic change therefore ICLARM and its collaborators will also attempt to contribute to the conservation of fish genetic resources by disseminating information on fish biodiversity to researchers and conservation managers, particularly in developing countries.

One important aspect of documenting fish genetic resources is to quantify their distribution and biological characteristics. For example, Fishbase will eventually contain an inventory of all recorded collections of Nile tilapia (*Oreochromis niloticus*) throughout its native range. Colour pictures of fish and maps will be provided showing its distribution. Compiling such information for a large number of species can only be done by integrating information from a variety of sources such as the scientific literature, research vessel and other fishery records, museum catalogues, and other verified records such as those from angling and the literature for aquarium hobbyists. Many of these data already exist in database form and Fishbase has successfully tapped several of these sources. Examples are the catalogue of fishes from the Museum of Natural History, Paris with information on size, locality, depth and date of sampling (Hureau 1991), an FAO database on international introductions of inland aquatic species (Welcomme 1988) and a database with records on occurrence and abundance for several thousand species throughout the tropics from 1975 to the present from the Dr Fridtjof Nansen Project, based in Bergen, Norway (Strømme 1992).

THE AIMS AND USES OF Fishbase WITH REGARDS TO FISH GENETIC RESOURCES FOR AQUACULTURE

Fishbase aims to assist the conservation of fish species and their habitats by providing researchers and policymakers with lists and maps describing the status (including threats to survival) of the fish species that occur in their countries and the localities where these species have been recorded over the past 250 years. This information will assist the National Conservation Plans which have been suggested by IUCN (Anon, 1992). Fishbase will include information on all international introductions and transfers of inland fishes in collaboration with FAO (Letter of Agreement between FAO and ICLARM on introduced species, November 1989). In light of the complementary nature of the comparative advantages and mandates of ICLARM and FAO, this need can best be met through mutual cooperation of the two organizations. FAO will continue to gather information through questionnaires sent to Directors of Fisheries Institutes, field projects and national and regional centers. ICLARM will also gather information as it arises through literature surveys and by queries to members of the proposed ICLARM/UNDP Fish Genetics Collaborative Research Network.

Fishbase also aims to help the *ex situ* conservation of fish genetic resources by serving as a directory of fish germplasm collections, with information on the origin and composition of founding stocks and their subsequent management. This will help fish breeders to exchange information. Moreover, public aquaria and hobby aquarists need information on where they can obtain specimens from those who breed fish in captivity, thus reducing collecting pressure on threatened wild stocks (Maitland and Evans 1986).

Fishbase will also assist the application of genetics in aquaculture. It will provide genetic data, including histories of founding population and broodstock management, accumulated inbreeding, and genetic characterization of strains and hybrids. The biggest and most extensive body of data available on genetic variability of fish populations is that derived from electrophoretic analysis of protein polymorphisms.

Initial efforts are focused on summarizing and entering data on protein polymorphisms from electrophoretic studies, using the nomenclature recommended by the International Union of

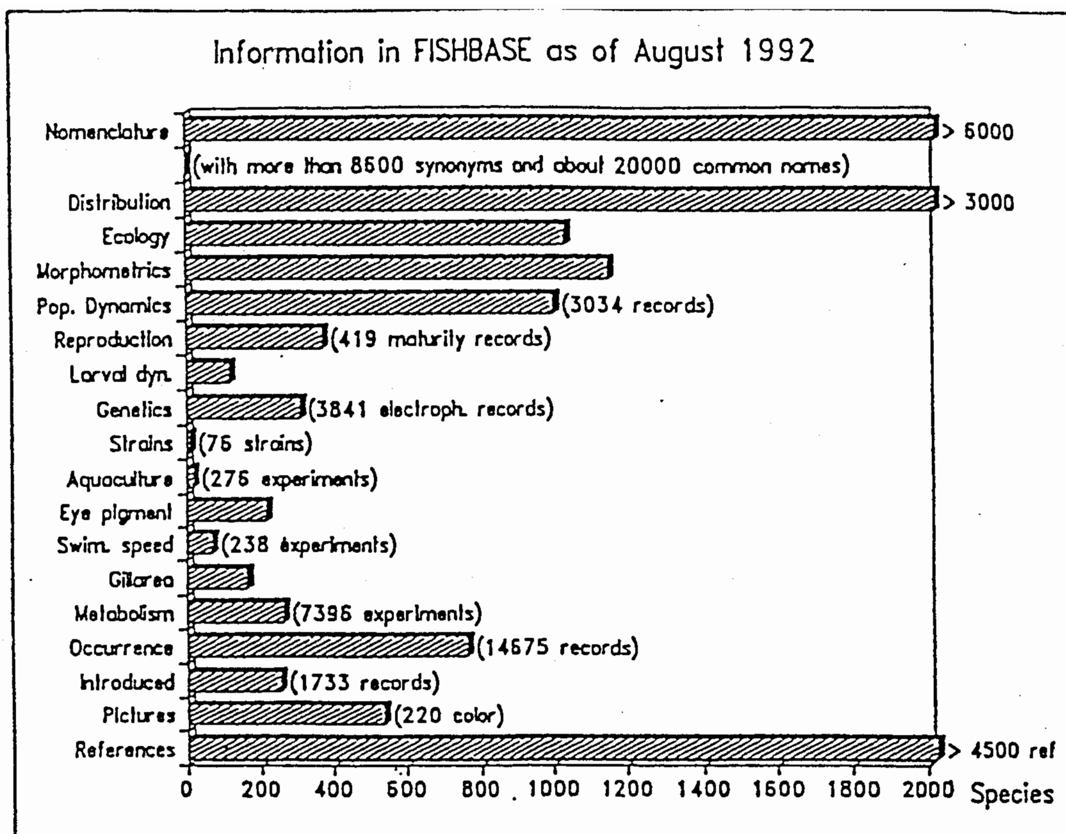

Figure 1 The status of entries into Fishbase.

Biochemistry's Nomenclature Committee (Shaklee *et al*, 1990). Fishbase documents information on the enzymes, the total number of loci studied, tissues and the buffer systems used. Fishbase lists heterozygosity values and proportions of polymorphic loci for a given species or strain in different localities, providing users with a view of the overall level of genetic diversity for that species. Aside from electrophoretic data, Fishbase also records karyotypes and DNA content which are potentially useful in the identification of species or hybrids.

The genetic improvement of farmed fish requires breeding programs to enhance traits of high economic importance (such as growth rate, age at maturity, carcass quality and many more). From selective breeding programs, Fishbase will record heritabilities and responses to selection.

THE CURRENT STATUS OF Fishbase

As of August 1992, Fishbase has documented the scientific names, synonyms and about 20,000 common names for more that 6,000 species. Figure 1 gives an overview of the coverage of different areas in terms of species and records. For example, Fishbase contains information on the population dynamics for 1,000 species, comprising about 3,000 records of growth parameters (there can be several records for the same species).

Fishbase also contains about 4,000 records of allele frequencies for different species and strains of fish. Figure 1 shows, however, that progress in entering data relevant to fish genetic resources for aquaculture has so far been limited, as it has for all aspects of aquaculture. This is because of the difficulty of extracting good summary data from non-standardized aquaculture experiments (the majority), even for the relatively small number of farmed fish species (about 123; FAO 1991). Moreover, there are few other databases in aquaculture from which Fishbase can draw information. These constraints are being addressed by increased efforts to extract and standardize data.

Awareness of the need to characterize fish breeding stocks and even pedigree individuals is very recent. Aquaculture lags far behind agriculture and animal husbandry in this. However, as fish genetic improvements research expands, both in selective breeding, hybridization and genetic management (eg production of polyploid and transgenic fish), the need to characterize broodstock genetically, using

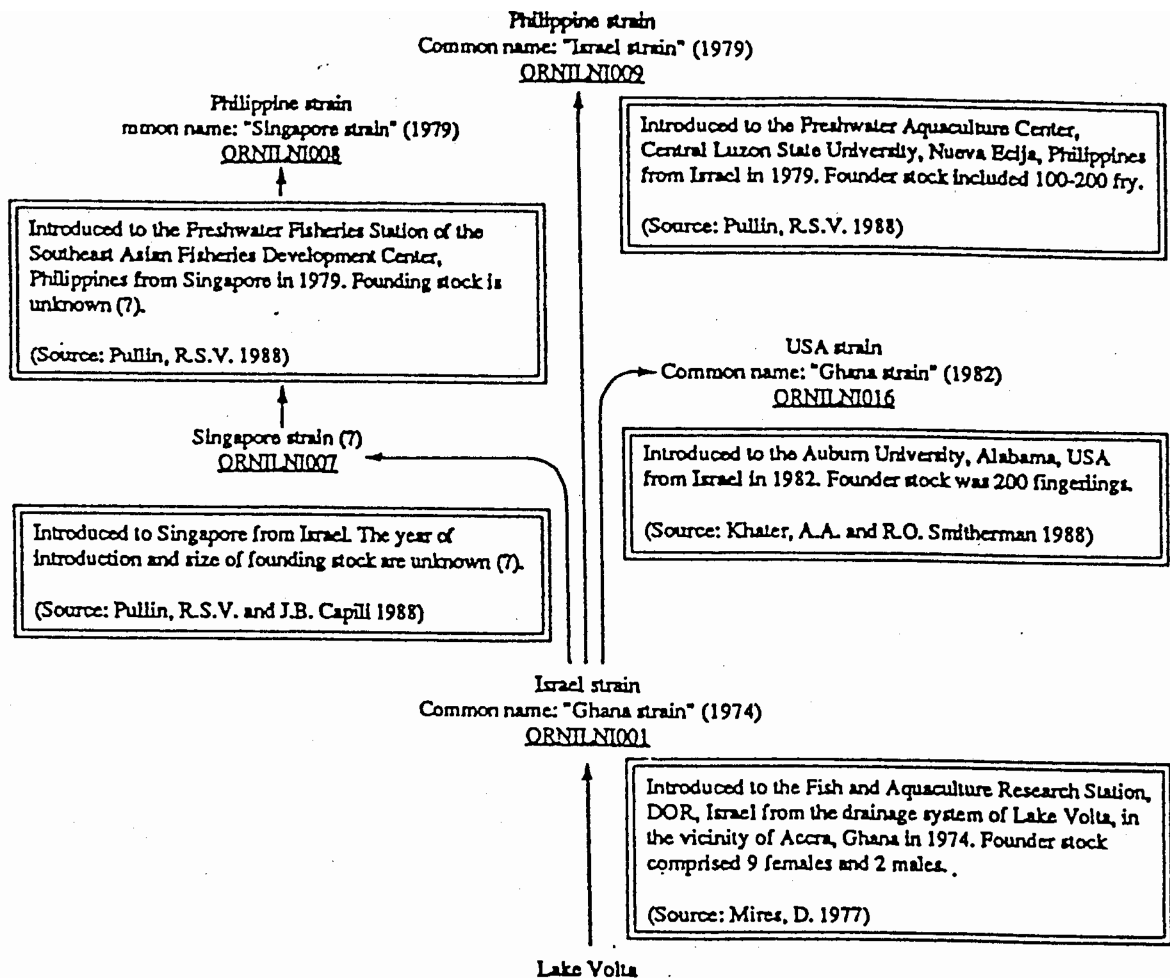

Figure 2 Fishbase record for the "Ghana" strain of Nile tilapia (*Oreochromis niloticus niloticus*).

standardized terminology, will be as important for fish as it is for crops and livestock. North American strains of rainbow trout are already being catalogued (Kincaid 1981) and there are scattered records of recognized strains or landraces of other farmed species; for example, carps (Jhingran and Pullin 1988; Komen 1990) and tilapias (Khater and Smitherman 1988; Eknath *et al*. 1991). Attempts to describe strains of other species groups are also beginning.

There is no current definition of a strain that is acceptable to all. According to Kincaid (1981), a strain is any fish population that exhibits certain reproducible characteristics (eg physiological, morphological, behavioral or cultural performance traits) that are significantly different from those of other populations of the same species or from stocks collected from such a population and maintained as a pure breeding population. Fishbase will provide accurate information on the culture performance traits of genuine strains. This is important to the fish breeders and farm managers. Little data has been available to them in the past and they have been forced to rely on their own experience and word of mouth for information. Progress will, of course, be only as rapid as the speed at which Fishbase can access, screen and process reliable information.

BEGINNING A STRAIN REGISTRY - THE TILAPIAS AS AN EXAMPLE

Documentation of tilapia strains is now underway in Fishbase and will serve as the Tilapia Strain Registry that was recommended by The Second International Symposium on Tilapia in Aquaculture (ISTA II) in 1987, Bangkok, Thailand (Pullin 1988). A strain registry with information on tilapias and carps and other strains of fish utilized in aquaculture has been established in Fishbase in collaboration with FAO (Letter of Agreement between FAO and ICLARM on strain registry, November 1989). Both Organisations will cooperate on creating a standardized nomenclature for strain designation. Gathering of information for the strain registry will be done in the manner as earlier described for introductions of inland fishes. Figure 2 shows

an example of the history of the "Ghana strain", its source of origin and subsequent transfers to many countries. For this, as for several important farmed or potentially farmable tilapias the subspecific name is important.

For the tilapia strain registry, information such as source of origin, year of introduction, composition of founding stock, distinct traits and other important information are gathered. In Fishbase, strains are documented when there is *published* information on the new strain and when the strain has a proven trait or traits distinct from the founding stock(s). The effective breeding number (Ne)(Smitherman and Tave 1988) is also recorded.

Fishbase is attempting to standardize strain nomenclature by assigning a unique straincode composed of 7 letters and 3 digit-number. An example is ORNILNI001 for *Oreochromis niloticus niloticus* strain. The first two letters refer to the first two letters of the genus; letters 3-5 refer to the first three letters of the species; and letters 6-7 refer to the first two letters of the subspecies. The 3 digit-number is sequential. For unknown subspecies, letters 6-7 will be designated as XX and for hybrids, letters 6-7 will be HX.

Parallel to this collection of information on farmed fish strains, Fishbase is gathering information on the wild genetic resources of tilapias and collections of museum specimens, through collaboration with the Zoologisches Institut und Museum of the University of Hamburg.

THE FUTURE

Flishbase is expected to become a major source of reference and a teaching and research tool for those working with fish genetic resources. The number of institutions and individuals collaborating in Fishbase is encouraging but more linkages are needed. Fishbase gives prominent credit to all sources of data that it uses and it increases awareness and use of sources of data as well as being a vehicle for linking these to other information through powerful relational software. ICLARM invites cooperation with individuals and institutions that have interests in helping to build and to use Fishbase, especially in the areas of aquaculture and fish genetic resources, where reliable information is most urgently needed. For example, ICLARM is planning a conference on the Genetic Resources of Asian Carps for Aquaculture, patterned after that held for the tilapias (Pullin 1988) from which a large breeding program and genetic characterization studies were initiated (Eknath *et al*, 1991a; Pullin *et al*, 1991).

ACKNOWLEDGEMENTS

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REFERENCES

Anon (1992). Global biodiversity strategy. Policy-makers' guide. World Resources Institute (WRI), The World Conservation Union (IUCN), United Nations Environment Programme (UNEP).

Eknath, AE, Macaranas, JM, Agustin, LQ, Velasco, RR, Ablan, MCA, Pante, MJR and Pullin, RSV (1991). Biochemical and morphometric approaches to characterize farmed tilapias. Naga, The ICLARM Q. 14(2):7-9.

Eknath, AE, Tayamen, MM, de Vera, MP, Danting, JC, Reyes, RA, Dionisio, EE, Capili, JB, Bolivar, HL, Abella, TA, Circa, AV, Bentsen, HB, Gjerde, B, Gjedrem, T and Pullin, RSV (1991). Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. Aquaculture (in press).

FAO Fishery Information, Data and Statistics Service (1991). Aquaculture production (1986-1989). FAO Fish Circ (815) Rev 3 141 p.

Froese, R (1990). Fishbase: an information system to support fisheries and aquacultural research. Fishbyte 8(3):21-24.

Hureau, JC (1991). Las base de données GICIM: Gestion informatisée de collection ichthyologiques du Muséum, p 225-227. In: Atlas Preliminaire des Poissons d'Eaux Douce de France. Conseil Supérieur de la Pêche, Ministère de l'Environment, CEMAGREF et le Muséum National d'Histoire Naturelle, Paris, 232 p.

Jhingran, VG and Pullin, RSV (1988). A hatchery manual for the common, Chinese and Indian major carps. ICLARM Studies and Reviews 11, 191 pp. Asian Development Bank, Manila, Philippines and International Center for Living Aquatic Resources Management, Manila, Philippines.

Khater, AA and Smitherman, RO (1988). Cold tolerance and growth of three strains of *Oreochromis niloticus*, p 215-218. In: Pullin, RSV, Bhukaswan, T, Tonguthai, K and Maclean, JL (Eds). The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 pp. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

R. SMITH MEMORIAL LIBRARY
DOCUMENTATION CENTER ICLARM

Kincaid, HL (1981). Trout strain registry. FWS/NFC-L/81-1, US Fish and Wildlife Service, Kearneysville, WV.

Komen, J (1990). Clones of common carp: new perspectives in fish research. PhD Dissertation. Agricultural University Wageningen, Wageningen, Netherlands. 169 pp.

Maitland, PS and Evans, D (1986). The role of captive breeding in the conservation of fish species. Int Zoo Yb 24/25:66-74.

Mires, D (1977). Theoretical and practical aspects of the production of all male tilapia hybrids. Bamidgeh 29:94-101.

Palomares, MLD, Moreau, J, Reyes-Marchant, P, Froese, R and Pauly, D (1991). Fishbase: une base de données sur les poissons. Fishbyte 9(2):58-61.

Pauly, D and Froese, R (1991). Fishbase: assembling information on fish. Naga, The ICLARM Q 14(4):10-11.

Pullin, RSV (Ed) (1988). Tilapia genetic resources for aquaculture. ICLARM Conference Proceedings 16, 108 pp International Center for Living Aquatic Resources Management, Manila, Philippines.

Pullin, RSV and Capili, JB (1988). Genetic improvement of Tilapias: problems and prospects, p 259-266. In: Pullin, RSV, Bhukaswan, T, Tonguthai, K and Maclean, JL (Eds) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, 623 p Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines.

Pullin, RSV (1990). Down-to-earth thoughts on conserving aquatic genetic diversity. Naga, The ICLARM Q 13(1):5-8.

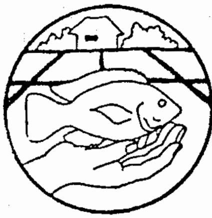
Pullin, RSV, Eknath, AE, Gjedrem, T, Tayamen, MM, Macaranas, JM and Abella, TA (1991). The genetic improvement of farmed tilapias (GIFT) project: the story so far. Naga, The ICLARM Q 14(2):3-6.

Shaklee, JB, Allendorf, FW, Morizot, DC and Whitt, GS (1990). Gene nomenclature for protein-coding loci in fish. Trans Am Fish Soc 119:2-15.

Smitherman, RO and Tave, D (1988). Genetic considerations on acquisition and maintenance of reference populations of tilapia. Aquabyte 1(1):2.

Strømme, T (1992). NAN-SIS: Software for fishery survey data logging and analysis. User's manual. FAO Computerized Information Series (Fisheries). No 4 Rome FAO 103 p.

Welcomme, RL (1988). International introductions of inland aquatic species. FAO Fish Tech Pap (294):318 p.



Biochemical and Morphometric Approaches to Characterize Farmed Tilapias

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JULIE M. MACARANAS
LIZA Q. AGUSTIN
RAVELINA R. VELASCO
MA. CARMEN A. ABLAN
MA. JOSEFA R. PANTE
ROGER S.V. PULLIN

The first introduction of tilapias to the Philippines in the form of *Oreochromis mossambicus* failed to start a commercially successful culture industry there because this species was unattractive to consumers and its early reproduction caused overcrowding in fishponds. Tilapia culture became popular only after the introduction of Nile tilapia (*Oreochromis niloticus*) in the 1970s. But, the tilapia industry in the Philippines, as elsewhere in Asia, suffers from poor or variable fish growth performance.

Collaborative research efforts between the Marine Science Institute of the University of the Philippines (UPMSI) and ICLARM, initiated in early 1980s, have focused on genetic characterization of farmed tilapias, broodstock management practices, and their implications for the future of the Philippine tilapia industry. These studies revealed the poor genetic status of farmed strains: narrow genetic base (descendants of a few introductions of small numbers of fish, mostly through intermediate countries); poor broodstock management resulting in inbreeding; and widespread introgression of genes from undesirable feral *O. mossambicus*. The general conclusion was that any genetic improvement efforts using the existing tilapia genetic resources would start at a disadvantage and may not bear fruit.

A new base population with a wider genetic base is being established through the collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT) by combining germplasm recently brought from Africa with the farmed strains in the Philippines (see genesis of the GIFT project, p. 3).

Biochemical (electrophoresis and mitochondrial DNA) and morphological analysis are important tools for characterization of strains.

Electrophoresis: Basic Concepts

Electrophoresis is the most useful technique for studying the genetic composition of individuals and populations at the level of individual genes. A gene is a specific length of DNA (deoxyribonucleic acid) occupying a position on the chromosome called a locus. One of several alternate forms of a gene constitutes an allele. Diploid organisms carry two alleles of each gene, one from each of the parents. Individuals having different alleles at one or more given loci are heterozygotes, while those with identical alleles are homozygotes. Alleles are distinguished by their protein products (usually enzymes from various tissues) in an electrical field. Different forms of the same enzyme, distinguishable by their mobility (or bands)in a starch gel medium (zymogram), are called isozymes. A locus is monomorphic if only one form of allele is known (one band on the zymogram) and polymorphic if two or more alleles (two or more bands) are found. Allelic isozymes are called allozymes. Isozyme analysis thus provides a tool for precise identification of the genotype of individuals for a given locus. Proteins can also be made to separate according to their isoelectric points through a procedure called isoelectrofocusing.

Characterization at the population or strain level essentially involves determination of the frequency of occurrence of each allele at a number of different loci. Several important estimates are used to detect relative levels of genetic variability and relationships among populations: percentage of loci that are polymorphic (a locus is considered polymorphic if the frequency of the most common allele does not exceed 0.95), average number of alleles per locus, frequency of heterozygotes (heterozygosity) and genetic distance. Genetic distance is measured in terms of the gene frequencies averaged over all loci across different populations. It is based on the concept that two populations which have diverged through time have accumulated a number of substitutions per locus. The

more distinct the differences between populations the greater the genetic distance between them. The genetic distances between populations become clear when they are layed out as a 'dendrogram' constructed by certain routine statistical procedures. These measures provide valuable insights into evolutionary

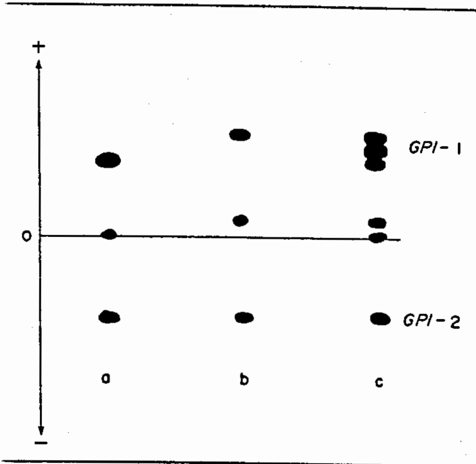

Fig. 1. ***Allozyme expression of GPI in*** **Oreochromis niloticus** ***(a),*** **O. mossambicus** ***(b) and in introgressed hybrids between both species (c). GPI-2 has a common allele for both species while GPI-1 displays a faster moving allele in*** **O. mossambicus** ***compared to*** **O. niloticus.** ***Introgressed hybrids are three-banded at GPI-1.***

processes, including mixing (introgression), genetic drift (random fluctuations in allele frequencies, particularly in small populations) mutation, migration and selection.

Evidence for Introgression

Electrophoretic studies on Philippine tilapias, initiated by our group in 1983, provided evidence for widespread mixing (introgression) of less desirable feral *O. mossambicus* populations with farmed Nile tilapia stocks. Of the 20 loci examined, six were found to be diagnostic markers of introgression. A typical zymogram of an isozyme – glucose phosphate isomerase (GPI) is shown in Fig. 1. GPI in tilapia is controlled by two loci: GPI-1 and GPI-2. Locus GPI-2 has the same allele for both *O. niloticus* and *O. mossambicus*, but the other locus (GPI-1) has different alleles in these species. Introgressed hybrid populations exhibit both alleles.

Extensive studies encompassing Nile tilapia populations from commercial farms, experimental stations, and government hatcheries in Luzon, Visayas and Mindanao indicated well-established introgression with *O. mossambicus*. In some populations, the degree of introgression was very high, that is they had very small genetic distance from *O. mossambicus*. It also became apparent that the genetic variability observed in Nile tilapia populations was caused primarily by introgression of *O. mossambicus*.

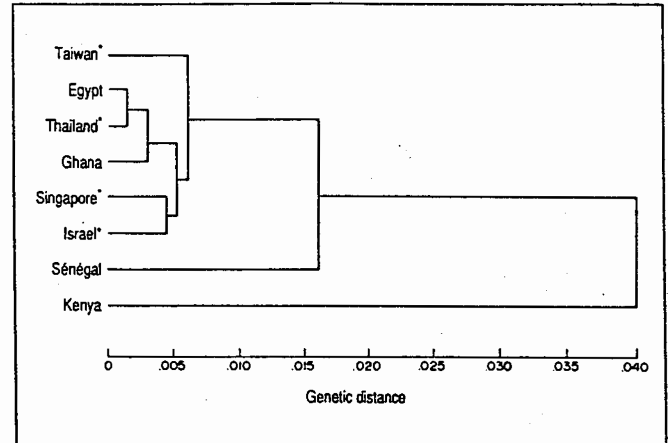

Fig. 2. ***Dendrogram constructed from genetic distance values shows three separable groups amongst eight tested strains of*** **O. niloticus** ***- a cluster of Philippine farmed strains (marked with asterisks; showing countries of origin of stocks used in the Philippines) with the Egypt and Ghana strains, Sénégal strain and the Kenya strain.***

Characterization of Strains

We analyzed progeny from the eight available *O. niloticus* strains, four African and four Philippine (see p. 3), at 30 loci. All strains shared alleles at 14 monomorphic and 16 polymorphic loci. A dendrogram constructed from genetic distance values (Fig. 2) reflects the close identity of the Philippine strains with those from Egypt and Ghana – confirming that the origin of Philippine strains is Egypt and Ghana. The wider separation of Kenya strain supports its recognition by Dr. E. Trewavas as a different subspecies (*O. niloticus vulcani*) from all others tested (*O. niloticus niloticus*).

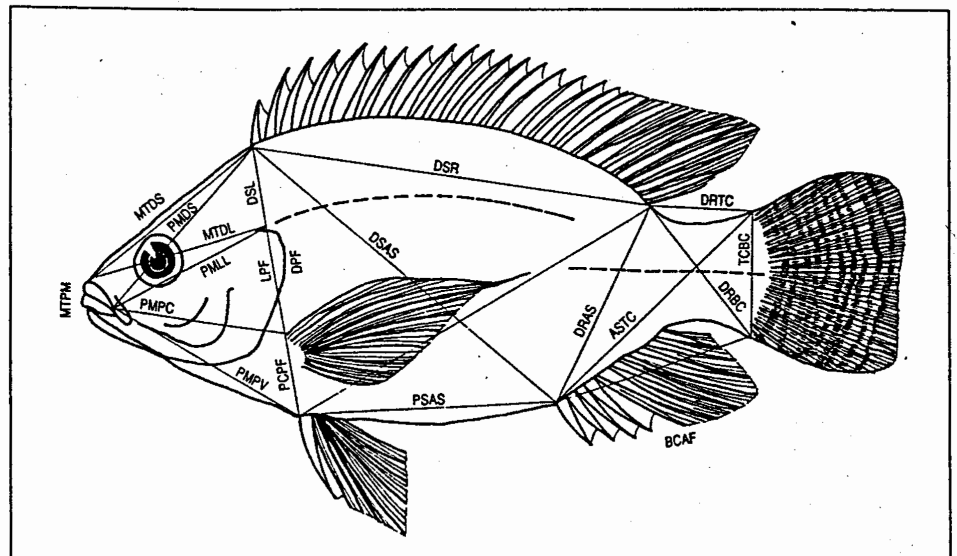

Fig. 3. ***Truss network of 21 landmark points on the body outline measured during morphometric characterization of the eight tilapia strains.***

Mitochondrial DNA (mtDNA)

Although protein electrophoresis has been a successful technique for describing

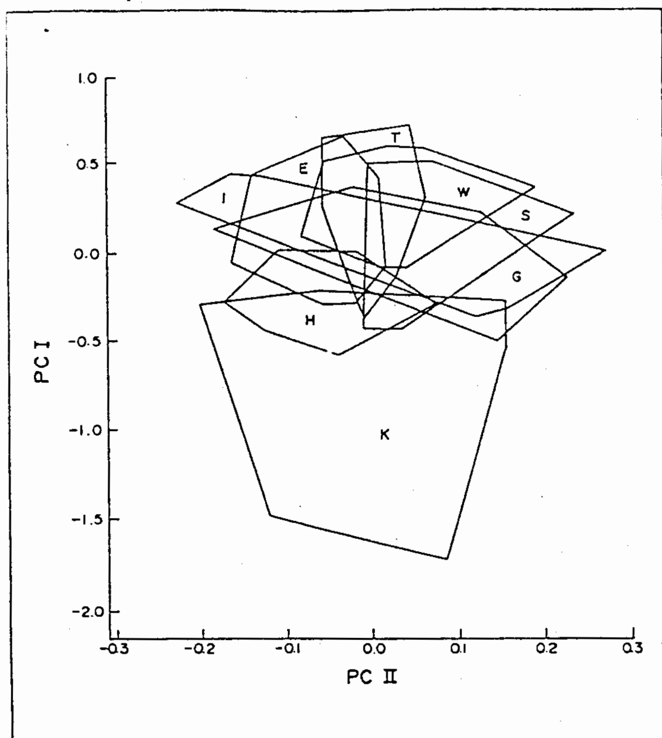

Fig. 4. *Plot of Principal Component I (PC I) and residuals of Principal Component II (PC II) of 21 morphometric characters of male and female* O. niloticus. *K-Kenya; H-Ghana; E-Egypt; S-Sénégal; I-Israel; G-Singapore; W-Taiwan; T-Thailand.*

the genetic structure of populations, it cannot detect all the genetic variation that may be of value. Its application is restricted to the part of the genome that codes for soluble enzymes. It resolves only the existing genetic variability and in many instances has failed to identify genetically discrete stocks.

Working at the DNA level (rather than the products of the genes) can tremendously increase our understanding of genetic variation and get a magnified view of the genetic distances between populations. About 99% of the DNA resides within the nucleus. Of the remaining 1%, it is the mtDNA that is of importance in quantifying genetic differences. An important property of mtDNA is its mode of inheritance which is strictly maternal. This property makes mtDNA a valuable tool for tracing female lineages within and among populations. The principal tools in mtDNA analysis are the restriction endonucleases – enzymes which break up the mtDNA into fragments of different lengths. These fragments are separated by electrophoresis and the patterns compared.

For the past 18 months, we have concentrated on standardizing procedures for extracting mtDNA. A technique devised by Chapman and Powers which makes use of phenol to extract protein from isolated mitochondria and ethanol to precipitate the mtDNA was found most suitable: mtDNA precipitates were obtained from fresh gonad and liver samples. Work is in progress using an array of restriction enzymes to induce fragmentation.

Morphometric Analysis: Basic Concepts

Morphometric characterization of strains of the same species often involves detection of subtle differences in variations of shape, independent of size. The truss network method (truss morphometrics) is a powerful technique to do this. The biases of traditional measurements (standard and total length, total height, etc.), namely, dense measurements in some areas of the body and a paucity elsewhere, is overcome in truss morphometrics by measuring distances between homologous (or landmark) points along the body (Fig. 3). The measurements, after appropriate data manipulation, are subjected to multivariate statistical analysis: discriminant analysis or principal component analysis (PCA). Discriminant analysis categorizes individuals based on *a priori* recognition of groups, e.g., sex or species. PCA, on the other hand, does not require *a priori* recognition of groups, and if there are several groups, data are pooled irrespective of groups. PCA constructs principal components (PC) which are linear combinations of the variables that describe the shape variations in the pooled sample.

Characterization of Tilapia Strains

In the GIFT project (see p. 3), a truss network of 21 landmark points on the body outline was used, aided by a digitizing tablet linked to a microcomputer. A computer program 'Computer Aided Monoscopic Analysis' (CAMA) was developed to calculate coordinates for these landmark points. PCA was performed using the statistical package SAS (Fig. 4). The Kenya strain, which has a relatively shorter and more streamlined mid-body region, separated out from the rest of the strains. Overall, however, the results indicate very little morphological differences among these eight strains.

Future Activities

Genetic characterization work will continue through the GIFT project. The emphasis in future will be on conservation and gene banking of potential genetic resources. DNA fingerprinting is also being contemplated because of its potential usefulness, for example in estimating inbreeding rates, in pedigree analysis and in detecting divergence of selected populations from the founder populations.

Further Reading

Bookstein, F.L., B. Chernoff, R. Elder, J. Humphries, G. Smith, and R. Strauss. 1985. Morphometrics in evolutionary biology. Special Publ. 15. The Academy of Natural Sciences of Philadelphia, Philadelphia.

Ferris, S. D., and W. J. Berg 1987. The utility of mitochondrial DNA in fish genetics and fishery management, p. 277–299. *In*. N. Ryman and F. Utter (eds.) Population genetics and fishery management. University of Washington, Seattle, Washington.

Hartl, D. 1980. Principles of population genetics. Sinauer Associates, Sunderland, Massachusetts.

ICLARM Contribution No. 722.

Attachment 3.

Protocol for maintenance of live genebank

Protocol for Reproduction of GIFT Reference Strains

Hans Bentsen

The reference strains should be maintained with a minimum of inbreeding and selection. The rate of inbreeding is determined by the number of breeders used in every generation and the family structure. With 25 breeding pairs (each breeder used in one mating only) and one male and one female replacement from each pair In each generation, the rate of inbreeding will be about 0.5% per generation (see Falconer equations 4.1 and 4.9). In addition, the rate of inbreeding in such a population may be kept at 0% per generation during the first 4 generations after the founder generation and approaching 0.5% per generation and only 6-7 generations by systematically avoiding mating of close relatives. A systematic mating design may also make the rate of inbreeding more homogeneous from generation to generation and between individuals within a generation. I suggest the following design:

1. Perform single pair mating of the original breeders (one female per male/one male per female) within each strain . Use 25 pairs per strain (or as many pairs as possible if the number of breeders is not sufficient to make 25 pairs).

2. Carry out separate rearing of each full sib group until tagging. Tag sufficient number of individual from each full sib group to make sure that at least one tagged male and one tagged female from each full sib group will be available and able to spawn at the time when the next replacement generation is to be produced. The new generation may then be communally stocked after tagging (all strains together if desired) and kept until they shall be used as breeders.

3. The original breeders may be discarded when the requirements under step 2 are fulfilled, or they may be kept alive as an extra security until step 4 below is completed.

4. To produce the next replacement generation, use one male and one female as breeders from each full sib group within each strain. If more than one male or/and female breeder is available in the full sib group, pick one random breeder of each sex (don't consider body size, spawning condition, etc. when you pick the breeders). If all males or/and females in a sib group are lost or unable to reproduce, and the original breeders are still available and

able to reproduce (step 3 above), a new full sib group may be produced. The mating(s) involving breeders from this group(s) (see step 5 below) may then be carried out within a certain delay.

5. Condition the breeder and stock them for single pair mating within each strain separately according to the following design (sib group 1 is the progeny of pair 1 of original breeders etc. up to e.g. sib group 25):

   Female from sib group 1 with male from sib group 2
   Female from sib group 2 with male from sib group 3
   Female from sib group 2 with male from sib group 4
   etc.
   etc.
   Female from sib group 25 (or the last sib group if less than 25) with male from sib group 1

   This will result in the same number of the breeding pairs in each strain as in the previous generation.

6. If one (or more) of the breeding pair is not producing offspring, and a replacement of the same sex from the same sib group are available, the female and/or the male be replaced. If more than one replacement is available, pick the replacement at random (see step 4 above). However, since age differences between the sib groups are not very important, you should allow plenty of time for the breeding pairs to produce offsprings before you replace the breeders. This is important to avoid as much as possible to carry out selection that may change reproductive-behavior, etc.

7. Repeat step 2 above to produce the third generation of breeders.

8. Repeat step 3 with the second generation of breeders and step 4 to pick the third generation of breeders.

9. After conditioning, stock the third generation of breeders for single pair mating within each strain according to the following design (NOTE: Sib 1 is now the progeny of the female from sib group 1 in the second generation etc. up to e.g. sib group 25):

   Female from sib group 1 with male from sib group 3
   Female from sib group 2 with male from sib group 4
   Female from sib group 3 with male from sib group 5

etc.

etc.

Female from sib group 24 (or the second last sib group if less than 25)
with male from sib group 1

Female from sib group 25 (or the last sib group if less than 25) with male from sib group 2.

10. Repeat step 6 to try to maintain the number of full sib groups from each strain in the fourth generation.

This procedure may then be repeated (mating female from sib group 1 with male from sib group 4 etc. to produce the fifth generation, mating female from sib group 1 with male from sib group 5 etc. to produce the sixth generation and so on. Remember that sib group 1 is always the progeny of the female from sib group 1 in the previous generation etc. This may go on until a female from sib group 1 is mated to a male from sib group 25 (or the highest sib group number). If we (or the reference strains) are still alive, we may then start from step 5 again.

If 25 pairs of original breeders (founder stock) may not be achieved because of too low number of males or too low number of females, a male may be mated to two females or a female to two males to increase the number of pairs. This will increase the effective population size slightly, but not as much as if all breeders were use in one mating only. The pairs should then be numbered in a way that results in a maximum number of generations between each time descendants from the same breeder are mated to each other. This will be achieved by numbering the pairs such that the number of the first pair were the breeders is used is approximately n/2 lower or higher than the number of the second pair (where n is the total number of pairs in the strain, e.g. 25). A breeder could then be used e.g. in pair number 1 and 13.

If the number of males and females among the original breeders in one strain (founder stock) are both lower than 25, you may still settle with as many pairs as the least numerous sex. If you want to use some breeders in more than one mating to increase the number of pairs, the maximum number of pairs should be determined by the most numerous sex to avoid that the mating schemes becomes too complicated. Repeated use of both males and females from the founder stock of a strain will not help much to increase the effective population size.

If all males and all females in a sib group in the following generations are lost before they are used for reproduction or are not available for reproduction or unable to reproduce, and the parents of that sib group are not available for production of a new sib group, then the sib group number should be deleted from the mating design in this and later generations, thus reducing the number of breeding pairs (and the effective population size). Both females and males from sib groups following below the lost sib in the mating design will then have to be moved one position upwards in the design.

This may also be done if all males or all females in a sib group are lost or unable to reproduce, and the parents of that group are not available. Alternatively, a replacement may be picked from another sib group. The replacement should then be picked from a sib group that has not recently been used to produce the sib group number where a breeder is missing (e.g. at least not during the previous 4-5 generations) and that will not be used (according to the mating design of the future generations) to produce this sib group in the near future (e.g. at least not in the following 4-5 generations).

Attachment 4.

Published manuscripts on the Project Activity

"Evaluation of culture performance of the eight strains in a wide range of farming systems under various agro-climatic conditions" (Generation 1 experiment)

Aquaculture, 111 (1993) 171–188
Elsevier Science Publishers B.V., Amsterdam

AQUA 30052

Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments*

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ABSTRACT

Eknath, A.E., Tayamen, M.M., Palada-de Vera, M.S., Danting, J.C., Reyes, R.A., Dionisio, E.E., Capili, J.B., Bolivar, H.L., Abella, T.A., Circa, A.V., Bentsen, H.B., Gjerde, B., Gjedrem, T. and Pullin, R.S.V., 1993. Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. *Aquaculture*, 111: 171–188.

Results from two consecutive generations of testing to evaluate the growth performance of eight different strains of Nile tilapia (*Oreochromis niloticus*) in eleven different farm environments are reported. The eight strains include four new strains recently imported to the Philippines from Egypt, Ghana, Kenya and Senegal; and four established Asian farmed strains popularly known in the Philippines as 'Israel', 'Singapore', 'Taiwan' and 'Thailand' strains.

The test environments were chosen to cover a wide range of Philippine tilapia farming systems, from simple ponds, as used by backyard farmers, to more intensive systems: fertilized ponds, ponds fertilized with on-farm agricultural residues, rice–fish systems, cages, and three tilapia hatcheries (satellite stations) located in different regions of the island of Luzon.

During the first generation trials in 1989, individually tagged fingerlings bred from the founder populations (total 7652) were communally reared in all test environments for about 90 days. In 1990, the second generation trials were made as a part of a complete diallele crossing experiment (8×8 strains). Data on 3420 individually tagged fingerlings of pure strains communally reared in eight test environments were used for the study.

The results indicated highly significant differences among the growth performances of the eight strains. Moreover, with the exception of the Ghana strain, the newly introduced African wild strains

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performed as well as or better than the most widely farmed Asian strains. The importance of strain × test environment interaction over the investigated range of test environments was low. The implications of these results for developing a breeding program for tilapias in the Philippines are discussed.

INTRODUCTION

Tilapias are widely recognized as one of the most important species for farming in a wide range of aquaculture systems from simple small-scale waste-fed fish ponds to intensive culture systems (Pullin, 1985). They form the mainstay for many resource-poor fish farmers. Annual production exceeds 50 000 mt in several Asian countries (Smith and Pullin, 1984). There is significant tilapia culture in Bangladesh, China, Indonesia, the Philippines, Sri Lanka, Thailand and Vietnam. Interest in tilapia culture is also increasing elsewhere in the Indian subcontinent and Pacific-rim countries. Tilapia has been dubbed the 'aquatic chicken' (Maclean, 1984). Among the wide variety of cultured tilapias, the most widely farmed is the Nile tilapia (*Oreochromis niloticus*).

The natural tilapia genetic resources are restricted to Africa but the main culture industries are at present in Asia, and use stocks of poor genetic material: descendants of a few introductions of small numbers of fish, mostly through intermediate non-tropical countries, and probably suffering from genetic founder and bottleneck effects (Pullin and Capili, 1988); genetic deterioration of cultured stocks due to widespread introgression of genes from other less desirable feral tilapia species, *Oreochromis mossambicus* (Macaranas et al., 1986); and possible inbreeding. Therefore, any substantial efforts aimed at genetically improving these Asian stocks would start with disadvantages and may not bear fruit.

A collaborative research project called the 'Genetic Improvement of Farmed Tilapias' (GIFT), co-financed by the Asian Development Bank (ADB) and the United Nations Development Program/Division for Global and Interregional Programs (UNDP/DGIP), began in April 1988. The GIFT project is being executed by the International Center for Living Aquatic Resources Management (ICLARM) in cooperation with the National Freshwater Fisheries Technology Research Center of the Bureau of Fisheries and Aquatic Resources (BFAR/NFFTRC), Freshwater Aquaculture Center of the Central Luzon State University (FAC/CLSU), Marine Science Institute of the University of the Philippines (UP/MSI) and the Institute for Aquaculture Research (AKVAFORSK).

The GIFT project's approach is to bring well documented tilapia germplasm from Africa to Asia where the species are already farmed, for establishing base populations from which genetically improved tilapia strains for farming will be developed. A sequential approach has been followed in the

GIFT project, from systematic documentation of the poor status of Asian farmed stocks and identification of sources of natural tilapia germplasm in Africa; evaluation of promising strains of *O. niloticus*; and then the establishment of base populations and plans to develop a more productive tilapia.

The objectives of the study reported here were to compare the growth performance of different strains of *O. niloticus* in different farm environments, estimate the importance of strain×test environment interactions, and then decide whether or not specialized strains for each farm environment will be necessary and the implications of this for a breeding program.

MATERIALS AND METHODS

Test strains

Based on the recommendations of the Workshop on Tilapia Genetic Resources for Aquaculture (Pullin, 1988), four important areas for collection of Nile tilapia germplasm were identified: Egypt, the Nile Delta system; Ghana, the upper Volta system; Kenya, Lake Turkana; and Senegal, the extreme west of its distribution.

The details of tilapia germplasm collections in Africa are given in Table 1.

TABLE 1

Details of Nile tilapia germplasm collections in Africa

Strain code	Collection sites	Date	Numbers collected
E1	Egypt (first collection)	May 1988	
	1. Lake Manzallah		30 breeders
	2. Creeks along desert road to Port Said		25 breeders
	3. Lakes around Alexandria		70 fingerlings
E2	Egypt (second collection)	Aug. 1989	
	1. Abassa		60 breeders
	2. Ismailia		90 breeders
Gh	Ghana		
	Volta River System	Oct. 1988	220 fingerlings
Ke	Kenya		
	First generation progeny from a founder stock collected in Aug. 1988	Aug. 1989	800 fingerlings
Se	Senegal		
	1. Dagana	Oct. 1988	120 breeders
	2. Dakar-Bangos		40 breeders
	3. Mbane		40 fingerlings

TABLE 2

Origin of the four farmed Nile tilapia strains in the Philippines

Strain code	Popular name	Origin
Is	Israel	Derived from founder stocks of Ghanaian origin kept in Israel. The original founder stock (1974) was 9 females and 2 males. Fry from 100–200 single pair matings were shipped to the Philippines in 1979.
Si	Singapore	Descended from a founder stock of Ghanaian origin shipped to Singapore from Israel and from there to the Philippines in 1979.
Th	Thailand	Egyptian origin. Introduced to the Philippines from Thailand in 1987. The Thailand founder stock was introduced from Japan in 1965 (50 fish formed the founder stock; however, the number which actually survived to breed is not clear).
Tw	Taiwan	Descended from founder stocks introduced to the Philippines from Taiwan in 1983–84; previous history not certain but most likely of Ghanaian origin.

All imported fish were held in a completely isolated quarantine facility at the BFAR/NFFTRC complex for periods ranging from 3 to 7 months. These collections represent the first ever direct transfers of *O. niloticus* from Africa to Southeast Asia. Nile tilapia collected from Egypt, Ghana and Senegal belong to the sub-species *Oreochromis niloticus niloticus*, whereas the Kenya stock is *O. n. vulcani* (Trewavas, 1983).

During the first generation growth trials, only three African strains were available: Egypt — first shipment (E1), of which only two breeding pairs survived, Ghana (Gh), and Senegal (Se). Second generation growth trials used all four African strains, including Kenya (Ke) and a second shipment from Egypt (E2).

From farmed Nile tilapia stocks in the Philippines, four strains were used: 'Israel' (Is), 'Singapore' (Si), 'Taiwan' (Tw) and 'Thailand' (Th), maintained separately in earthen ponds ever since their introduction to the BFAR/NFFTRC complex. The origin of Is, Si, and Tw strains is Ghana, while the Th strain originates from Egypt (Pullin and Capili, 1988; Macaranas et al., 1993) (Table 2).

Test environments

The test environments were chosen to cover a wide range of Philippine tilapia farming systems: fertilized ponds (with and without supplementary feeding), ponds fertilized with on-farm agricultural residues (ipil-ipil leaves, and leaves and vines of sweet potato), rice–fish systems, cages (different stocking densities; with and without feeding), and three hatcheries (BFAR satellite stations) located in different regions of the island of Luzon. Test environments are described in Table 3 and Table 4.

TABLE 3

Description of test environments

Environment code	Description
S1	BFAR satellite station located in the lowlands near Laguna Lake, southern Luzon. Pond culture. Standard[a] management and fertilization.
S2	BFAR satellite station located in the coastal region of north-west Luzon. Pond culture. Standard[a] management and fertilization.
S3	BFAR satellite station located in the highlands of central Luzon. Pond culture. Only two applications of fertilizer (chicken manure and inorganic fertilizer during the grow-out period).
P1	On-station[b]. Pond culture. Standard[a] management and fertilization.
P2	On-station[b]. Pond culture. Standard[a] management and fertilization. Supplementary feeding (70% rice bran and 30% fish meal) at 5% body weight twice daily.
C1	BFAR satellite station located in the lowlands near Laguna Lake, southern Luzon. Cage culture in a farm reservoir without fertilization or feeding.
C2	On-station[b]. Cage culture in reservoir without fertilization. Feeding (70% rice bran and 30% fish meal) at 20% body weight once daily. One replicate in first generation harvested after 57 days.
C3	On-station[b]. Cage culture in ponds (Standard[a] management and fertilization) with supplementary feeding (70% rice bran and 30% fish meal) at 10% body weight twice daily.
W1	On-station[b]. Pond culture. Fertilized with chicken manure (1000 kg/ha) every second week.
W2	On-station[b]. Pond culture. Fertilized with untreated ipil-ipil leaves (*Leucena* sp.) at 50 kg dry matter/ha daily.
W3	On-station[b]. Pond culture. Fertilized with untreated leaves and vines of sweet potato (*Ipomea batata*) at 50 kg dry matter/ha daily.
W4	On-station[b]. Pond culture. Fertilized with carabao manure (1000 kg/ha) plus inorganic fertilizer 16–20–0 (50 kg/ha) every second week.
RF	On-station[b]. Rice–fish culture. Trench refuge system (0.75 m wide×0.5 m deep). Plots planted to IR-70 variety of rice.

[a]Inorganic fertilizer (16–20–0) at 50 kg/ha and chicken manure at 1000 kg/ha every second week.
[b]BFAR/NFFTRC and FAC/CLSU facilities in Muñoz, lowlands of central Luzon.

Breeding of test strains

Single-pair mating (25 breeding pairs from each strain) was done in 1-m^3 hapas installed in breeding ponds. The pre-maxilla of males was clipped before stocking to avoid possible injuries to females. To tackle the problem of asynchronous spawning and consequently the problems of initial age and size effects, collection of fry was done in batches. Fry collected during short episodes of spawning (3–7 days) were pooled and reared separately for each strain and batch. The identity of different batches was also maintained by tagging. The fry were reared until they reached about 3 to 5 g when they were individually tagged with modified Floy fingerling tags. The number of fingerlings for tagging from each batch within strain was determined by the number of females contributing progeny in each batch.

TABLE 4

Salient features of test environments

Code[a]	Generation[b]		No. of replicates	Area	Stocking density (no./m²) Generation		Fertilizer[c] application	Feeds[c]	Natural[d] food production	Water t (°C) Generat
	First	Second			First	Second				First
S1	+	+	1	1287 m²	0.6	2	standard	none	adequate	27–29
S2	+	+	1	1300 m²	0.6	2	standard	none	adequate	30–37
S3	+	+	1	1300 m²	0.6	2	inadequate	none	inadequate	17–22
P1	+	+	4	1200 m²	0.6	2	standard	none	adequate	27–30
P2	–	+	2	500 m²	–	5	standard	yes	adequate	–
C1	+	–	1	3×3×1.5 m	50	–	none	none	inadequate	29–32
C2	+	+	2	5×5×2 m	30	33	none	yes	inadequate	27–30
C3	+	–	3	5×5×2 m	22	–	standard	yes	adequate	27–30
RF	+	–	4	400 m²	0.5	–	standard	none	adequate	28–31
W1	+	–	2	100 m²	0.6	–	see Table 3	none	adequate	28–31
W2	+	+	2	100 m²	0.6	2	see Table 3	none	adequate	28–31
W3	+	–	2	100 m²	0.6	–	see Table 3	none	adequate	28–31
W4	–	+	2	100 m²	–	2	see Table 3	none	adequate	–

[a]See Table 3.
[b]+ included; – not included.
[c]See Table 3.
[d]Based on subjective criteria: adequate — green water; inadequate — relatively clear water.

After tagging, fry from different test strains were communally stocked in the test environments. The total numbers of fingerlings stocked in each of the different test environments during the two consecutive generations of testing are presented in Tables 5 and 6. During the first generation trials in 1989, individually tagged fingerlings bred from the founder populations (total 7652) were communally reared in all test environments for about 90 days. In 1990, the second generation trials were made as a part of a complete diallele crossing experiment (8×8 strains). Data on 3420 individually tagged fingerlings of pure strains communally reared in eight test environments were used for the study.

Regular sampling (about 30% of the population) was carried out every 21 days to monitor growth performance. Following a rearing period of about 90 days (range: 85–92 days across test environments) all fish were harvested. The numbers of individuals without tags, easily identified by scars on their bodies, were also recorded. Following the termination of the first generation growth trials, the fish harvested were used as breeders for the second generation experiment.

Data analysis

Body weights at harvest were analyzed according to the following generalized linear models (GLM) procedure in the first generation (model 1) and the second generation (model 2):.

$$Y_{ijklm}=a+E_i+G_j+S_k+G_j\cdot E_i+S_k\cdot E_i+B_l(E_i)+e_{ijklm} \text{ (model 1)}$$

$$Y_{ijklm}=a+E_i+G_j+S_k+B_l+G_j\cdot E_i+S_k\cdot E_i+B_l\cdot E_i+e_{ijklm} \text{ (model 2)}$$

where:

Y_{ijklm} is the final body weight of the mth individual,
a is a constant,
E_i is the fixed effect of the ith test environment,
G_j is the fixed effect of the jth strain,
S_k is the fixed effect of the kth sex,
B_l is the fixed effect of the lth batch,
e_{ijklm} is a random error for the mth individual.

First order interaction terms between the test environment and the other fixed effects were included in both models. Because of incomplete distribution of batches across test environments, a nested analysis of batches within environments was carried out in the first generation. Strain Si was not tested in environment C3 during the first generation. Consequently, the analysis across environments had to be carried out in two runs, excluding either Si or the C3 variable. Because of heavy mortality during the second generation, test environment S3 was excluded from analysis.

The replicate trials within environments P1, P2, C2, C3, W1, W2, W3, W4,

and RF were not significantly different. They were pooled after applying a multiplicative correction factor (range 0.9962 to 1.0102) generated by dividing the mean for a given test environment by the mean of each replicate. Because of the unequal variances in the sex by environment subcells, the observations were weighted by the reciprocal of the within-subcell variances during the GLM analyses.

The least square mean (LSM) values generated from the above models were used for further analysis.

RESULTS

Mortality and tag loss

The total numbers of individually tagged fingerlings, mortality and estimated tag losses after a rearing period of about 90 days, during the two consecutive generations are presented in Table 5 and Table 6. Tag losses ranged between 0% to 20% in the first generation. Mortalities reported for the second generation include tag losses.

In the first generation, mortality was relatively low (<10%) in test environments C1 and C2, medium (10–25%) in test environments S1, S2, P1 and C3, and high (>30%) in test environments S3, W1, W2, W3 and RF. In the second generation, total mortality (including tag loss) was medium in test environments S1, S2, P1 and P2, and high in test environments C2, W2 and W4. There was a near total mortality in test environment S3. Mortality does

TABLE 5

First generation: numbers of tagged fingerlings stocked, percentage mortality (including tag loss) of strains within each test environment, and estimated total mortality (excluding tag loss) and estimated total tag loss in different test environments

Test environment	No. of fish stocked	Mortality of strains							Estimated total mortality	Estimated total tag loss
		El	Gh	Se	Is	Si	Tw	Th		
S1	829	28	24	24	38	39	27	38	10	20
S2	702	25	32	21	26	28	29	15	12	13
S3	680	60	59	72	74	72	73	75	59	10
P1	2714	35	27	32	42	34	37	40	25	10
C1	693	5	6	5	7	3	5	4	5	0
C2	104	19	7	14	12	14	27	7	4	10
C3	596	28	24	30	28	–	28	24	16	11
W1	212	42	54	50	50	56	63	42	49	2
W2	214	50	22	30	28	32	39	33	32	1
W3	218	45	33	39	52	39	35	40	38	2
RF	690	55	53	36	57	55	50	53	51	0
Total	7652	36	31	32	38	37	37	34	27	7

TABLE 6

Second generation: numbers of tagged fingerlings stocked, percentage mortality (including tag loss) of strains, estimated total mortality (includes tag loss) in different test environments

Test environments	No. of fish stocked (pure strains)	Mortality of strains									Total mortality
		E1	E2	Gh	Ke	Se	Is	Si	Tw	Th	
S1	360	30	18	8	10	13	13	15	15	15	15
S2	360	18	28	30	10	13	23	25	23	8	20
S3	360	100	80	98	90	78	100	100	88	68	89
P1	720	18	20	28	25	19	23	44	21	23	24
P2	720	54	14	26	21	18	16	39	23	8	23
C2	180	85	20	70	35	40	0	70	55	25	43
W2	360	68	43	73	60	50	60	63	63	73	60
W4	360	68	35	58	38	50	40	35	50	50	47
Total[a]	3420	43	15	36	27	26	24	40	32	26	

[a]Excluding test environment S3.

TABLE 7

Degrees of freedom (d.f.), marginal mean squares (MS) and percent contribution for the effects in model 1 (first generation) and model 2 (second generation)

Effects	First generation			Second generation		
	d.f.	MS[a]	Percent[b]	d.f.	MS[a]	Percent[b]
Environments	10	2924.9	73.3	6	227.5	19.3
Strains	6	63.4	1.6	8	18.5	1.6
Sex	1	864.6	21.6	1	844.3	71.7
Batch	–	–	–	3	17.3	1.5
Environment × strains	59	12.1	0.3	48	3.9	0.3
Environment × sex	10	123.9	3.1	6	62.4	5.3
Environment × batch	–	–	–	18	3.1	0.3
Batch (environment)	15	3.4	0.1	–	–	–
Error	4866	0.8		2058	0.95	
R^2		0.89			0.82	

[a]Type III MS; all significant at $P<0.001$.
[b]Based on total marginal MS for all independent variables.

not appear to be strain-specific. However, in the first generation, the Gh strain registered relatively lower mortality rates across different test environments. In the second generation, mortality was lowest in strain E2 and highest in strains E1 and Si.

Growth

The marginal mean squares (MS) from models 1 and 2 are presented in Table 7. The MS for all effects in the model were significant ($P<0.001$). Their magnitudes, however, were highly variable. In the first generation, environment and sex effects accounted for 73% and 22% of the variation respectively. The same effects explained 19% and 72% of the variation during the second generation. This apparent reversal of importance of these two effects is because of the range of test environments included during the two generations: in the first generation, the environments ranged from extremely poor test conditions to more normal and representative farming conditions (see Tables 3 and 4).

The batch variable, among other factors, accounts for initial age and size effects. The batch effect is negligible (0.1%) in the first generation. In the second generation the magnitude of batch effect was almost equal to that of the strain effect (1.5%). The number of batches and the range of body weights of fingerlings at stocking and at harvest, within each batch, during the first and second generations, are presented in Table 8.

LSM of final body weights across test environments were 53.2 g and 50.1 g

TABLE 8

LSMs and standard errors (in parentheses) of initial and final body weights (g) of different batches across test environments during the first and second generation growth trials

Batch	First generation[a]		First generation[b]		Second generation	
	Initial	Final	Initial	Final	Initial	Final
1	5.8 (0.04)	70.0 (0.8)	—	—	—	—
2	4.9 (0.03)	70.0 (0.7)	—	—	4.9 (0.32)	45.8 (0.6)
3	3.9 (0.04)	73.3 (0.7)	6.0 (0.04)	29.9 (0.3)	4.7 (0.34)	51.1 (2.3)
4	—	—	6.4 (0.06)	30.4 (0.3)	3.5 (0.06)	40.7 (0.4)
5	—	—	—	—	4.2 (0.08)	41.7 (0.5)

[a]Test environments: S1, S2, S3, and P1.
[b]Test environments: C1, C2, C3, W1, W2, W3, and RF.

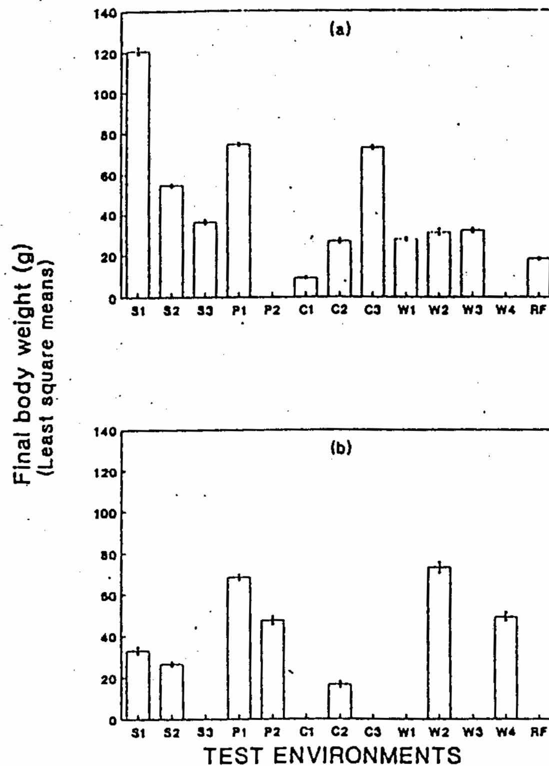

Fig. 1. Histogram of environment means (LSM of final body weights of all strains) during (a) first generation, and (b) second generation, across different test environments. Vertical bars are standard errors. See Table 3 and 4 for explanation of environment codes.

for males and 37.6 g and 35.2 g for females during the first and second generations respectively.

LSM of final body weights were highly variable between the test environments (environment means, Fig. 1). They range from 120.8 g in S1 to 9.4 g in C1 in the first generation and from 73 g in W1 to 16.6 g in C2 in the second generation. The growth performance in some of the test environments was far below what is expected under normal farming conditions. The rank of the test environments was also highly variable from the first to the second generation. This was caused partly by the fact that the two experiments were carried out in different seasons, and partly by other uncontrolled environmental conditions. In test environments S1 and S2, growth was depressed during the second generation due to dense phytoplankton blooms.

LSM of final body weight of the different strains across test environments (genotype means) during the first and second generations are presented in Fig. 2. The trends were similar in the two generations. Some of the African

TABLE 9

First generation: rank-order and significance of ranks for final body weight of strains, across and within test environments (Ra
ments sharing the same superscript letters are not significantly different $P < 0.05$; significant re-rankings of strains compared to r
ments are in boxes)

Strain	Rank	Rank within test environments								
		S1	S2	S3	P1	C1	C2	C3	W1	W2
El	1^{a}	1^{a}	1^{a}	1^{a}	1^{a}	2^{b}	5^{bc}	1^{a}	1^{a}	1^{a}
Th	2^{b}	2^{b}	2^{b}	5^{ab}	3^{bc}	3^{b}	2^{ab}	4^{b}	6^{cd}	2^{b}
Tw	3^{b}	3^{c}	4^{b}	3^{ab}	2^{b}	4^{b}	3^{bc}	2^{b}	2^{ab}	3^{b}
Se	4^{bc}	4^{c}	$\boxed{6^{c}}$	2^{a}	5^{cd}	$\boxed{1^{a}}$	$\boxed{1^{a}}$	3^{b}	4^{bcd}	5^{bc}
Is	5^{c}	5^{cd}	5^{b}	4^{ab}	4^{cd}	6^{cd}	6^{bc}	5^{b}	3^{abc}	6^{bc}
Si	6^{d}	6^{d}	3^{b}	7^{b}	6^{d}	7^{d}	7^{c}	–	7^{d}	4^{bc}
Gh	7^{e}	7^{e}	7^{d}	6^{ab}	7^{e}	$\boxed{5^{bc}}$	4^{bc}	6^{c}	5^{cd}	7^{c}
Mean weight (g)		120.8	54.7	36.4	74.6	9.4	26.8	72.8	27.8	31.4
N (total)		578	525	210	1757	658	89	439	103	143
Environment rank		1	4	5	2	11	9	3	8	7

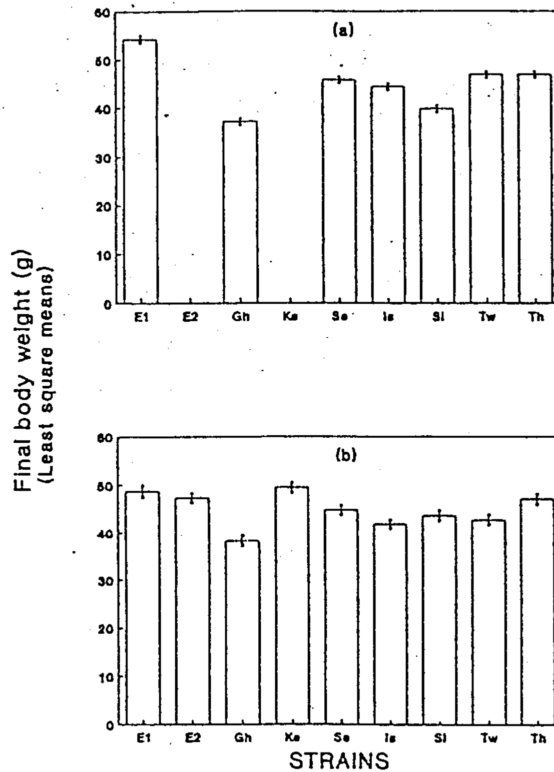

Fig. 2. Histogram of genotype means (LSM of final body weights of each strain across different test environments) during (a) first generation, and (b) second generation. Vertical bars are standard errors. See Tables 1 and 2 for explanation of strain codes.

strains performed better or as well as the best of the 'domesticated' Philippine strains. During the first generation, E1 was the fastest growing strain and Gh the slowest. In the second generation, the Ke strain emerged as the fastest growing strain and Gh was again the slowest. LSM of strains E1, E2 and Ke were not significantly different, however. Growth performances of other strains were intermediate.

Of particular importance for the present study is the amount of variation explained by the term strain×test environment, the magnitude of which is low (0.3% of the total variation in both generations). This interaction term contains both rank interaction and magnitude interaction (i.e., the best strains are relatively more superior in some environments than in others).

The relative rankings of strains in each of the different test environments during the first and second generations are presented in Tables 9 and 10, respectively. Rank interaction seems to be of minor importance ($P<0.05$), except that strain Se ranks higher in extremely poor environments (C1, C2 and

TABLE 10

Second generation: rank-order and significance of ranks for final body weight of strains across and within test environments (Ranks within test environments sharing the same superscript letters are not significantly different $P<0.05$; significant re-rankings of strains compared to rank across test environments are in boxes)

Strain	Rank	Rank within test environments							
		S1	S2	S3*	P1	P2	C3	W2	W4
Ke	1^{a}	1^{a}	1^{a}	–	$\boxed{4^{bc}}$	2^{ab}	1^{a}	4^{a}	1^{a}
E1	2^{a}	5^{bc}	3^{ab}	–	1^{a}	1^{a}	$\boxed{9^{c}}$	2^{a}	4^{ab}
E2	3^{ab}	2^{b}	7^{bc}	–	2^{b}	3^{ab}	4^{abc}	1^{a}	2^{ab}
Th	4^{ab}	3^{b}	$\boxed{2^{a}}$	–	3^{b}	4^{abc}	2^{ab}	3^{a}	6^{bc}
Se	5^{bcd}	4^{b}	5^{abc}	–	5^{bc}	6^{bcd}	3^{abc}	6^{ab}	5^{ab}
Si	6^{cde}	9^{c}	6^{abc}	–	7^{bc}	5^{abcd}	7^{c}	5^{ab}	3^{ab}
Tw	7^{de}	6^{bc}	4^{ab}	–	6^{bc}	7^{bcd}	6^{c}	8^{ab}	7^{bc}
Is	8^{e}	7^{bc}	8^{bc}	–	8^{c}	8^{cd}	5^{bc}	7^{ab}	8^{bc}
Gh	9^{f}	8^{bc}	9^{c}	–	9^{d}	9^{d}	8^{c}	9^{b}	9^{c}
Mean weight (g)		33.1	26.5	–	68.3	47.2	16.6	73.0	49.0
N (total)		288	306	–	547	576	108	144	191

*Heavy mortality; excluded from analysis.

RF during the first generation, Table 9). The re-ranking of strain E1 in environment C2 during the second generation (Table 10) might be explained by the fact that only three fish in this particular cell survived, and that mortality may not be random. The remaining significant re-rankings do not seem to be systematic, but occur randomly, as can be expected in a large experiment like the present study.

The relative growth performance of each of the strains across different environments, expressed as a deviation of LSM of body weights of strains at harvest from the respective environment means during the first generation is presented in Fig. 3. This reveals: consistency in growth performance of individual strains across test environments with slight changes in relative ranking; and the potential for growth and the magnitude of possible differential growth performance among strains across a wide range of environments. Strain E1 was consistently the best performing strain and Gh the poorest performing strain.

DISCUSSION

Asian fish farming systems are, and will probably remain, diverse. They range from simple backyard, resource-limited fish ponds to relatively inten-

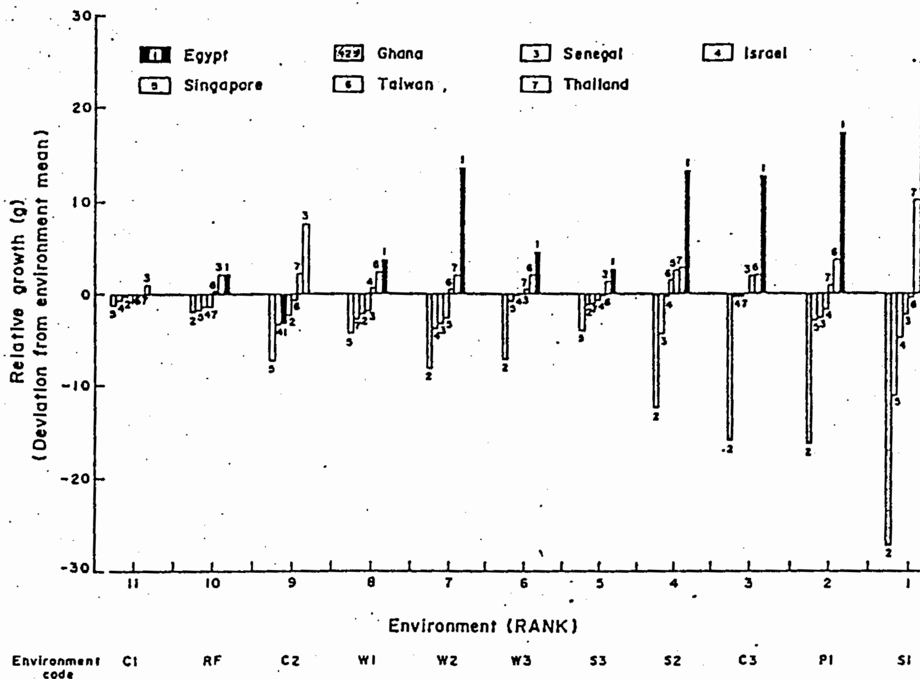

Fig. 3. First generation: relative growth performance of seven strains of *Oreochromis niloticus* in different test environments. Relative growth performance has been calculated as a deviation of LSM of final body weights of each strain from the respective environment means (from Table 7). See Tables 3 and 4 for explanation of environment codes.

sive fish farming systems. Along this continuum, there are other farming systems that include systems utilizing different fertilizer inputs such as on-farm crop residues and other animal wastes, rice–fish culture, cage culture, etc. Rigorous evaluation of promising strains across these diverse farming systems, which is further compounded by various agroclimatic conditions under which each of the different strains is to be tested, is a major task attempted here. The relative performance of a number of potential strains in each of the different target environments and the magnitude of genotype×environment interaction will determine whether or not specialized strains are necessary for each of the farming systems.

Results here from two consecutive generations of testing to evaluate the growth performance of eight strains of Nile tilapia in different test environments have indicated highly significant differences in growth performance among the strains. With the apparent exception of the Gh strain, the 'wild' African strains, after only one or two generations in captivity, performed as well as or better than the established 'domesticated' strains presently used by Philippine fish farmers. Among the established Philippine farmed stocks, the

strain Is is at present the most widely cultured strain in the Philippines. From results here, it is not the best strain. Earlier growth experiments by Pante et al. (1990) and Capili et al. (1990) involving only three of the four Philippine farmed stocks (Is, Si and Tw) reared in tanks and cages did not show consistent differences in their growth performances. This was largely due to methodological problems encountered during testing (e.g. initial weight differences) and also poor growth and survival of the strains in the environments tested.

The strain×test environment interaction was low but significant (Table 7). The interaction seems to be mainly magnitude interaction (Fig. 3), while rank interaction was less important (Tables 9 and 10). The test environments during first generation growth trials ranged from extremely poor growth conditions to representative tilapia farming conditions. Even in poor test environments, the strain showing the best growth performance in representative farming conditions, strain E1, also exhibited above average growth performance. In environments causing extremely slow growth (less than 30 g final body weight after a grow-out period of 90 days), strain Se ranked higher than the other strains. Since these test environments may not be regarded as normal farm environments, it may be concluded that the best strains across all test environments will also be the best strains in a wide variety of applied tilapia farming environments. Moreover, the total gene action realizable through additive genetic variation in the strain that will be eventually released for culture will be there to be expressed to whatever degree the farm environments allow (Fig. 3).

The possibility of different rank-order of strains in extremely poor environments should be investigated further by extending the grow-out period until the market size is obtained. In an experiment where fish from two slow growth environments in the first generation (S3 and C1) were transferred to normal farming conditions and reared for a further period of 120 days, the rank-order did not differ significantly ($P<0.05$) from those observed here across test environments (Reyes and Eknath, in prep.). One of the important test environments causing poor growth was the rice–fish system (RF). Since the grow-out period in this system is restricted to the flooded period of the rice culture, an extra growth period before stocking or after harvesting of rice might be required. Both strategies should be investigated and strain ranking at market size should be evaluated before firm conclusions on possible strain×test environment interaction in this particular farming system can be drawn.

Interestingly, the effect of differences in initial size and age on subsequent growth performance of fingerlings under communal stocking — a topic of considerable debate — was of limited importance (Tables 7 and 8). However, the batch collection procedure should be recommended to correct for possible variation caused by age differences, initial size differences, effects of rearing separately in hapas until tagging, etc., especially if the difference in

time between collecting the first and the last batch is substantial. The initial size difference alone has been shown to be a poor predictor of later growth performance, when comparisons were made across three batches collected at intervals of 1 week (Palada-de Vera and Eknath, 1993).

Overall, the results here suggest that the growth performance of the strains is relatively consistent across the diverse range of test environments and that development of specialized strains for each of the farming systems is not necessary. The primary objective of the GIFT project is to build a base population with a broader genetic base and initiate a genetic improvement program to develop a more productive tilapia. The approach is to test these strains in crossbreeding to evaluate non-additive genetic effects (heterosis). This will determine the breeding strategy — purebreeding or crossbreeding — to be followed. If heterosis turns out to be of lesser importance for economically important traits, then the best performing strains and their crosses will be pooled together to form a mixed base population ('synthetic' strain). These aspects will be examined in subsequent papers.

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REFERENCES

Capili, J.B., Luna, S.B. and Palomares, M-L.D., 1990. A multivariate analysis of the growth of three strains of tank-reared tilapia *Oreochromis niloticus*. In: R. Hirano and I. Hanyu (Editors), The Second Asian Fisheries Forum, Asian Fisheries Society, Manila, Philippines, pp. 425–428.

Macaranas, J.M., Taniguchi, N., Pante, M-J.R., Capili, J.B. and Pullin, R.S.V., 1986. Electrophoretic evidence for extensive hybrid gene introgression into commercial *Oreochromis niloticus* (L.) stocks in the Philippines. Aquacult. Fish. Manage., 17: 249–258.

Macaranas, J.M., Eknath, A.E., Augustin, L.Q., Velasco, R.R., Ablan, M.C.A. and Pullin, R.S.V., 1993. Genetic improvement of farmed tilapia: documentation and genetic characterization of strains. Aquaculture, 111: 296.

Maclean, J.L., 1984. Tilapia — the aquatic chicken. ICLARM Newsletter, 7(1): 17.

Palada-de Vera, M.S. and Eknath, A.E., 1993. Predictability of individual growth rates in tilapia. Aquaculture, 111: 147–158.

Pante, M.J.R., Macaranas, J.M., Capili, J.B., Tayamen, M.M., Bimbao, M-A.P., Ablan, M.C.A. and Pullin, R.S.V., 1990. Development of research methods to evaluate Nile tilapia (*Oreochromis niloticus*) strains in the Philippines. Aquaculture, 85: 324–325.

Pullin, R.S.V., 1985. Tilapias: 'everyman's fish'. Biologist, 32(2): 84–88.

Pullin, R.S.V. (Editor), 1988. Tilapia genetic resources for aquaculture. ICLARM Conference Proceedings 16. International Center for Living Aquatic Resources Management, Manila, Philippines, 108 pp.

Pullin, R.S.V. and Capili, J.B., 1988. Genetic improvement of tilapias: problems and prospects. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Philippines, pp. 259–266.

Smith, I.R. and Pullin, R.S.V., 1984. Tilapia production booms in the Philippines. ICLARM Newsletter, 7(1): 7–9.

Trewavas, E., 1983. Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. Br. Mus. (Nat. Hist.), London. 583 pp.

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Growth and reproduction of individually tagged Nile tilapia (*Oreochromis niloticus*) of different strains*

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ABSTRACT

Bolivar, R.B., Eknath, A.E., Bolivar, H.L. and Abella, T.A., 1993. Growth and reproduction of individually tagged Nile tilapia (*Oreochromis niloticus*) of different strains. *Aquaculture*, 111: 159–169.

Growth and reproduction traits were observed in seven strains of *Oreochromis niloticus* from Egypt, Ghana, and Senegal (bred from wild collected stocks recently imported to the Philippines from Africa), and 'Israel', 'Singapore', 'Taiwan' and 'Thailand' (strains maintained for aquaculture purposes in the Philippines), respectively. Twenty individually tagged fish were stocked separately for each strain in 1-m^3 fine mesh hapas installed in outdoor concrete tanks and weighed every 2 weeks from 60 days to 210 days.

There were no significant differences ($P<0.05$) in growth among strains with the exception of Ghana which showed a significantly lower body weight at 210 days ($P<0.05$). Based on ages at first spawning, three phenotypes of females were distinguished: early spawning, late spawning and females that did not reproduce during the experiment (virgin females). Growth performances of late spawning and virgin females were nearly equal to the male growth performance in some of the strains tested. This indicates that if reproduction can be delayed in the females, average growth rates comparable to those of an all-male population might be achieved.

Phenotypic correlations observed between age and female body weight at first spawning ranged from 0.77 to 0.99; age and number of eggs at first spawning, 0.30 to 0.81; and female body weight at first spawning with the number of eggs produced, 0.64 to 0.76. Overall, the correlations between these traits were significant ($P<0.05$).

INTRODUCTION

Farmed tilapias often reach maturity before they are large enough to be harvested. This results in uncontrolled reproduction and subsequent reduc-

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tion in growth rate. Consequently, faster growth rate and delayed age at maturation are two important breeding goals.

Evaluation of different tilapia strains for aquaculture purposes has been a subject of many investigations (for a review, see Tave, 1988). The importance of choosing appropriate strains for building base populations has been emphasized. In the Philippines, collaborative research efforts during the past 10 years revealed the poor genetic status of farmed stocks and the necessity to broaden the genetic base upon which a national breeding program could be built (Pullin, 1988; Pullin and Capili, 1988). A program of systematic documentation, evaluation and utilization of tilapia genetic resource to build a national breeding program, commenced with the Genetic Improvement of Farmed Tilapias (GIFT) project (Pullin et al., 1991; Eknath et al., 1993). The chosen strategy was to combine new germplasm from Africa with the farmed strains available in the Philippines. This study was conducted to compare the growth and reproductive performance of three new wild-collected African strains and four 'domesticated' strains of Nile tilapia (*Oreochromis niloticus*) and to determine the phenotypic correlations between growth and reproduction traits in these seven strains.

MATERIALS AND METHODS

Strains of *O. niloticus* currently being screened by the collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT) were used in this study: Egypt, Ghana, Senegal (bred from wild-collected fish, recently imported to the Philippines from Africa) and four established Asian farmed strains popularly known in the Philippines as 'Israel', 'Singapore', 'Taiwan' and 'Thailand' strains. The origins of these strains were described by Eknath et al. (1993). Single-pair mating for each strain was done in 1-m^3 breeding hapas installed in earthen ponds. Fry were collected and reared in hapas to a size of about 3 to 5 g (approximately 60 days from swim-up fry). A random sample of 20 fish from each strain was individually tagged using Floy fingerling tags attached by a nylon loop through the dorsal musculature. Each strain was stocked separately in 1-m^3 hapas installed in outdoor concrete tanks ($1 \times 2.5 \times 1$ m) at the Freshwater Aquaculture Center. Two hapas were arranged per tank with a flow-through water system. This experiment was conducted from July 1989 to February 1990.

The fish were fed daily with pelleted feeds (70% rice bran and 30% fish meal) at the rate of 20% fish body weight per day up to 90 days of culture and reduced to 10% thereafter. The amount of feed given was adjusted every 2 weeks. The breeding condition of females was observed daily, based on the presence of eggs or fry in the mouth or on the condition of the genital papilla which still may be swollen even a few days after egg release. Numbers of eggs

or fry were counted and weight of spawners recorded. Individual weights of all fish were recorded every 2 weeks.

The age at first spawning of females was highly variable, and ranged from as early as 30 days to as late as 180 days (Table 1). Small-scale tilapia farmers in the Philippines usually practise a grow-out period of about 3 months. Early or 'precocious' spawning in such small grow-out ponds before harvest is common. Therefore, based on the observed ages at first spawning, three categories or 'phenotypes' of females were distinguished in the seven strains: early spawning, females spawning 30–60 days after stocking; late spawning, females spawning 90–180 days after stocking; and virgin females, females that did not spawn during the experiment (210 days). The numbers of each of the three female phenotypes and males within strains are given in Table 2. The numbers of females were rather small. This was due to: (i) the relatively small

TABLE 1

Ages at first spawning and the numbers of females spawning at each period in seven strains of *Oreochromis niloticus*

Strains	Ages at first spawning (day)								
	Early spawning			Late spawning					
	30	45	60	90	120	135	150	165	180
Egypt	0	0	1	0	0	0	0	0	0
Ghana	0	3	3	2	1	0	1	0	0
Senegal	0	5	1	1	0	0	0	0	0
Israel	0	1	1	0	0	0	0	0	0
Singapore	0	1	2	0	0	1	0	2	1
Taiwan	1	1	0	0	1	0	0	0	0
Thailand	0	1	1	1	1	0	0	0	0

TABLE 2

Numbers of early and late spawning females, virgin females and males in seven strains of *Oreochromis niloticus* after 210 days of rearing in hapas in tanks

Strains	Early	Late	Virgin	Total females	Total males
Egypt	1	0	12	13	3
Ghana	6	4	3	13	7
Senegal	6	1	7	14	5
Israel	2	0	6	8	8
Singapore	3	4	4	11	5
Taiwan	2	1	7	10	10
Thailand	2	2	6	10	9

numbers of fish stocked initially to facilitate frequent observations of all individuals; (ii) the impossibility of determining the sex of the fish at stocking because of small size — although skewed sex ratios were eventually observed in only three strains; and (iii) the fact that tilapias generally do not spawn readily in small containers.

The following traits were studied for females only: body weight at stocking, age at first spawning, age at second spawning, number of eggs produced at every spawning episode, body weight at every spawning and final body weight. Pearson correlation analysis was done to determine relationships between these traits. The pre-spawning and post-spawning growth rates for early- and late spawning females also were examined. Final body weights of all strains and the different phenotypes of females were analyzed using generalized linear models (GLM) procedure.

RESULTS

Growth

Growth curves of the three female phenotypes and males within 'wild type' African strains and 'domesticated' Philippine strains are presented in Fig. 1 and Fig. 2, respectively. Least Square Means (LSM) of body weight of different female groups and males are shown in Table 3a. There were significant differences in initial weights between strains. This, however, did not affect later growth performance (GLM analysis; $P<0.05$).

There were no significant differences ($P<0.05$) in growth between strains with the exception of Ghana which had significantly lower body weight at 210 days ($P<0.05$). LSM of body weight at 210 days were not significantly different among males and among early spawning females. Within late spawners and virgin females, Singapore and Senegal strains, respectively, were significantly heavier than all other strains. Across all strains, LSM of males were significantly higher than virgin females, late spawning and early spawning females. The virgin females were also significantly heavier than the late spawning females ($P<0.01$). There was, however, no significant difference between late and early spawners.

A matrix of statistical differences in LSM of body weights between different female phenotypes and males, within strains, is presented in Table 3b. Except for the Israel and Taiwan strains, the growth of virgin females equalled that of males. For the Singapore, Taiwan and Thailand strains, growth of late spawning females also equalled that of males. While mean body weights at first spawning of early spawners were not significantly different, these were variable among late spawners (Table 4).

Pre-spawning and post-spawning growth rates of early and late spawning females, and growth rate of virgin females and males are presented in Table 5. Overall, there were no significant differences ($P<0.05$) in pre- and post-

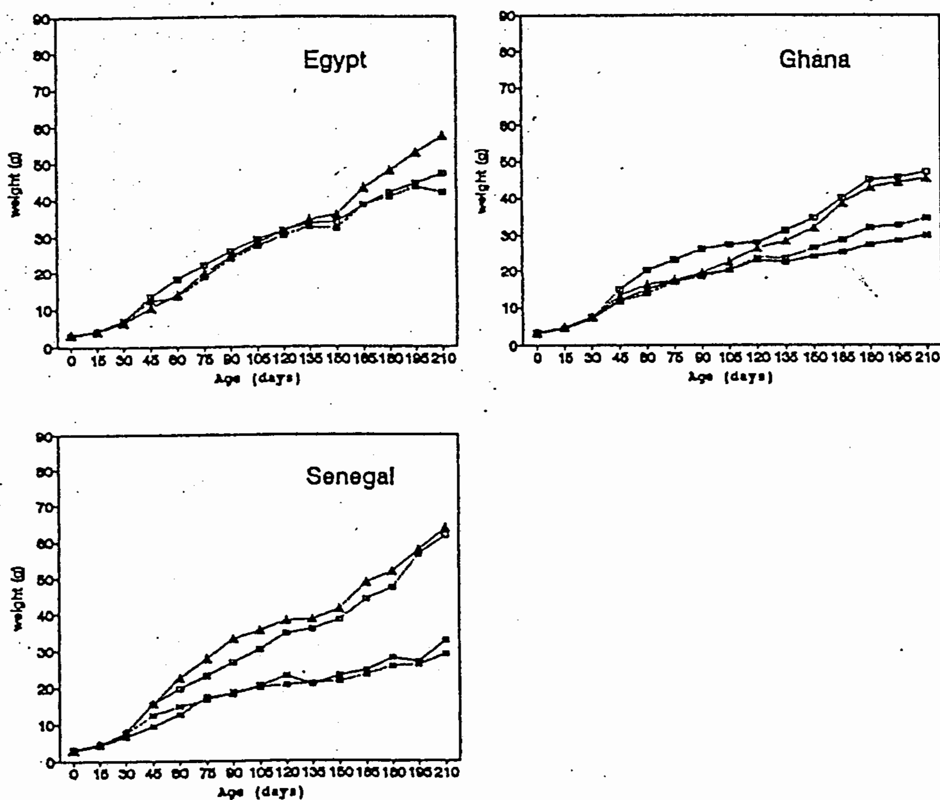

Fig. 1. Progeny of wild-collected African strains of *O. niloticus* — growth curves of early spawning females (■···■), late spawning females (■——■), virgin females (□···□) and males (▲···▲).

spawning growth rate of early spawners. While growth rate of virgin females and post-spawning growth rate of late spawners were not significantly different ($P<0.05$), the post-spawning growth rate of the latter was considerably lower and equalled those of early spawning females. Growth rates of virgin females and males were not significantly different ($P<0.05$). Among strains, except for the Senegal strain, the pre- and post-spawning growth rates were not significantly different among early spawning females. Among late spawning females, significant differences were observed only in Ghana and Thailand strains. Between virgin females and males, significant differences in growth rates were observed only for the Israel and Taiwan strains.

Age at first spawning

The ages at first spawning and the numbers of females, spawning at each period, within strains and two female phenotypes (early spawning and late spawning) are presented in Table 1. The total numbers of the three female

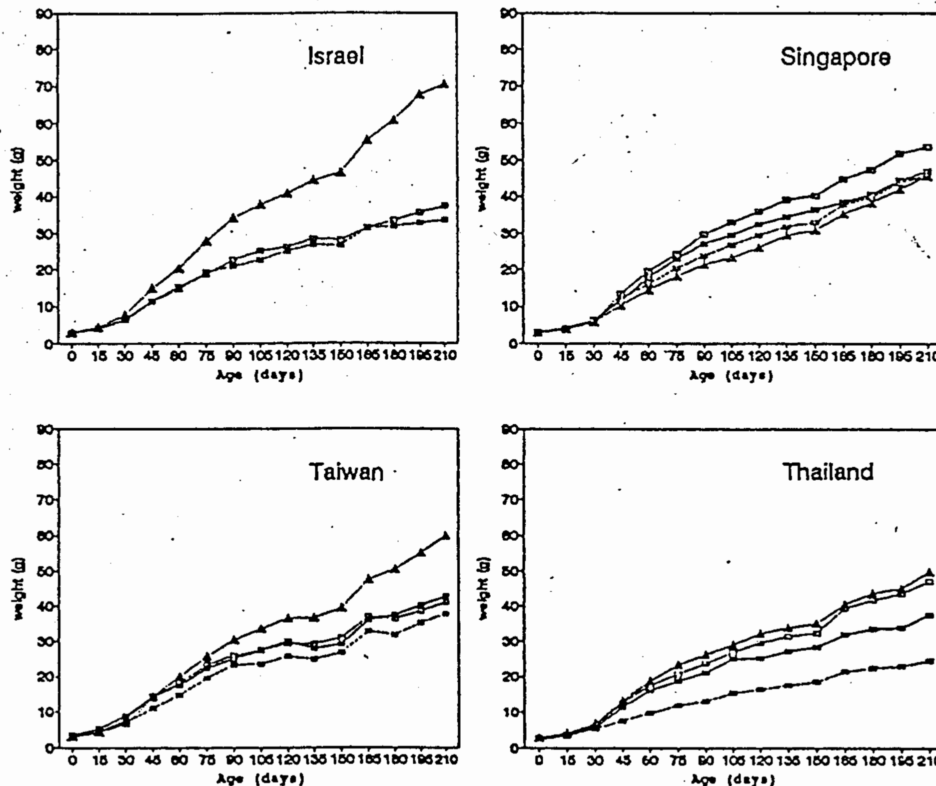

Fig. 2. 'Domesticated' strains of *O. niloticus* — growth curves of early spawning females (◘···◘), late spawning females (◘——◘), virgin females (□···□) and males (▲···▲).

phenotypes and males are presented in Table 2 (for a discussion on the rationale for categorization of female phenotypes, see Materials and Methods).

The proportions of early spawning, late spawning and virgin females to the total number of females observed across all strains at 210 days were 28%, 15% and 57%, respectively. Of the spawning females, 65% were early spawners (n=22) and 35% were late spawners (n=12). The modal ages at first spawning of early- and late spawning females were 45 days and 135 days, respectively.

Highest proportions of early spawning females were observed in Ghana and Senegal strains (46% and 42%, respectively), the lowest in the Egypt strain (only one female), while the rest showed intermediate values (25–30%). The proportion of virgin females was highest for the Egypt strain, lowest for Ghana and Singapore and intermediate for other strains. Late spawning females were observed only for the Ghana, Senegal, Singapore, Taiwan and Thailand strains.

The number of eggs produced by late spawning females (244±27 eggs/female; approximately 10 901 eggs/kg body weight) was significantly higher

TABLE 3a

Least Square Means (LSM) of final body weights (g) of early spawning females, late spawning females, virgin females and males in seven strains of *Oreochromis niloticus* (standard errors in parentheses)

Strains	All sexes	Females			Males
		Early	Late	Virgin	
Egypt	49.02[a]	41.90[a]	—	47.55[a]	57.27[a]
	(3.34)	(9.57)	—	(2.76)	(5.53)
Ghana	38.16[b]	34.60[a]	29.92[a]	47.20[a]	45.42[a]
	(3.58)	(4.79)	(4.79)	(6.77)	(4.78)
Senegal	51.46[a]	29.04[a]	32.70[a]	61.48[b]	63.60[a]
	(3.16)	(4.28)	(9.57)	(3.62)	(4.28)
Israel	51.29[a]	33.60[a]	—	37.53[a]	70.45[a]
	(4.47)	(6.77)	—	(5.53)	(4.79)
Singapore	47.97[a]	45.96[a]	47.02[b]	53.47[a]	45.54[a]
	(3.55)	(5.53)	(4.79)	(4.79)	(4.28)
Taiwan	51.08[a]	32.60[a]	42.70[a]	41.02[a]	59.80[a]
	(3.16)	(9.57)	(9.57)	(3.91)	(3.03)
Thailand	45.65[a]	26.90[a]	41.65[a]	47.02[a]	49.80[a]
	(3.07)	(6.77)	(6.77)	(3.91)	(3.19)
Overall mean		34.03	41.86	48.21	55.99
		(2.53)	(3.79)	(1.85)	(1.80)

LSM with the same superscript are not significantly different ($P<0.05$) from the reference strain: Egypt (early, virgin and males) and Ghana (late).

TABLE 3b

Matrix of statistical differences in body weight at 210 days (from Table 3a) between female phenotype (early, late and virgin female) and males: 1 — early and late spawners, 2 — early and virgins, 3 — early and males, 4 — late and virgins, 5 — late and males, and 6 — virgin females and males

Strains	1	2	3	4	5	6
Egypt		ns	ns			ns
Ghana	ns	ns	ns	*	*	ns
Senegal	ns	*	*	*	*	ns
Israel		ns	*			*
Singapore	ns	ns	ns	ns	ns	ns
Taiwan	ns	ns	*	ns	ns	*
Thailand	ns	*	*	ns	ns	ns
Overall	ns	*	*	*	*	*

*Significant at $P<0.05$; ns, not significant.

($P<0.01$) than that of the early spawning females (131±15 eggs/female, approximately 8953 eggs/kg body weight). The number of eggs produced by the same female increased steadily during subsequent spawning.

TABLE 4

Mean body weights (g) at first spawning and at harvest for early and late spawning females in seven strains of *Oreochromis niloticus* after 210 days of rearing in hapas in tanks (standard errors in parentheses)

Strains	Early	Late
Egypt	6.50	—
Ghana	9.90	19.20
	(1.22)	(2.85)
Senegal	8.10	18.20
	(0.57)	
Israel	9.70	—
	(2.40)	—
Singapore	11.30	36.20
	(3.17)	(4.75)
Taiwan	8.00	29.80
	(2.59)	
Thailand	8.30	23.60
	(0.35)	(2.83)

TABLE 5

Growth rates (g/day) of early and late spawning females, virgin females and males in seven strains of *Oreochromis niloticus* after 210 days of rearing in hapas in tanks (standard errors in parentheses)

Strains	Early		Late		Virgin	Males
	Pre-s[1]	Pos-s[2]	Pre-s	Pos-s		
Egypt	0.22	0.17	—	—	0.21	0.26
	(0.04)	(0.04)	—	—	(0.02)	(0.02)
Ghana	0.17	0.14	0.17	0.10	0.21	0.20
	(0.02)	(0.02)	(0.02)	(0.02)	(0.04)	(0.01)
Senegal	0.15	0.09	0.20	0.12	0.28	0.29
	(0.02)	(0.02)	(0.04)	(0.04)	(0.02)	(0.01)
Israel	0.12	0.14	—	—	0.17	0.32
	(0.03)	(0.03)	—	—	(0.03)	(0.01)
Singapore	0.15	0.09	0.22	0.19	0.24	0.20
	(0.03)	(0.02)	(0.02)	(0.02)	(0.03)	(0.01)
Taiwan	0.09	0.14	0.23	0.20	0.18	0.27
	(0.03)	(0.04)	(0.04)	(0.04)	(0.02)	(0.01)
Thailand	0.09	0.11	0.26	0.11	0.21	0.22
	(0.03)	(0.03)	(0.03)	(0.03)	(0.02)	(0.01)
Mean	0.14	0.13	0.21	0.14	0.22	0.25
	(0.01)	(0.01)	(0.01)	(0.02)	(0.01)	(0.01)

[1]Pre-spawning growth rates. [2]Post-spawning growth rates.

TABLE 6

Correlation coefficients among growth and reproduction traits in five strains of *Oreochromis niloticus* after 210 days of rearing in hapas in tanks

Traits	Overall	Strains				
		Ghana	Senegal	Singapore	Taiwan	Thailand
INBWT-AGESP1	−0.25	−0.46	−0.80**	−0.03	0.55	0.69
INBWT-BWSP1	−0.16	−0.51	−0.79**	0.15	0.64	0.87
INBWT-EGGSP1	−0.15	−0.35	−0.82**	−0.57	0.94	0.62
INBWT-FBDWT	−0.43**	−0.46	0.21	−0.02	0.50	−0.26
BWSP1-AGESP1	0.92**	0.97**	0.77*	0.92**	0.99*	0.87
FBDWT-AGESP1	0.44**	0.38	0.06	0.03	0.99	0.34
BWSP1-EGGSP1	0.32*	0.64*	0.76*	−0.08	0.87	0.71
BWSP1-FBDWT	0.49**	0.47	−0.19	0.34	0.98	0.26
FBDWT-EGGSP1	0.15	0.40	−0.17	−0.18	0.77	0.20

*$P<0.05$; **$P<0.01$. [1]INBWT — initial body weight, AGESP1 — age at first spawning, BWSP1 — body weight at first spawning, EGGSP1 — number of eggs at first spawning, FBDWT — final body weight.

Correlations among traits

Only five strains were included in the correlation analysis because only one spawning female was observed from the Egypt strain and only two from the Israel strain. The phenotypic correlations among growth and reproduction traits are presented in Table 6. Overall correlation coefficients were positive and significant for age at first spawning with body weight at first spawning (0.92), age at first spawning with number of eggs at first spawning (0.33), age at first spawning with final body weight of females (0.44), and body weight at first spawning with the number of eggs at first spawning (0.49).

DISCUSSION

Although the sample size used in this study was relatively small, the general observations on growth performance conform to the major experiments conducted under the GIFT project (Eknath et al., 1993). Significant differences in growth performance among strains could not be detected here because of the relatively stressful conditions of rearing in small hapas. This is consistent with the observations of Reyes and Eknath (1991). Initial size did not seem to affect later growth performance (Palada-de Vera and Eknath, 1993). Moreover, the Ghana strain was the poorest performing strain, as found throughout the GIFT project experiments.

The observation of three possible phenotypes among females with respect to first spawning may have been influenced by environmental and behavioral interactions. However, no aggressive behavior by males was observed. In platyfish, Kallman and Schriebman (1973) found specific genes responsible for

early/late maturity. A similar genetic mechanism may be present in tilapia. This needs further investigation. It was interesting to note that the growth performance of late spawning and virgin females was similar to that of males in some of the strains tested. This indicates that if reproduction can be delayed in the females, average growth rates comparable to those of an all-male population might be achieved.

The phenotypic correlations obtained in this study support the general observations on the relationship among growth and reproductive traits of female *O. niloticus*. Several authors have reported an increase in number of eggs produced by larger, older tilapia females (Botros, 1969; Peters, 1983; Siraj et al., 1983; Watanabe and Kuo, 1985; Galman et al., 1988). However, Cisse (1988) found no significant correlations between the weight of a spawner and the number of eggs per spawning. Strain-specific differences in reproduction performance were reported by Smitherman et al. (1988). One of the immediate plans of the on-going GIFT project is to study growth and reproduction under commercial production conditions with the ultimate goal of producing late-maturing tilapias with high growth potential, the prospects for which appear to be promising (Kronert et al., 1989; Oldorf et al., 1989).

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REFERENCES

Botros, G.A., 1969. A comparative study on the fecundity of *Tilapia nilotica* L. and *T. zillii* Gerv. from Lake Mariut (Egypt). Rev. Zool. Bot. Afr., LXXIX: 3–4.

Cisse, A., 1988. Effects of varying protein levels on spawning frequency and growth of *Sarotherodon melanotheron*. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, The Philippines, pp. 329–333.

Eknath, A.E., Tayamen, M.M., Palada-de Vera, M.S., Danting, J.C., Reyes, R.A., Dionisio, E.E., Capili, J.B., Bolivar, H.L., Abella, T.A., Circa, A.V., Bentsen, H.B., Gjerde, B., Gjedrem, T. and Pullin, R.S.V., 1993. Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. Aquaculture, 111: 171–188.

Galman, O.R., Moreau, J. and Avtalion, R., 1988. Breeding characteristics and growth performance of Philippine red tilapia. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, The Philippines, pp. 169–175.

Kallman, K.D. and Schreibman, M.P., 1973. A sex-linked gene controlling gonadotrop differentiation and its significance in determining the age of sexual maturation and size of the platyfish, *Xiphophorus maculatus*. Gen. Comp. Endocrinol., 21: 287–304.

Kronert, U., Horstgen-Schwark, G. and Langholz, H.-J., 1989. Prospects of selecting for late maturity in tilapia (*Oreochromis niloticus*). I. Family studies under laboratory conditions. Aquaculture, 77: 113–121.

Oldorf, W., Kronert, U., Balarin, J., Haller, R., Horstgen-Schwark, G. and Langholz, H.-J. 1989. Prospects of selecting for late maturity in tilapia (*Oreochromis niloticus*). II. Strain comparison under laboratory and field conditions. Aquaculture, 77: 123–133.

Palada-de Vera, M.S. and Eknath, A.E., 1993. Predictability of individual growth rates in tilapia. Aquaculture, 111: 147–158.

Peters, H.M., 1983. Fecundity, egg weight and oocyte development in tilapia (Cichlidae, Teleostei). ICLARM Translations 2, International Center for Living Aquatic Resources Management, Manila, Philippines, 28 pp.

Pullin, R.S.V. (Editor), 1988. Tilapia genetic resources for aquaculture. ICLARM Conference Proceedings 16. International Center for Living Aquatic Resources Management, Manila, The Philippines, 108 pp.

Pullin, R.S.V. and Capili, J.B., 1988. Genetic improvement of tilapias: problems and prospects. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 259–266.

Pullin, R.S.V., Eknath, A.E., Gjedrem, T., Tayamen, M., Macaranas, J. and Abella, T.A., 1991. The Genetic Improvement of Farmed Tilapia (GIFT) Project. The Story So Far. NAGA The ICLARM Quarterly, 14(2): 3–6.

Reyes, R.A. and Eknath, A.E., 1991. Environmental effects on expression of genetic potential for growth in seven strains of Nile tilapia (*Oreochromis niloticus*) and its implications for applied breeding programs. Paper presented at the Fourth International Symposium on Genetics in Aquaculture, Wuhan, China, 29 April to 3 May 1991.

Siraj, S.S., Smitherman, R.O., Castillo-Galluser S. and Dunham, R.A., 1983. Reproductive traits for three year classes of *Tilapia nilotica* and maternal effects of their progeny. In: L. Fishelson and Z. Yaron (Compilers), International Symposium on Tilapia in Aquaculture Proceedings. Tel Aviv University, Tel Aviv, Israel, pp. 210–218.

Smitherman, R.O., Khater, A.A., Cassell, N.I. and Dunham, R.A., 1988. Reproductive performance of three strains of *Oreochromis niloticus*. Aquaculture, 70: 29–37.

Tave, D., 1988. Genetics and breeding of tilapia: a review. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture. ICLARM Conference Proceedings 15, Department of Fisheries, Bangkok, Thailand, and International Center for Living Aquatic Resources Management, Manila, Philippines, pp. 285–293.

Watanabe, W.O. and Kuo, C.-M., 1985. Observations on the reproductive performance of Nile tilapia (*Oreochromis niloticus*) in laboratory aquaria at various salinities. Aquaculture, 49: 315–323.

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ABSTRACT

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Aquaculture, 111: 147–158.

Growth curves of 1370 individually tagged fingerl
days) of seven strains of *Oreochromis niloticus*: Egyp
and 'Thailand', reared communally in fertilized ea
weeks during a 90-day production cycle. Predictabi
relation of individual growth performance and also
Positive correlations were observed between size a
correlations, however, decreased towards the end of
weight was highest when body weights of males and
tively. Onset of sexual maturation seemed to reduc
tween sexes set in early in the life cycle, before sexing
Sexing of individuals by size-grading had low succe
result of this study is that growth performance was n

INTRODUCTION

Identification of relatively fast growin
has important applications in genetic se
management decisions pertaining to si
(Gunnes, 1976; Askew, 1978; Malecha e
1987). For selection and size-grading to
derstand the growth characteristics of i
under communal stocking in targeted fi
portantly, relative growth rates of indi

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production cycle should be predictable with a high degree of accuracy (Eknath and Doyle, 1990).

In tilapias, the widely recognized phenomenon of divergent growth performance of males and females (Lowe-McConnell, 1982) adds another dimension to this task of determining predictability of individual growth rates. Sexual maturation further compounds the problem.

The objectives of the present study were to determine: (i) if males and females of Nile tilapia (*Oreochromis niloticus*) could be separated by size-grading relatively early in the production cycle, when sexing based on external morphology is not possible, (ii) if mortality is dependent on initial size, (iii) the size (and age) at which divergence of growth performance between sexes occurs, (iv) predictability of individual growth performance during a production cycle, and (v) the effects of initial size (and age) differences on later growth performance of individuals.

MATERIALS AND METHODS

This study was a part of a major experiment designed to estimate the magnitude of genotype×environment interaction when seven strains of Nile tilapia (*Oreochromis niloticus*) were reared in eleven different test environments (Eknath et al., 1993).

Altogether 1370 individually tagged fingerlings of three newly imported African strains (Egypt (E1), Ghana (Gh), Senegal (Se)) and four established Philippine farmed stocks (locally known as 'Israel' (Is), 'Singapore' (Si), 'Taiwan' (Tw), and 'Thailand' (Th)) were communally reared in fertilized earthen ponds at the Bureau of Fisheries and Aquatic Resources, National Freshwater Fisheries Technology Research Center, Muñoz, Nueva Ecija, the Philippines. The origin of strains and experimental set-up are described in detail by Eknath et al. (1993).

Single-pair mating (25 breeding pairs of each strain) was done in 175 1-m^3 hapas installed in breeding ponds. The progeny from each strain were reared separately in hapas until they reached a mean body weight of 3–5 g. The fish were individually tagged with modified Floy fingerling tags and reared in earthen ponds for 90 days. The age and initial body weights of tagged fingerlings at stocking ranged from 78 to 119 days, and 1.8 to 14.6 g, respectively. Regular sampling to record individual body weights was done every 21 days.

Predictability of individual growth rates by strain and sex was determined by Pearson correlation analysis between body weights at initial stocking, at successive samplings, and at harvest. Based on the mean ($\bar{X}$) and standard deviation (σ) of initial body weights (BW), the fish were classified into four size groups, as follows:

Size group 1 (SG1) $BW < -1\sigma$ (range 1.8–3.1 g)

Size group 2 (SG2) $\bar{X} > BW > -$

Size group 3 (SG3) $+1\sigma > BW > \bar{X}$

Size group 4 (SG4) $BW > +$

Mortality, sex-ratio and growth tra
monitored.

RESULTS

Sex-ratio and size-specific mortality

Sex-ratios of fish at harvest were not s
1:1 in the different size groups (Table
observed when comparing the sex-ratio
females among smaller groups and mor

In SG1 the mortality across all strain
groups. This was due to a size-depend
particularly E1 and Tw. Survival was h
as recorded here also included tag loss
to be random and not strain- or sex-spe

Sex-specific growth

The growth curves of different strain
growth curves of males and females of
invariably growing faster than females
divergence of growth rates between se
gence of growth rates occur at later ag
the fastest growing strain (E1) than i
arrows in Fig. 1). However, this patter
body weights at harvest are also highly
E1 were significantly heavier while thos
The other strains were intermediate.

Size-specific growth

The growth curves of different size
same strains followed similar trends. T
the different size groups in the entire p
effects through a general linear model
Fig. 2. The following GLM model was

$Y_{ijkl} = a + G_i + S_j + SG_k + e_{ijkl}$

where Y_{ijkl} is the final body weight of t
the effect of the ith strain, S_j is the effe
kth size group, and e_{ijkl} is a random err

TABLE 1

Initial body weights at stocking (g), and percentage mortality (includes tag loss) and sex-ratio at harvest, of the different size groups of *Oreochromis niloticus* grown in earthen ponds at the BFAR/NFFTRC complex. See text for explanation of strain abbreviations

Size group	Size range (g)	n	Mean weight (g)	Strain: Mortality (%)							Pooled[a]	n M:F	Sex ratio (pooled) M:F
				El	Gh	Se	Is	Si	Tw	Th			
1	1.8–3.1	82	2.9	73	23	25	56	43	100	23	39	20:28	0.7:1 ns
2	3.2–4.8	755	4.0	33	19	32	35	30	35	31	30	271:258	1:1 ns
3	4.9–6.6	350	5.6	28	23	24	36	20	24	24	26	135:124	1:1 ns
4	6.7–14.6	183	8.3	37	0	33	35	18	36	34	33	68:54	1.2:1 ns
Pooled[b]	1.8–14.6	1370	4.9	34	20	30	37	28	30	30	30	494:464	1:1 ns

ns: not significantly different from 1:1 sex-ratio ($P<0.05$).
[a]Within size group, across all strains.
[b]Within strain, across all size groups.

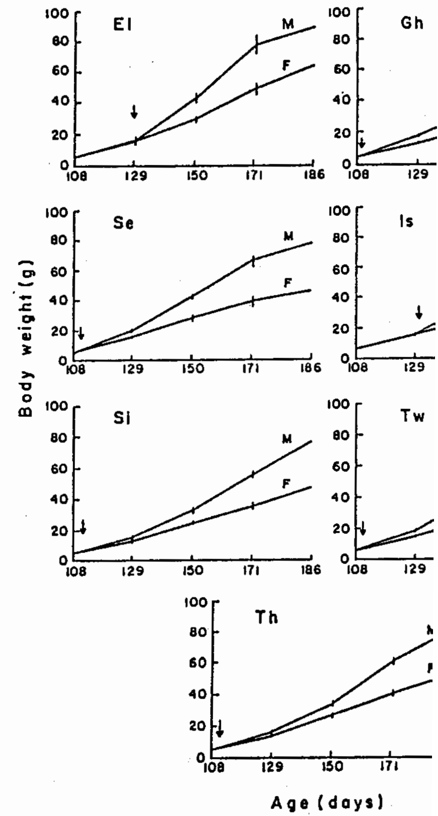

Fig. 1. Growth curves of males (M) and females
grown in earthen ponds at the BFAR/NFFTRC c
divergence of growth curves. Vertical bars are stan

It is clear that the smaller size group
catch up with the largest size groups. I
harvest were not significant ($P<0.01$)
groups were not standardized. Howe
performance were shown to be negl
(Eknath et al., 1993).

Predictability of individual growth

Pearson correlation coefficients betw
ing and body weights at successive sam

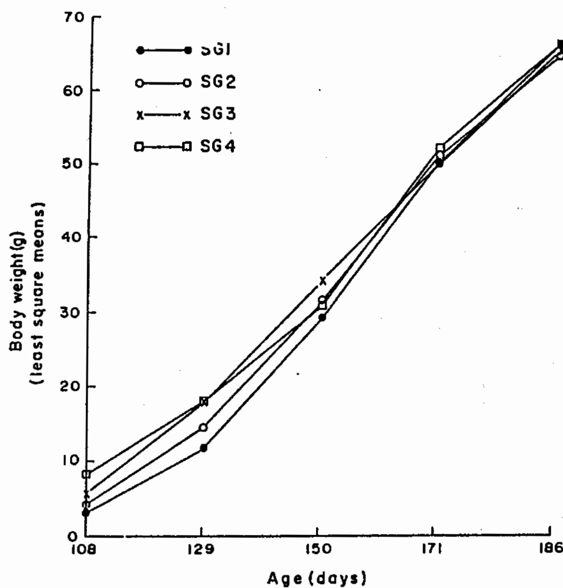

Fig. 2. Growth curves of different size groups of individuals, corrected for strain and sex effects (see text), grown in earthen ponds at the BFAR/NFFTRC complex. See text for explanation of size groups (SG).

are presented in Table 2. In almost all cases, correlation coefficients were not significantly different from zero. Correlations with body weights at later ages than 129 days (21 days after stocking) seemed to differ significantly from zero only at random. Both positive and negative correlations were observed.

Highly significant correlations were observed among body weights at later ages, specifically from age 150 days onwards, when males and females had attained mean body weights of about 25 g and 33 g, respectively. For the entire population, corrected for strain effects, highly significant correlations were observed when males and females had reached mean body weights of 37 and 26 g, respectively. However, in the Gh and Th strains, the correlations between body weights at age 150 and 186 days were not significant (Table 3). This was probably due to strain-specific effects of reproduction on growth.

DISCUSSION

Objective discrimination of sexes of tilapias at an early age is important in genetic experiments to correct for sex-ratios and sex-related differences in growth performance. Since morphological differences between sexes occur

TABLE 2

Correlation coefficients between body weights at stoc males and females of different strains of *Oreochromis* NFFTRC complex. See text for explanation of strain

Strain	Sex	Average age (days)	
		129	1
El	F	0.84**	
	M	0.89**	
Gh	F	0.16	
	M	0.50	
Se	F	0.52	-
	M	0.33	-
Is	F	0.37	-
	M	0.29	-
Si	F	0.85**	
	M	0.48*	
Tw	F	0.53	-
	M	0.46**	
Th	F	0.11	-
	M	0.60**	

**Significant at $P<0.01$;
*Significant at $P<0.05$.

relatively late in the life history of fish, u ity (Chervinski, 1983), discriminatory and shape, mouth parts and gonadal de croscopic examination of gonads to dete tilapias (Alvendia-Casauay and Cariño, tion because of destructive sampling. Th for discrimination of the sexes has beer 1988), but the discriminatory power of weak at early ages. Moreover, it may h where rapid screening of large numbers

The a priori expectation of the present uals at stocking may be predominantly f ble 1). The idea was that by size-gradin designed mechanical graders (Pruginin (slower growing) females could be se stocking. Selective separation of sexes m g but separation is not likely to reach 1C problem of early discrimination of sexe ducing sex-specific marker genes (for ex terns) should be investigated.

TABLE 3

Mean body weights (g) at average age 150 days, and correlation coefficients between body weights at age 150 days and subsequent ages, of males (M) and females (F) of different strains of *Oreochromis niloticus* grown in earthen ponds at BFAR/NFFTRC complex. See text for explanation of strain abbreviations

Strain	Sex	Mean body weight (age 150 days)	Correlation coefficients at:	
			Average age 171 days	Average age 186 days
El	F	29.2	0.55+	0.66**
	M	43.1	0.98**	0.81**
Gh	F	22.6	0.64+	0.14
	M	33.6	0.50*	0.15
Se	F	28.2	0.37	0.55**
	M	42.0	0.21	0.03
Is	F	25.0	0.78**	0.62**
	M	35.4	0.71**	0.52**
Si	F	24.6	0.29+	0.72**
	M	32.9	0.91**	0.60**
Tw	F	25.7	−0.23	0.61**
	M	40.0	0.67**	0.31*
Th	F	27.0	0.34+	0.03
	M	34.1	0.58**	0.11
Pooled	F	26.2	0.37*	0.53*
	M	37.0	0.71*	0.45*

**Significant at $P<0.01$;
*Significant at $P<0.05$;
+Significant at $P=0.1$.

Evidence for size-specific mortality is sketchy, although relatively smaller individuals have been shown to suffer greater mortality rates than larger individuals (Askew, 1978; Newkirk, 1981). Except for El and Tw strains, the higher mortality rates among SG1 individuals did not appear to be strain-specific. Mortality is also not sex-specific as reflected by the 1:1 sex ratio at harvest.

Divergent growth performance of sexes in tilapias is well known (Lowe-McConnell, 1982), but the age or size at which divergence of growth performance occurs has not been unequivocally established. Results presented here indicate that this phenomenon may be strain-specific. Also, the fastest growing strain El had a later onset of divergence than did the slowest growing strain Gh (Fig. 1).

A widely held assumption, based mostly on growth experiments in small containers and often involving rearing of very few numbers of individuals in isolation, is that social hierarchies in farmed fish persist throughout the rearing period. This implies that individual growth rates relative to the rest of the

population are constant for life — slow
Therefore, size-grading of fish, and som
fish culture. However, the few studies
show little or no effect on the subseque
see Gunnes, 1976; McGinty, 1985). Si
penditure and possible discarding of va
with later growth performance (Newkir
growth characteristics of individuals un
simulated farm environments, as carrie

Correlations among growth at succes
have often been found to be low or eve
growth, maturation and environmental
During certain phases of growth, howe
lations (or predictability) have been re
relative growth performance of individ
(mean body weight less than 10 g) is
formance and potential size at harvest.
is greatly increased, however, when th
least 25 and 33 g, respectively, and th
between the sexes has been firmly establ
centrate on this more predictable phase

In comparing the relative growth perf
of fish, the confounding effects of intera
initial age and size differences between
ity among locations and seasons, hav
However, for the purposes of efficient
and reliable prediction of expected gene
communal testing (for a comprehensi
Wohlfarth and Moav, 1985) has been
(for example, Dunham et al., 1982;
problem of behavioral interactions amc
ing and at the same time to avoid replic
proposed the use of 'internal reference
ever, does not fully resolve the proble
between the reference population and
the number of experimental units requ
number of strains tested and the com
crease with the number of strains inclu

The effect of initial size and age diffe
ance has remained a major problem wit
Moav, 1972). The ideal solution would
beginning of experiments. This is not p
lated to the spawning behavior, early g

several techniques have been used to correct for initial size effects: method of minimum variance, computation of constants relating mean initial weight and mean weight gain, and multiple nursing (Wohlfarth and Moav, 1985). Of these, the technique of multiple nursing (manipulation of feeding and stocking to force fish to a common size) originally developed for common carp (Moav and Wohlfarth, 1968) has been recommended for a variety of fish species. The potential problem of compensatory growth following multiple nursing, however, should be investigated. Compensatory growth does not seem to be a problem with common carp (Wohlfarth and Moav, 1985) and catfish (Dunham et al., 1982).

The problems of initial age and size effects are more pronounced in tilapias because of their asynchronous spawning behavior and also because the number of individuals at each spawning is relatively small. Techniques for correction of these effects include: selective sampling of fish of known age (Tave and Smitherman, 1980) or size (Teichert-Coddingtion, 1983) and weight-specific selection or 'collimation' (Doyle and Talbot, 1986). The inadvertent changes brought about by such techniques on the genetic variance of the population and possible indirect selection remain to be evaluated.

A practical approach to this problem is the collection of fry in batches (pooling of fish produced over short periods of time into groups) and differential marking of batches — a technique well known among animal breeders and routinely applied in the Genetic Improvement of Farmed Tilapias (GIFT) project in the Philippines.

Against the background of debate about initial size effects on fish growth experiments, this study has shown that the growth performance of *O. niloticus* appears unaffected by initial size differences within the size range and conditions investigated here.

ACKNOWLEDGEMENTS

This work forms a part of the collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT), co-financed by the Asian Development Bank (RETA 5279) and the United Nations Development Programme Division for Global and Interregional Programmes (INT/88/019). Dr. Hans Bentsen provided critical comments on earlier drafts of the manuscript. We are also grateful to Mr. Tereso Abella, Mr. Jay Maclean and Drs. Roger S.V. Pullin and Terje Refstie, for their comments and suggestions.

REFERENCES

Alvendia-Casauay, A. and Cariño, V.S., 1988. Gonadal sex differentiation in *Oreochromis niloticus*. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Sec-

ond International Symposium on Tilapia i
15, Department of Fisheries, Bangkok, Tha
Resources Management, Manila, Philippin
Askew, C.G., 1978. A generalized growth and
bivalve culture. Aquaculture, 14: 91–104.
Brzeski, V.J. and Doyle, R.W., 1988. A morph
In: R.S.V. Pullin, T. Bhukasaan, K. Tongu
ternational Symposium on Tilapia in Aqu
Department of Fisheries, Bangkok, Thaila
Resources Management, Manila, Philippin
Chervinski, J., 1983. Sexual dimorphism in til
Doyle, R.W. and Talbot, A.J., 1986. Effectiv
culture stocks. Aquaculture, 57: 27–35.
Doyle, R.W. and Talbot, A.J., 1988. Repeatab
R.S.V. Pullin, T. Bhukaswan, K. Tonguthа
national Symposium on Tilapia in Aquacu
partment of Fisheries, Bangkok, Thailand,
sources Management, Manila, Philippines,
Doyle, R.W., Field, C.A. and Basiao, Z., 1990
to compare the growth of genetic strains i
tors), The Second Asian Fisheries Forum,
499–502.
Dunham, R.A., Smitherman, R.O., Chappel
Communal stocking and multiple rearing
Maricult. Soc., 13: 261–267.
Eknath, A.E. and Doyle, R.W., 1990. Repeat
munal stocking: implications for size-grad
(Editors), The Second Asian Fisheries For
pp. 45–48.
Eknath, A.E., Tayamen, M.M., Palada-de Ve
Capili, J.B., Bolivar, H., Abella, T.A., Cir
and Pullin, R.S.V., 1993. Genetic improve
of eight strains of *Oreochromis niloticus* te
111: 171–188.
Gunnes, K., 1976. Effects of size grading you
growth. Aquaculture, 9: 381–386.
Lowe-McConnell, R.H., 1982. Tilapias in fis
McConnell (Editors), The Biology and C
ings 7, International Center for Living A
pines, pp. 83–113.
Malecha, S.R., Polovina, J. and Moav, R., 19
system for year-round culture of the fresh
sity of Hawaii Sea Grant Tech. Rep. UNI
McGinty, A.S., 1984. Suitability of communa
Carib. Food Crops Soc., 19: 259–266.
McGinty, A.S., 1985. Effects of size at stocki
hybrids. J. World Maricult. Soc., 16: 52–5
Moav, R. and Wohlfarth, G.W., 1968. Genet
44 (4); 12–29.
Newkirk, G.F., 1981. On the unpredictability
oyster a runt for life?. In: C. Claus, N. de

of Bivalve Molluscs. Eur. Maricult. Soc. Spec. Publ. No. 7, EMS, Bredene, Belgium, pp. 211–218.

Pruginin, Y. and Shell, E.W., 1962. Separation of sexes of *Tilapia nilotica* with a mechanical grader. Prog. Fish Cult., 24: 37–40.

Tave, D. and Smitherman, R.O., 1980. Predicted response to selection for early growth in *Tilapia nilotica*. Trans. Am. Fish. Soc., 109: 439–445.

Teichert-Coddington, D., 1983. Bidirectional mass selection for rapid prematuration growth in *Tilapia nilotica*. M.S. thesis, Auburn University, Alabama, USA, 33 pp.

Wickins, J.F., 1987. Effects of size, culling and social history on growth of cultured elvers, *Anguilla anguilla* L. J. Fish Biol., 31: 71–82.

Wohlfarth, G.W. and Moav, R., 1972. The regression of weight gain on initial weight. I. Methods and results. Aquaculture, 1: 7–28.

Wohlfarth, G.W. and Moav, R., 1985. Communal testing, a method of testing the growth of different genetic groups of common carp in earthen ponds. Aquaculture, 48: 143–157.

Attachment 5.

Published manuscripts on the Project Activity

"Estimation of the magnitude of non-additive genetic effects (heterosis) and thereby determine the breeding strategy: pure breeding and crossbreeding"

(Generation 2 experiment)

1
2
3
4
5 GENETIC IMPROVEMENT OF FARMED TILAPIAS: GROWTH PERFORMANCE
6 IN A COMPLETE DIALLEL CROSS EXPERIMENT
7 WITH EIGHT STRAINS OF *OREOCHROMIS NILOTICUS***
8
9
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Abstract

A complete diallel cross experiment was carried out with eight strains of Nile tilapia (*Oreochromis niloticus*). The strains represented four wild populations collected from various locations in Africa and four populations that had been reproduced over a large number of generations for tilapia farming in Asia. Growth performance was recorded in a total of 23 739 individually tagged progeny of the 64 different strain combinations after a grow-out period of about 90 days in seven different test environments representing applied farming systems in the Philippines. Least square means of body weight at harvest were computed for each strain combination within and across test environments, and additive genetic effects, maternal (reciprocal) effects and non-additive genetic effects (heterosis) were estimated. The least square mean heterosis of both reciprocals of all strain crosses across all test environments was 4.3 percent, and the cross that expressed the largest non-additive genetic effect showed a least square mean heterosis of 14.0 percent. However, only seven out of the 22 crosses that expressed a significant heterosis were better performing than the best pure strain, and the largest gain was then about 11 percent. The least square mean heterosis within test environments ranged from 0 to 9.6 percent, and the largest heterosis was observed in some of the test environments with poor growth performance. Significant reciprocal effects were observed, showing that some strains performed better as sire strains and others as dam strains. Significant genotype (strain combination) by test environment interactions were also detected The interactions were mainly associated to an expected variation in the magnitude of the differences between genotypes that was proportional to the mean performance in the test environments. However, some examples of re-ranking of the genotypes were observed, mainly associated to the non-additive component of performance. It was concluded that specific crossing schemes, possibly involving specialised sire and dam lines, may improve growth performance in Nile tilapia, but that this improvement will be quite marginal and should be accompanied by selection within the parent strains. Furthermore, the growth performance of such hybrids may be more sensitive to genotype by environment interactions affecting the non-additive component of performance. A regular selection program based on additive genetic performance will normally result in a comparable genetic improvement after a short period of repeated selection, and may then continue beyond the results obtained in the

1 present cross experiment without the complications caused by the laborious dissemination
2 procedures required by a crossbreeding program and the problems caused by a possibly
3 increased environmental sensitivity in the hybrids.
4
5 *Keywords*: Nile tilapia; *Oreochromis niloticus*; cross-breeding; heterosis; genotype by
6 environment interaction
7
8
9 **1. Introduction**
10
11 Tilapias are widely recognized as one of the most important fish species for freshwater
12 aquaculture in a wide range of farming systems from simple small-scale waste-fed fish ponds
13 to intensive culture systems (Pullin, 1985). The adaptability and tolerance of tilapias to a
14 wide range of environments has resulted in a rapid expansion of tilapia farming among
15 resource-poor fish farmers in Asia since the introduction of the species into Asian
16 aquaculture around 1965, and the interest in tilapia farming is increasing in other parts of the
17 world (Pullin, 1994). Among the wide variety of tilapias, Nile tilapia (*Oreochromis niloticus*)
18 is the most common in aquaculture.
19
20 The natural genetic resources of tilapias are restricted to Africa. The Nile tilapia stocks used
21 in Asian aquaculture seems to be descendants of a few introductions of small numbers of
22 fish, mostly through intermediate non-tropical countries, and probably suffering from genetic
23 founder and bottleneck effects (Pullin and Capili, 1988). Since then, the stocks seem to have
24 been affected by uncontrolled introgression of genes, sometimes from feral populations of
25 less desirable tilapia species (Macaranas et al., 1986), and no systematic efforts have been
26 made to control inbreeding. Furthermore, the performance of Asian farmed stocks has not
27 been scientifically evaluated and compared to that of wild African stocks. The need for a
28 systematic effort to secure and to improve further the genetic quality of farmed stocks of Nile
29 tilapia is widely recognized. The long-term goal should be to supply the tilapia farming
30 industry with domesticated breeds that perform as well as the traditional breeds of terrestrial
31 farm animals when compared to their wild ancestors (Bentsen, 1990, Eknath et al., 1991b).

1
2 A collaborative research project called the «Genetic Improvement of Farmed Tilapias»
3 (GIFT) was started in April 1988 (Pullin et al., 1991). The project has been executed by the
4 International Center for Living Aquatic Resources Management (ICLARM) in cooperation
5 with the National Freshwater Fisheries Technology Research Center of the Bureau of
6 Fisheries and Aquatic Resources (BFAR/NFFTRC), Freshwater Aquaculture Center of the
7 Central Luzon State University (FAC/CLSU) and the Institute of Aquaculture Research
8 (AKVAFORSK). During the first phase of the project, the Marine Science Institute of the
9 University of the Philippines (UP/MSI) was an additional collaborator.
10
11 The GIFT project began by comparing the growth performance and survival of four Asian
12 farmed strains and four African wild strains of Nile tilapia in various farm environments on
13 the island of Luzon, Philippines. Relative performance and strain genotype by test
14 environment interactions were evaluated (Eknath et al., 1993). A complete diallel cross
15 experiment was then carried out to study the components of heterosis according to the well
16 established models from farm animal breeding (see e.g. Fimland, 1983) and to further study
17 genotype by environment interactions. This experiment is reported here.
18
19

20 **2. Material and methods**

21

22 *2.1. Strains*

23

24 Broodstock from eight strains of Nile tilapia (*O. niloticus*) was used to produce all 64
25 possible reciprocal strain crosses and pure strains in a complete diallel cross design. The
26 strains used were four strains collected in 1988-1989 from the wild in Egypt (E2), Ghana
27 (Gh), Kenya (Ke) and Sénégal (Se) and four farmed strains introduced to the Philippines in
28 1979-1984 from Israel (Is), Singapore (Si), Thailand (Th) and Taiwan (Tw). The areas for
29 collection of the wild strains were identified based on the recommendations of a workshop on
30 tilapia genetic resources for aquaculture (Pullin, 1988). Further details on the origin and the
31 collection of the strains are given by Eknath et al., 1993. Except from the E2 and Ke strains,

the broodstocks used in the present study was taken at random from a pool of progeny originating from 25 pairs of wild caught (Gh and Se) or randomly collected (Is, Si, Th and Tw) breeders per strain. The Ke broodstock was taken at random from a pool of progeny from a mass spawning of a large number of wild caught breeders. The E2 broodstock was a random sample from 150 wild caught breeders.

2.2. Test environments

Test fish from all the 64 genetic groups were stocked for growout in eight different farm environments in May-June 1990. The test environments included fertilized ponds (1300 m^2) with a stocking density of 2 fingerlings per m^2 on three BFAR satellite stations in the Philippines; one located in the lowlands near Laguna de Bay, southern Luzon (S1), the second located in the coastal region of north-west Luzon (S2) and the third located in the highlands of central Luzon (S3). In addition, five different farming systems were used for testing at the BFAR/NFFTRC and FAC/CLSU facilities at Muñoz, Nueva Ecija in the lowlands of central Luzon; standard fertilized ponds (1200 m^2) with a stocking density of 2 fingerlings per m^2 (P1), standard fertilized ponds (500 m^2) with supplementary feeding (rice bran/fish meal mixture) and a stocking density of 5 fingerlings per m^2 (P2), cage culture in 5x5x2 m cages in a non-fertilized reservoir with feeding (rice bran/fish meal mixture) and a stocking density of 33 fingerlings per m^2 (C2), and 1000 m^2 ponds fertilized with ipil-ipil (*Leucaena* sp.) leaves (W2) or with carabao (buffalo) manure (W4), both with a stocking density of 2 fingerlings per m^2. Test environments P1, P2 and C2 in Muñoz were duplicated. Further details about the management and the test environments are given by Eknath et al., 1993.

2.3. Mating design and rearing of fry

Male and female breeders were first separated for about two weeks in 2x2x1.5 m hapas (stocking density of 10 fish per m^2) and were fed twice a day with a mixture of 70% rice bran and 30% fish meal at 10% of the body weight. After this conditioning period, six to thirteen 1x1x1 m breeding hapas for each of the 64 strain combinations were stocked with

1 one male and two females per hapa. A total of 742 breeding hapas were installed in one 4500
2 m^2 earthen pond. Seven days after stocking the breeders, all hapas were inspected and fry
3 were collected over a period of two to three days. Female breeders that had spawned were
4 removed from the breeding hapas, and the fry were transferred to 1x1x1 m fine mesh rearing
5 hapas at a stocking density of 200-250 fry per hapa. Fry from the same strain combination
6 were pooled together and reared separately. The collection of fry was then repeated four
7 times at intervals of 12 to 25 days (Table 1). Fry collected during the same period is referred
8 to as a batch. For each strain combination, the different batches collected during the breeding
9 period were also reared separately. The total numbers of males and females contributing
10 progeny to each of the 64 strain combinations are shown in Table 2. The lower number of
11 females that were successfully spawned within some strain combination subcells was due to
12 lower spawning freqencies among the females in the subcells, and not due to lower numbers
13 of females stocked for mating. After 21 days of rearing in fine mesh hapas, the fry were
14 transferred to 1x1x1 m hapas with larger mesh size (B net hapas) at a stocking density of 100
15 fry per hapa, still keeping the strain combinations and batches separate.
16
17 *2.4. Tagging and stocking of test fish*
18
19 After a rearing period of 10 to 20 weeks depending on the batch, the first test environment
20 was stocked with tagged fingerlings. The last test environment was stocked about five weeks
21 later. Within each strain combination, efforts were made to maintain a similar distribution of
22 batches at stocking in each of the test environments. In total, about 360-370 fingerlings from
23 each of the 64 strain combinations were randomly sampled within batches and individually
24 tagged with Floy fingerling tags, and individual body weights were recorded. The mean body
25 weight of the different batches at tagging is shown in Table 1. The number of fingerlings
26 tagged from each batch within each strain combination was determined by the number of
27 females contributing progeny in each batch. Equal numbers of fingerlings from each strain
28 combination were then pooled together before they were communally stocked in the tests
29 environments as shown in Table 3. After a growout period of about 90 days, all fish were
30 harvested and individual body weights were recorded. The numbers of individuals that had
31 lost the tags, easily identified by scars below the base of the dorsal fin, were also recorded.

1
2
3 *2.5. Data analysis*
4
5 Body weights at harvest were analyzed across all environments according to the following
6 generalized linear model (GLM) (SAS,1990). Because of heavy mortality (Table 3),
7 environment S3 was excluded from the analysis:
8
9 (model 1)

10 $$Y_{ijkln} = a + E_i + S_j + B_k + G_l + E_i{*}S_j + E_i{*}B_k + E_i{*}G_l + e_{ijkln}$$

11
12 where:

13 Y_{ijkln} is the body weight at harvest of the *n*th individual
14 a is a constant
15 E_i is the effect of the *i*th test environment (i = 1,2,...,7)
16 S_j is the effect of the *j*th sex (j = male or female)
17 B_k is the effect of the *k*th batch (k = 1,2,...,5)
18 G_l is the effect of the *l*th strain combination (l = 1,2,...,64)
19 e_{ijkln} is a random error for the *n*th individual
20
21 The model was used to estimate the marginal contribution of each of the model effects (Type
22 III sums of squares and the marginal increase of R^2 when the effect was included in the
23 model) and to test the significance of the effects. Least square means (LSM) of body weight
24 at harvest were also computed across and within test environments. First order interactions
25 between test environment and sex, batch or strain combination were included in the model to
26 check for environment specific effects of sex, age at stocking or strain combination genotype.
27 Replicates within test environments did not differ significantly, but the means and standard
28 deviations of body weight at harvest of the replicates tended to vary proportionally. To
29 maintain the common coefficient of variation, the records within replicated test environments
30 were consequently pooled together after applying a multiplicative correction factor generated
31 by dividing the mean body weight at harvest of the pooled test environment with the mean

1 body weight at harvest of each replicate. Because of unequal variances in the test
2 environment by sex subcells, the observations were weighted by the reciprocal of the within-
3 subcell variances during the GLM analysis..
4
5 The interaction term between strain combination and test environment was used to test for
6 genotype by environment interactions. However, this estimate will be affected by
7 proportional interactions (i.e. increased differences between strain combinations as the mean
8 body weight at harvest of the test environment increases). Non-proportional genotype by
9 environment interactions were investigated by computing Pearson correlation coefficients
10 between LSM of performance of the strain combinations in the different test environments
11 and their performance across test environments (LSM of E_i*G_l and G_l according to model 1)
12
13 Estimates of non-additive genetic effects can be obtained from model 1 as the difference in
14 LSM of body weight at harvest between each strain cross and the mean of the parent strains.
15 However, the number of breeders contributing progeny to each of the strain combinations
16 was highly variable and sometimes quite low (Table 2), and the additive genetic performance
17 of the progeny may consequently be affected by random sampling of breeders. Such
18 sampling errors and possible reciprocal effects (effects of using the strains as dams versus
19 using them as sires) will confound the estimates of non-additive genetic effects obtained
20 from LSM of body weight at harvest according to model 1.
21
22 Estimates that are less sensuitive to the random sampling of breeders used to produce each
23 strain combination subcell may be obtained by decomposing the effects of the strain
24 combinations in model 1 into the general additive genetic effect and the general reciprocal
25 effect of each of the eight parent strains across strain combinations, and the non-additive
26 genetic effect of each of the 28 strain crosses across both reciprocals. This was done by
27 applying the following generalized linear model (see e.g. Fimland, 1983) within each test
28 environment:
29
30 (model 2)

31 $$Y_{jklmn} = a + S_j + B_k + \sum_l b_{A(l)}*A_l + \sum_l b_{R(l)}*R_l + \sum_m b_{D(m)}*D_m + e_{jklmn}$$
32

1
2 where a, S_j and B_k are as in model 1 and:

3 Y_{jklmn} is as Y_{ijkln} in model 1

4 $b_{A(l)}$ is the regression coefficient showing the additive genetic effect of genes
5 originating from the lth strain (l = 1,2,...,8)

6 A_l is the proportion of genes in the nth individual originating from the lth strain
7 (A_l = 0.0, 0.5 or 1.0)

8 $b_{R(l)}$ is the regression coefficient showing the general reciprocal effect of the lth
9 strain (l = 1,2,...,8)

10 R_l is the proportion of the genes of the dam of the nth individual originating
11 from the lth strain (R_l = 0.0 or 1.0)

12 $b_{D(m)}$ is the regression coefficient showing the mean non-additive genetic effect of
13 both reciprocals of the mth cross between two different strains (m =1,2,...,28)

14 D_m is the proportion of the non-additive genetic effect of the mth strain
15 combination (both reciprocals) expressed in the nth individual (D_m = 0.0 or 1.0

16 e_{jklmn} is as e_{ijkln} in model 1

17
18 applying the following restrictions to the scores describing the genetic composition of each
19 individual:
20
21 $\sum_l A_l = \sum_l R_l = 1.0$
22
23 $\sum_m D_m = 1.0$ for crosses or $\sum_m D_m = 0.0$ for purebreds
24
25
26 The data were precorrected and weighted as under model 1. An analysis according to model
27 2 provides estimates and significance tests of the contrasts between the strains for additive
28 genetic effects ($b_{A(l)}$) and for general reciprocal effects ($b_{R(l)}$) for body weight at harvest in
29 each test environment separately. The general reciprocal effect of a strain is the difference in
30 performance of the progeny when the strain is used as a dam strain compared to when it is
31 used as a sire strain, and is presently coded entirely as a maternal effect (R_l= 1.0 when the lth
32 strain is used as rthe dam strain). In addition, the estimates will be affected by random

differences in additive genetic performance between the sires and the dams sampled from the strain to produce each of the reciprocals.

The estimates of additive genetic effects and general reciprocal effects obtained from model 2 will be based on the performance of all purebred and crossbred progeny of each strain within each test environment instead of on the narrow sample of parent broodstock (Table 2) represented in each strain combination subcell of model 1. This will reduce substantially the sensitivity of the estimates to errors caused by random sampling of parent broodstock.

The model will also provide unbiased estimates and significance tests of the non-additive genetic deviations of body weight at harvest from the mean of the parent strains ($b_{D(m)}$) for each of the 28 possible strain crosses (both reciprocals pooled together) within each test environment. Compared to model 1, the estimates from model 2 will be based on offspring from about twice as many breeders per strain cross (i.e. both reciprocals), thus reducing the sampling error that may cause confounding of additive and non-additive genetic effects. The general reciprocal effects of each strain will also be excluded from the estimates of the non-additive genetic effects. The general non-additive effect of a strain may be computed as the mean non-additive effect of all strain crosses involving the strain.

Under the following restriction:

$$\sum_{l} b_{A(l)} = \sum_{l} b_{R(l)} = 0$$

the general least square mean of body weight at harvest across strains and reciprocals excluding non-additive genetic effects (*LSM(A+R)*) may be computed within each test environment according to the following formula:

$$LSM(A+R) = a + (\sum_{j} S_j)/n_S + (\sum_{k} B_k)/n_B$$

1 where S_j and B_k are the estimates obtained from model 2 and n_S and n_B are the number of
2 sexes and batches respectively.
3
4 Least square mean contributions to body weight at harvest in each test environment of the
5 progeny of the lth strain when used as sires ($LSM(A+R)_{s=l}$) or as dams ($LSM(A+R)_{d=l}$), still
6 excluding non-additive genetic effects, may then be computed according to the following
7 formulas:
8
9 $LSM(A+R)_{s=l} = ½\ LSM(A+R) + ½\ b_{A(l)}$
10 $LSM(A+R)_{d=l} = ½\ LSM(A+R) + ½\ b_{A(l)} + b_{R(l)}$
11
12 where $b_{A(l)}$ and $b_{R(l)}$ are the estimates obtained from model 2.
13
14 The least square mean of body weight at harvest within each test environment of the progeny
15 of sires from strain $l=x$ and dams from strain $l=y$ ($LSM(A+R)_{xy}$), still excluding non-additive
16 genetic effects may then be computed as follows:
17
18 $LSM(A+R)_{xy} = LSM(A+R)_{s=x} + LSM(A+R)_{d=y}$
19
20 If $b_{D(xy)}$ is the non additive genetic effect ($b_{D(m)}$ according to model 2) of the cross between
21 strain x and strain y ($m=xy=yx$), and if $b_{D(xy)}$ is set to zero when $x=y$ (purebred groups), the
22 least square mean of body weight at harvest of each strain combination (LSM_{xy}) within each
23 test environment (comparable to the least square means of the E_i*G_l subcells of model 1) may
24 be computed as follows:
25
26 $LSM_{xy} = LSM(A+R)_{xy} + b_{D(xy)}$
27
28 where strain $l=x$ is the sire strain and strain $l=y$ is the dam strain. Furthermore, percent
29 heterosis (H_{xy}) for each strain cross within each test environment may be computed as
30 follows:
31

$H_{xy} = (b_{D(xy)}*100)/LSM(A+R)_{xy}$

Least square mean estimates across test environments of $b_{A(i)}$, $b_{R(i)}$, $b_{D(m)}$, general non-additive effects, the various *LSM* parameters and H_{xy} may be obtained by simply computing their mean values across test environments. However, to test the significance of the contrasts between strains, crosses or strain combinations of these pooled parameters, records from all test environments were analyzed simultaneously according to a modified model 2, replacing all variables with their interaction terms with test environments. The proper contrasts may then be defined and tested.

The estimates according to model 2 provide an opportunity to analyze genotype by environment interactions for additive ($LSM(A+R)_{xy}$) and non-additive ($b_{D(m)}$) genetic effects on body weight at harvest separately. Pearson correlation coefficients based on estimates of these parameters in the different test environments and across test environments were computed as described for total performance according to model 1.

The agreement between the least square mean solutions of body weight at harvest of the strain combinations according to model 1 (G_i or E_t*G_i) and according to model 2 (LSM_{xy} across or within test environments) was investigated by computing Pearson correlation coefficients between the solutions from the two models.

3. Results

3.1. Mortality and tag loss

The overall mortality and tag loss in each of the eight test environments are shown in Table 3. The mortality was relatively low in test environments S1, S2, P1 and P2, medium in test environment W4, and relatively high in test environments C2 and W2. There was an almost total mortality in test environment S3, probably caused by low temperature and insufficient application of fertilizer, resulting in a restricted food supply. Consequently, test environment S3 was excluded in the further analysis.

A complete analysis of the mortality records from the present experiment and the correlations with growth performance will be reported separately. However, the Pearson correlation coefficient between the overall LSM of body weight at harvest and mortality in the 64 strain combinations was of the order of -0.41 for mortality across test environments and -0.34 for the mortality in the stressful S3 test environment. This means that the faster growing strain combinations showed lower mortalities. The mortality (including tag loss) of the different strain combinations across all eight test environments varied from 15 to 40 percent.

Tag loss across test environments (excluding test environment S3) was 4.0 percent, and only three of the test environments (P2, W2 and W4) experienced tag loss above 2.0 percent (Table 3). Those three test environments also showed the highest body weights at harvest (Fig. 1), and this may indicate an increased rate of tag loss as the size of the fish exceeds a certain limit.

3.2. Growth performance

Model 1 explained 78 percent of the total variation in body weight at harvest (Table 4). The marginal sums of squares of the effects in model 1 and the marginal contribution of each effect to the proportion of the variance explained by the model (R^2) are shown in Table 4. All effects were highly significant ($p<0.0001$).

The main source of variation in body weight at harvest was the effect of test environments. The marginal increase in R^2 associated to the test environment variable was 53 percent units. The least square means (LSM) of body weight at harvest in the different test environments according to model 1 are shown in Fig. 1 and ranged from 20.0 grams in C2 to 71.6 grams in W2.

The marginal increase in R^2 associated with the sex variable was 13 percent units. The LSM of body weight at harvest for males and females across test environments according to model 1 were 53.9 and 35.8 grams, respectively. In addition, the marginal increase in R^2 by

1 including the interaction term between test environments and sex was about 4 percent units.
2 The interaction between sex and test environments was largely an expected proportional
3 interaction (increased sex differences measured in grams body weight at harvest in test
4 environments with higher growth rates), even if the female to male ratio of LSM of body
5 weight at harvest ranged from 0.61 (environments P1 and P2) to 0.75 (environments S1 and
6 C2).
7
8 The effect of the age of the fingerlings at stocking (the batch variable) on body weight at
9 harvest was quite limited, as may be seen from Table 4 and Fig. 2. The effect of the
10 interaction term between test environment and batch was negligible and mainly proportional
11 in nature. Nevertheless, the batch variable was included in the further analysis as a correction
12 term to adjust for unequal distribution of the batches across the subcells of the models.
13
14 The marginal increase in R^2 caused by including the strain combination variable in model 1
15 was 1.8 percent units. This means that the random variation in body weight at harvest
16 between individuals within the strain combinations was large compared to the systematic
17 variation between strain combinations. However, the LSM of body weight at harvest across
18 test environments according to model 1 ranged from 37.3 grams in the purebred Gh x Gh
19 group to 54.8 grams in the progeny of E2 sires x Kc dams (Table 5), showing that the strain
20 combinations can differ widely in mean growth performance.
21
22 The genotype (strain combination) by test environment interaction effect was also limited (a
23 marginal R^2 contribution of 1.8 percent units). The interaction seemed to be mainly
24 proportional in nature, as suggested by the positive and medium to high Pearson correlation
25 coefficients between LSM of body weights at harvest of the strain combinations in the
26 different test environments according to model 1 (Table 6). Test environment C2 (cage
27 culture) seemed to represent an exception, showing lower and partly non-significant
28 correlation coefficients with most other test environments. The correlation coefficients
29 between LSM of body weight at harvest in each test environment and LSM of body weight at
30 harvest across test environments, both according to model 1, were high (0.73-0.89) except
31 for test environment C2 (0.45).

3.2.1. Effects of strains

Model 2 explained 74 percent of the total variation in body weight at harvest, as compared to 78 for model 1. The reduction in R^2 is expected, since model 1 will account for the variation caused by the random sampling of breeders within the test strains to produce the strain combinations (Table 2). Model 2 will include most of this variation in the error term. The Pearson correlation coefficient between least squares means of body weight at harvest in the strain crosses across test environments according to model 1 (G_l) and model 2 (LSM_{xy}) was 0.94 (Fig. 3). Pearson correlation coefficients within test environments varied from 0.89 to 0.95, except in test environment C2 where the correlation coefficient was 0.85.

The scoring of the variables in model 2 will result in estimates of the additive genetic effects on body weight at harvest of each strain across strain combinations as if all genes were transmitted from male breeders (R_l is scored as 0.0 when *l*th strain is used as a sire strain and 1.0 when it is used as a dam strain). This is based on the assumption that differences between strains in reciprocal effects on body weight at harvest in a species like *O. niloticus*, where the females are mouthbrooders, would most likely be caused by different maternal abilities of the strains.

The additive genetic deviations of the eight strains ($b_{A(l)}$) from the general least square means (*LSM(A+R)*) across and within test environments are shown in Table 7. The ranking of the strains within each test environment was quite consistent with the ranking across test environments. Changes in the rank order of strains within test environments compared to the rank order across test environments were non-significant and seemed to occur at random. Across test environments, the E2 and Ke strains showed the largest positive and the Is and Gh strains showed the largest negative additive genetic deviations for body weights at harvest.

The reciprocal effects in model 2 provide estimates of the effect on body weight at harvest of using each strain as a dam strain instead of as a sire strain. It should be noted that the general

maternal effect (across strains) may not be estimated from the present experimental design. As previously stated, the restriction $\Sigma\, b_{R(l)} = 0$ (ie. that the average reciprocal effect of all strains is zero) was implemented to obtain estimates according to model 2.

The deviations of the reciprocal effects on body weight at harvest ($b_{R(l)}$) of the eight strains from the overall least square means ($LSM(A+R)$) across and within test environments are shown in Table 7. Apart from a significantly more positive reciprocal effect of the E2 strain relative to other strains in test environment S1 and the Th strain in test environment S2, the reciprocal effects were consistent across environments (no significant rank order changes). The dams from the Is and Tw strains produced the best performing offspring when compared to sires from the same strain across test environments, closely followed by dams from the Kc strain. Dams from the E2 strain clearly produced the poorest performing offspring when compared to sires from the same strain.

The least square mean contributions across test environments to body weight at harvest of progeny from sires and progeny from dams of each of the strains, excluding non-additive genetic effects, ($LSM(A+R)_{s=i}$ and $LSM(A+R)_{d=i}$ as previously defined) are shown in Fig. 4. The ranking of the strain contributions depended on whether the genes of the strain were inherited from a sire or a dam. Unlike the ranking of the additive genetic contributions from sires, the E2 strain ranked significantly lower than the Kc and Tw strains when used as dams, and the Is strain ranked significantly higher than the Gh, Si and Th strains when used as dams.

The least square mean estimates of body weight at harvest of the pure strains across test environments based on the sire and dam contributions of each strain ($LSM(A+R)_{xy}$ when $x=y$) is shown in Fig. 5. The Kc strain showed the best pure strain performance, followed by the E2 and Tw strains, while the Gh strain showed the poorest performance.

3.2.2. Effects of strain crosses

The non-additive genetic effects measured in grams body weight at harvest were estimated by the $bD_{(m)}$ parameters according to model 2 as a deviation from the additive and reciprocal effects of the parent strains ($LSM(A+R)_{xy}$) for each of the 28 possible strain crosses (both reciprocals pooled) within each test environment. The least squares mean of the non-additive genetic effects across test environments for each strain cross is shown in Table 8. Significant non-additive genetic effects (all positive) were detected in 11 out of the 28 strain crosses. Five of those were among the seven crosses involving the E2 strain. The Is and Th strains each showed significant non-additive genetic effects in four out of the seven crosses involving the strain. Significant non-additive genetic effects were only detected in one out of the six crosses between the farmed Asian strains.

According to Table 7, the least squares mean of non-additive genetic effects across all strain crosses and test environments was 1.50 grams ($p<0.001$), as compared to a least squares mean of additive and reciprocal effects of 43.66 grams. Corresponding estimates were 2.31 grams and 44.28 grams for crosses between African wild strains, 0.27 grams and 43.04 grams for crosses between Asian farmed strains, and 1.66 grams and 43.66 grams for crosses between African and Asian strains.

The least squares mean of general non-additive genetic effects of each strain (computed across all test environments and all strain crosses involving the strain) are shown in Table 7 and Fig. 5. The E2 strain clearly showed the largest general non-additive genetic effect, and the effect was also consistently significant when analyzed within test environments (Table 7) except for in test environment C2. The Sc, Is and Th strains also showed a highly significant, positive general non-additive genetic effect across test environments, and a similar but mostly non-significant tendency within test environments. The general non-additive effect of the Si strain across test environments was close to zero. However, the Si strain showed the largest general non-additive genetic effect of all the strains in test environment C2. In all other test environments except S1, the general non-additive genetic effects of the Si strain were non-significant and tended to be negative. The general non-additive effects of the Gh and Ke strains were also more pronounced in the C2 test environment than in most other test environments.

Percent heterosis on body weight at harvest in each of the 56 strain crosses (H_m) was computed within each test environment as previously described. Mean percent heterosis for each strain cross across all test environments are shown in Table 9. Since percent heterosis measures the non-additive genetic effects relative to the additive genetic and reciprocal effects, the strain crosses showing the largest non-additive genetic effects will not necessarily show the largest heterosis when expressed in percent. The largest mean percent heterosis across test environments was observed in the crosses between the Th and the Gh strains (14.0 and 13.7 percent) followed by the crosses between the E2 and Se strains (11.6 and 12.3 percent). The crosses between the E2 strain and the Ke, Se and Th strains and between the Gh and Se strains all showed about 10 percent heterosis across test environments. All other crosses showed less than 7 percent mean heterosis across test environments.

Mean percent heterosis (Table 10) varied considerably from one test environment to another (from -0.05 percent in test environment P2 to 9.62 percent in test environment C2), and the correlation coefficients between additive and non-additive components of body weight at harvest within test environments were mostly low and non-significant. As a result, mean percent heterosis as previously defined may be different from the ratio between least squares means of non-additive and additive genetic (including reciprocal) components computed across crosses and test environments (mean percent heterosis will depend on how the additive and non-additive components are distributed within the cross by test environment cells). Mean percent heterosis across all test environments was 4.30 (Table 10). The crosses between the African wild strains showed 5.68 percent mean heterosis as compared to 1.77 percent for the crosses between Asian farmed strains. Crosses between African and Asian strains showed 4.74 percent mean heterosis.

The strain crosses showing the largest percent heterosis within each test environment were all among the strain crosses showing the largest percent heterosis across test environments (Table 9), except for in the C2 test environment where the crosses between the Si strain and the Ke and Th strains were among the strain crosses showing the largest percent heterosis. This suggests that the non-additive genetic effects were affected by genotype by test

1 environment interaction (see below). Maximum heterosis achieved within each test
2 environment was mostly of the same magnitude as across test environments (13-19 percent),
3 except for in test environments C2 and W2 where maximum heterosis was 39 and 26 percent
4 respectively.
5
6 Table 10 also shows a trend towards larger mean percent heterosis in test environments
7 resulting in low mean body weight at harvest (S1 and C2) and vice versa (P2), and a
8 significant negative correlation coefficient between additive and non-additive genetic
9 components in the test environment with the lowest body weight at harvest (C2). This may
10 suggest that the relative impact of non-additive gene effects was larger at the lowest growth
11 rates. However, the trend was not completely consistent. Mean percent heterosis in one of the
12 test environments resulting in low body weights at harvest (S2) was lower than in the test
13 environment resulting in the highest body weight at harvest (W2), and the correlation
14 coefficient between additive and non-additive genetic components was not significantly
15 different from zero in two of the test environments with low body weights at harvest (S1 and
16 S2).
17
18 *3.2.3. Genotype by environment interactions*
19
20 As previously shown in Table 6, the analysis according to model 1 revealed indications of a
21 strain combination by test environment interaction, mainly expressed by a low correlation of
22 body weight at harvest in the C2 test environment with the other test environments.
23 According to Table 7, this interaction seems to be more associated with the non-additive
24 genetic deviations than the additive or reciprocal deviations as estimated by model 2.
25
26 Table 11 shows Pearson correlation coefficients between the additive (including reciprocal)
27 genetic effects on body weight at harvest of the 64 strain combinations ($LSM(A+R)_{xy}$
28 according to model 2) in the different test environments and across all test environments. The
29 additive performance of the strain combinations showed significant positive correlation
30 coefficients between all test environments, mostly in the range of 0.5-0.8. The lowest
31 correlation coefficient was observed between the S1 and the W2 test environments (0.35).

The correlation coefficients with the C2 test environment did not differ from the other test environments. The correlation coefficients between the additive performance in each of the test environments and the least squares mean additive performance across test environments were all highly positive (0.72-0.95).

Table 12 shows Pearson correlation coefficients between non-additive genetic effects on body weight at harvest in the 28 strain crosses (b_{ij}, according to model 2) in the different test environments and across test environments. For all test environments except C2, the correlation coefficients for non-additive performance were positive and mostly significant in the range of 0.4-0.7. The correlation coefficients with the C2 test environment were all non-significant and tended to be negative. The correlation coefficients between non-additive performance in each of the test environments and least squares mean non-additive performance across test environments were highly positive and significant (0.70-0.86) for all test environments except C2, which showed no significant correlation with the non-additive performance across test environments.

4. Discussion

4.1. Data analysis

As previously mentioned, the statistical analysis according to model 2 was carried out to reduce the random errors caused by the sampling of breeders from the pure strains to produce the 64 strain combinations (Table 2), which may seriously affect the estimates obtained from model 1. From the bottom line of Table 2, it may be seen that the average number of breeders per strain contributing additive genetic effects to the experimental fish according to model 2 was 133 breeders, and that the estimates of the reciprocal effects according to model 2 were based on an average of 58 male breeders versus 75 female breeders per strain, thus reducing the sampling error substantially.

Model 2 assumes that the additive genetic effects and the reciprocal effects of the strains are expressed equally in all strain combinations. This is in accordance with the definition of

additive genetic effects (the average genetic effect of a breeder on all progeny irrespective of the mating partner) and with the assumption that the most important reciprocal effect in a species like Nile tilapia, where the females brood the eggs and nurse the fry, would be the maternal abilities of the dam strain.

Model 2 is based on a well established methodology in farm animals (see e.g. Fimland, 1983). However, the performance components estimated by the model will not always be strictly additive or reciprocal. Since the experimental fish were all purebred or first generation crosses (F1), certain epistatic gene effects may be inherited as if they were additive or reciprocal effects. This can only be investigated by estimating recombination loss in progeny of F1 and later generations.

The possibilities of specific reciprocal effects (effects deviating from the general reciprocal effects estimated by model 2) in some of the strain crosses cannot be ruled out. However, the present experimental design will provide poor estimates of specific reciprocal effects, because of the restricted number of breeders contributing progeny to each strain combination (Table 2) and the lack of well defined genetic relationships between the breeders used in reciprocal crosses. Estimates of specific reciprocal effects based on the solutions from model 1 may result in severe confounding with random additive genetic differences between the breeders used to represent the strains.

The estimates of non-additive genetic effects according to model 2 are pooled estimates based on both reciprocals for each strain combination. The estimates will then be based on progeny from on average 16-17 breeders per strain in each strain cross or twice as many breeders as the average under model 1. Theoretically, the non-additive genetic effects in the reciprocals may differ as a result of epistatic gene effects involving sex linked genes. Such effects may not be distinguished from general or specific reciprocal effects in the present experiment.

The fact that the least squares solutions for body weight at harvest in the 64 genetic groups according to the two models were not completely in agreement (Fig. 3) and that the R^2-value

1 of model 1 was higher than that of model 2 (0.78 versus 0.74) does not necessarily mean that
2 model 1 offers more accurate estimates of the components of performance than model 2. As
3 stated above, model 1 will include a larger proportion of the random errors caused by
4 sampling of breeders into the estimates. This will increase the R^2-value of the model, but may
5 at the same time result in less representative estimates.
6
7 *4.2. Effects of sex and age at stocking*
8
9 The effects of sex on body size at harvest were slightly stronger in the present study than
10 when the same strains were compared as pure strains (Eknath et al. 1993). The overall female
11 to male body weight ratio was 0.65 in the present study and 0.70 in the pure strain
12 comparison test. However, the possibility of increased sex differences in body weight at
13 harvest in strain crosses compared to pure strains should be further investigated before any
14 conclusions are drawn.
15
16 The modest effect of age at stocking (the batch variable) on body weight at harvest agreed
17 well with previous results from the pure strain comparison test. Under the present fry rearing
18 conditions, age differences of more than two months at stocking for grow-out did not affect
19 body weight at harvest much (Fig. 2). Palada-de Vera and Eknath (1993) showed that groups
20 of tilapia fry differing in mean size at stocking from 2.9 to 8.3 grams did not differ
21 significantly in body weight at harvest after three months of communal rearing. The size
22 differences at stocking of the batches in the present experiment were smaller than this (Table
23 1). Nevertheless, the batch collection and recording procedure applied in the present study
24 provided an opportunity to check and, if necessary, correct for effects of age differences in
25 genetic studies with asynchronous spawning during the production of the experimental fish.
26
27 *4.3. Additive genetic performance*
28
29 The ranking of the additive genetic components of growth performance (including reciprocal
30 effects) of the test strains in the present experiment (Fig. 5) agreed well with the results from
31 the pure strain comparison test reported by Eknath et al. (1993). Again, it may be concluded

that the growth performance in culture of three of the four strains originating directly from wild caught, African broodstock (the E2, Ke and Se strains) were as good as or better than the Asian cultured strains. This suggests that the broodstock of Nile tilapia originally introduced into Asian aquaculture may have been of poor quality, or that generations of reproduction in culture may have reduced the growth performance because of inbreeding or negative selection (see e.g. Eknath and Doyle, 1990) or by introgression of less desired genetic material (see eg. Macaranas et al., 1986). In any case, the present results offer no evidence for the development of faster growing aquaculture strains of Nile tilapia merely as a result of generations of reproduction in captivity.

4.4. Maternal performance

The significant differences between the strains in general reciprocal effects (Table 7 and Fig. 4) suggest that the strains may differ in maternal performance in a way that may still be detected in the body weight of the progeny at harvest. Other possible sources of reciprocal effects (eg. sex linked or cytoplasmatic inheritance or paternal effects) may not be ruled out, but are less obvious. The average number of male and female breeders per strain used in the present experiment (58 and 75 respectively) was probably large enough to avoid that the estimates of reciprocal effects were severely confounded with differences in additive genetic performance between male and female breeders caused by random sampling.

The differences in reciprocal effects observed in the present experiment may justify the use of specialized sire and dam lines in a hybrid program. If the differences are caused by genetic variation in maternal performance, the possibilities to record and select for the trait should be investigated. Selection for the trait may be used both to improve growth performance in purebreeding programs and to form specialized dam lines for hybrid programs.

Furthermore, it may be seen from Table 2 that the dams from some of the strains were obviously much more willing to mate and produce progeny than the dams from other strains. During the mating period, 146 dams from the Ke strain and 91 dams from the Is strain produced progeny, as compared to only 35 dams from the Si strain. It is interesting to notice

that the strains with the highest frequency of reproduction among the dams were the same as the strains showing the largest positive reciprocal effects (effects of using the strain as dams in stead of as sires) on the growth performance of the progeny of (Table 7 and Fig. 4). This may suggest some kind of association between the readiness of the dams to spawn and maternal abilities.

4.5. Non-additive genetic performance

The general non-additive genetic effects on body weight at harvest of each of the test strains in crosses with all other strains and across all test environments were quite modest compared to the additive genetic plus reciprocal effects (Fig. 5). The magnitude of percent heterosis of the different strain crosses across test environments (Table 9) was in the range of or somewhat lower than that reported from previous studies with other freshwater species such as carps (Bakos, 1979, Wohlfarth, 1993) and channel catfish (Dunham, 1987) and somewhat higher than for anadromous salmonids (Ayles and Baker, 1983, Gjerde and Refstie, 1984, Gjerde, 1988). Within certain test environments (C2 and W2), maximum percent heterosis was comparable to the highest heterosis observed at certain occasions in the studies referred to above (30 percent and above). It remains to be seen if any possible systematic environmental conditions producing such increased non-additive genetic effects can be identified and utilized in a predictable way in applied aquaculture systems.

The indications of a larger percent heterosis at lower growth rates (Table 10) were not conclusive, and should be investigated further before any conclusions are drawn. However, Wohlfarth (1993) reported results from crossbreeding experiments with common carp that also suggested that the expression of non-additive genetic effects may be increased under environmental conditions causing low growth rates.

However, the benefits from using hybrids in applied aquaculture production are not determined by percent heterosis alone. The total performance of the hybrid should be compared to the best performing pure strain and not the mean of the parent strains to assess the gain in performance. The Gh sires x Th dams cross showed the largest percent heterosis

1 across test environments in the present study (14.0 percent, Table 9), but performed poorer
2 than the pure Kc strain. Within test environments, the maximum heterosis observed was 39
3 percent (the Th sires x Si dams cross in the C2 test environment), but the total performance
4 was considerably poorer than the pure Kc strain in the same test environment. The total
5 performance of the 64 strain combinations across test environments compared to the
6 purebred performance of the Kc strain is shown in Fig. 6. The body weight at harvest of the
7 best performing hybrid (the E2 sires x Kc dams cross) was about 11 percent higher than the
8 pure Kc strain.
9
10 The strains showing significant general non-additive genetic effects across test environments
11 (Table 7) did not express these effects consistently in all strain crosses (Table 8). Two of the
12 crosses involving the E2 strain, three of the crosses involving the Is or the Th strains and four
13 of the crosses involving the Sc strain showed no significant heterosis (least squares means
14 across test environments). This suggests that specific combining effects between the strains
15 may be more important than the general non-additive effects of each strain. The specific
16 combining effects may not be predicted from the general non-additive effects of the strains
17 involved in the strain cross. The only general trend observed in the present experiment was
18 the consistent lack of significant general non-additive genetic effects when analyzed across
19 test environments in all crosses involving the Si strain (Table 8). However, significant non-
20 additive genetic effects were observed within test environments for some crosses involving
21 the Si strain, in particular in test environment C2 (Table 7).
22
23 The Asian farmed strains used in the present study originate from rather narrow founder
24 stocks introduced into Asian aquaculture since 1965 (Pullin and Capili, 1988), and may
25 consequently be expected to show less genetic variation within each strain than the wild-
26 caught African strains. On the other hand, the Asian strains do not seem to have been
27 genetically isolated after the introduction of the founder stocks, and non-recorded
28 interbreeding with other stocks may have resulted in increased heterogeneity. Macaranas et
29 al. (1986, 1995) even found evidence of introgression from *O. mossambicus* in all the Asian
30 strains, and the heterozygosity levels based on allele frequencies in 30 protein loci were
31 higher in the Asian strains than in the African strains.

In general, crosses between homozygous strains are often expected to result in larger non-additive genetic effects than crosses between heterozygous strains (confer the use of inbred strains in hybrid programs for both farm animals and plants). The lower heterosis observed in the crosses between the Asian farmed strains in the present study compared to the crosses between the African wild strains (Table 9) supports the results from the isoenzyme studies referred to above, i.e. that the Asian farmed strains may not be considered more homozygous than the wild African strains.

It may also be argued that non-additive genetic effects are more likely to occur in crosses between genetically distant populations because of the increased possibilities of divergent fixation of interacting alleles. These effects may be dominance effects resulting in heterosis or epistasis breakdown resulting in recombination loss (see e.g. Hill, 1982). Nei genetic distances based on the same 30 protein loci ranked the Ke strain as the most distant strain (mean D = 0.041), followed by the Se strain (mean D = 0.016). The rest of the strains clustered within a genetic distance of 0.005 or less (Eknath et al., 1991a, Macaranas et al., 1995). There was no obvious relationship between these genetic distances and the non-additive genetic effects of the strain crosses in the present experiment (Fig. 5 and Table 8).

4.6. Genotype by environment interactions

The low proportion of the total variance (1.8 percent) explained by genotype by environment interactions in the present study agrees well with pevious reports on Atlantic salmon (1.4 - 3.7 percent, Gunnes and Gjedrem, 1978) and rainbow trout (1.2 - 5.5 percent, Gunnes and Gjedrem, 1981).

The body weight at harvest of the 64 strain combination genotypes seemed to be affected by a partly non-proportional strain combination genotype by test environment interaction in the C2 test environment compared to most other test environments (Table 6). However, this interaction seemed to be almost entirely associated with the non-additive genetic components of performance (Tables 11 and 12).

Because of the lower number of individuals stocked per strain combination (25) and the rather high average mortality (43.7%) in the C2 test environment (Table 3), it may be argued that the estimates of the non-additive genetic effects according to model 2 in the C2 test environment may be more affected by random errors than in the other test environments. However, the deviations in the C2 test environment from the pattern of general non-additive genetic effects shown in Table 7 (no significant effects of the E2 strain and a highly significant effect of the Si strain) did not seem to occur at random. None of the seven crosses involving the E2 strain showed significant non-additive genetic effects in the C2 test environment ($p>0.05$), while three of the crosses involving the Si strain showed significant ($p<0.05$ to $p<0.001$) and two showed nearly significant ($p<0.06$) non-additive genetic effects. This forms a systematically different pattern compared to the least squares means across test environments (Table 8).

These results suggest that the non-additive genetic component of growth performance in Nile tilapia may be more sensitive to environmental variation than the additive genetic component. If so, genetic improvement strategies exploiting the non-additive genetic components (eg. crossbreeding or hybrid programs) may require that the users of the improved material can provide a more uniform and well defined farm environment compared to strategies exploiting the additive genetic components of performance (eg. purebreeding programs with selection). Furthermore, performance testing for hybrid programs will then require a stronger similarity between the test environment and the target farm environments than additive purebreeding programs. Several specialized hybrids may have to be developed if the target farm environments are diverse, while one purebreeding program may serve larger variety of farm environments.

5. Conclusions

In general, the crosses between the Nile tilapia stains used in the present study expressed low levels of heterosis. A crossbreeding program based on the best performing strain combination

1 from the present study may increase body weight at harvest in farmed Nile tilapia by about
2 10 percent compared to the best performing pure strain across a range of applied farm
3 environments similar to those used by Philippine tilapia farmers. Specialized sire and dam
4 lines may be required to fully exploit this possibility, and the heterosis may be poorly
5 expressed in some farm environments because of genotype by environment interactions
6 affecting the non-additive genetic performance. Alternatively, specialized hybrids may have
7 to be produced for certain farm environments.
8
9 The dissemination of genetic gain from this type of crossbreeding program(s) to small scale,
10 resource-poor tilapia farmers, which is the target group of the GIFT project, could be a
11 demanding process. Hybrid fingerlings would probably have to be produced by a number of
12 specialized multiplier units that could maintain or be supplied with the pure parent strains.
13 The strains would have to be kept separately until the breeders were stocked for fry
14 production, and the breeders would have to be sexed before stocking. The target farmers
15 would have to return to the multiplier units to purchase hybrid fingerling before every cycle
16 of production, since on-farm reproduction using hybrid breeders would result in loss of
17 heterosis in the progeny.
18
19 The requirements and benefits of such a crossbreeding program should be compared with
20 those of purebreeding programs utilizing repeated selection to improve the additive genetic
21 performance (Gjedrem, 1985, Bentsen, 1990, Bentsen and Gjerde, 1994). Several selection
22 experiments with other farmed fish species have produced additive genetic gain after one or
23 two generations of selection comparable to the gain achieved by using the best performing
24 hybrid in the present study (Bondari, 1983, Gjerde, 1986, Dunham, 1987, Hershberger et al.,
25 1990). Unlike crossbreeding programs, additive genetic gain from purebreeding programs
26 may then be accumulated over generations, and the dissemination of the genetic gain to the
27 target farmers will be much simpler. Crossbreeding programs will therefore probably need to
28 incorporate purebreeding and selection within each of the parent strains to compete with the
29 long term gain achieved in a plain purebreeding program (Gjedrem, 1985). The present study
30 also suggests that additive genetic performance may be more robust towards environmental

variation (e.g. the diverse farming systems used by small-scale tilapia farmers in the Philippines).

Additive genetic parameters will now be estimated in a genetically mixed base population of progeny from breeders chosen from this diallel cross experiment, and the response to selection for body weight at harvest will be determined. The combined results from the sequence of experiments will be utilized to design an applied genetic improvement and dissemination program to serve the target group of the GIFT project (small-scale, resource-poor tilapia farmers).

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References

1
2 Ayles, G. and Baker, R., 1983. Genetic differences in growth and survival between strains
3 and hybrids of rainbow trout (*Salmo gairdneri*) stocked in aquaculture lakes in the Canadian
4 prairies. Aquaculture, 33:269-280.
5
6 Bakos, J., 1979. Crossbreeding Hungarian races of common carp, to develop more
7 productive hybrids. Advances in Aquaculture. Farnham, Surrey, U.K.: Fisheries News Books
8 Ltd, pp. 635-642.
9
10 Bentsen, H.B., 1990. Application of breeding and selection theory on farmed fish. Proc. 4th
11 World Congress on Genetics Applied to Livestock Production, Edinburgh, Scotland, Vol.
12 16:149-158.
13
14 Bentsen, H.B. and Gjerde, B., 1994. Design of fish breeding programs. Proc. 5th World
15 Congress on Genetics Applied to Livestock Production, Guelph, Canada, Vol. 19:353-359.
16
17 Bondari, K., 1983. Response to bidirectional selection for body weight in channel catfish.
18 Aquaculture, 33:73-81.
19
20 Dunham, R.A., 1987. American catfish breeding programs. In K. Tiews (Editor), Selection,
21 hybridization and genetic engineering in aquaculture. Schriften der Bundesforschungsanstalt
22 für Fischerei, Hamburg, Vol II:407-416.
23
24 Eknath, A.E and Doyle, R.W., 1990. Effective population size and rate of inbreeding in
25 aquaculture of Indian major carps. Aquaculture, 85:293-305.
26
27 Eknath, A.E., Macaranas, J.M., Agustin, L.Q., Velasco, R.R., Ablan, M.C.A., Pante, M.J.R.
28 and Pullin, R.S.V., 1991a. Biochemical and morphometric approaches to characterize farmed
29 tilapias. NAGA, The ICLARM Quarterly, 14(2):7-9.
30

1 Eknath, A.E., Bentsen, H.B., Gjerde, B., Tayamen, M.M., Abella, T.A., Gjedrem, T. and
2 Pullin, R.S.V., 1991b. Approaches to national fish breeding programs: Pointers from a tilapia
3 pilot study. NAGA, The ICLARM Quarterly, 14(2):10-12.
4
5 Eknath, A.E., Tayamen, M.M., Palada-de Vera, M.S., Danting, J.C., Reyes, R.A., Dionisio,
6 E.E., Capili, J.B., Bolivar, H.L., Abella, T.A., Circa, A.V., Bentsen, H.B., Gjerde, B.,
7 Gjedrem, T. and Pullin, R.S.V., 1993. Genetic improvement of farmed tilapias: The growth
8 performance of eight strains of *Oreochromis niloticus* tested in different farm environments.
9 Aquaculture, 111:171-188.
10
11 Fimland, E., 1983. Methods of estimating the effects of heterosis. Z. Tierzüchtg.
12 Züchtgsbiol., 100:3-8.
13
14 Gjedrem, T., 1985. Improvement of productivity through breeding scheemes. GeoJournal
15 10.3:233-241.
16
17 Gjerde, B., 1986. Growth and reproduction in fish and shellfish. Aquaculture, 57:37-55.
18
19 Gjerde, B., 1988. Complete diallel cross between six inbred groups of rainbow trout, *Salmo*
20 *gairdneri*. Aquaculture, 75:71-87.
21
22 Gjerde, B. and Refstie, T., 1984. Complete diallel cross between five strains of Atlantic
23 salmon. Livestock Prod. Sci., 11:207-226.
24
25 Gunnes, K. and Gjedrem, T., 1978. Selection experiments with salmon IV. Growth of
26 Atlantic salmon during two years in the sea. Aquaculture, 15:19-33.
27
28 Gunnes, K. and Gjedrem, T., 1981. A genetic analysis of body weight and length in rainbow
29 trout reared in seawater for 18 months. Aquaculture, 24:161-174.
30

Hershberger, W.K., Myers, J.M., Iwamoto, R.N., McAuley, W.C. and Saxton, A.M., 1990. Genetic changes in the growth of coho salmon (*Oncorhynchus kisutch*) in marine net-pens, produced by ten years of selection. Aquaculture, 85:187-197.

Hill, W.G., 1982. Dominance and epistasis as components of heterosis. Z. Tierzüchtg. Züchtungsbiol. 99:161-168.

Macaranas, J.M., Taniguchi, N., Pante, M-J.R., Capili, J.B. and Pullin, R.S.V., 1986. Electrophoretic evidence for extensive hybrid gene introgression into commercial *Oreochromis niloticus* (L.) stocks in the Philippines. Aquacult. Fish. Manage., 17:249-258.

Macaranas, J.M., Agustin, L.Q., Ablan, M.C.A., Pante, M.J.R., Eknath, A.E. and Pullin, R.S.V., 1995. Genetic improvement of farmed tilapias: biochemical characterization of strain differences in Nile tilapia. Aquaculture International, 3:43-54.

Palada-de Vera, M.S. and Eknath, A.E., 1993. Predictability of individual growth rates in tilapia. Aquaculture, 111:147-158.

Pullin, R.S.V., 1985. Tilapia: 'everymans fish'. Biologist, 32(2):84-88.

Pullin, R.S.V. (Editor), 1988. Tilapia genetic resources for aquaculture. ICLARM Conference Proceedings 16. International Center for Living Aquatic Resources Management, Manila, Philippines, 108 pp.

Pullin, R.S.V., 1997. World tilapia culture and its future prospects. In: R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (Editors), The Third International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings 41, in press.

Pullin, R.S.V. and Capili, J.B., 1988. Genetic improvement of tilapias: Problems and prospects. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Second International Symposium on Tilapia in Aquaculture, ICLARM Conference

1 Proceedings 15. Department of Fisheries, Bangkok, Thailand and International Center for
2 Living Aquatic Resources Management, Philippines, pp. 259-266.
3
4 Pullin, R.S.V., Eknath, A.E., Gjedrem, T., Tayamen, M.M., Macaranas, J.M., and Abella,
5 T.A., 1991. The genetic Improvement of farmed tilapias (GIFT) project: The story so far.
6 NAGA, The ICLARM Quarterly. 14(2):3-6.
7
8 SAS Institute Inc., 1990. SAS/STAT User's Guide, Version 6, 4th Edition. SAS Institute
9 Inc., Cary, NC, USA.
10
11 Wohlfarth, G.W., 1993. Heterosis for growth rate in common carp. Aquaculture, 113:31-46.
12

Table 1. Collection period of batches of Nile tilapia fry, number of fingerlings tagged per batch, and mean body weight with standard deviations (SD) of each batch at tagging.

Batch	Collection period	No. of fingerlings tagged	Body weight (g)	
			Mean	SD
1	Dec. 27-28, 1989	287	4.88	1.37
2	Jan. 10-11, 1990	5 491	5.51	2.19
3	Jan. 23-24, 1990	1 256	5.28	1.87
4	Feb. 21-23, 1990	7 903	3.26	1.30
5	Mar. 12-16, 1990	8 802	4.14	1.71
Total		23 739		

Table 2. The number of breeders (sires/dams) of Nile tilapia contributing progeny to each of the 64 strain combinations.

Sire strain	Dam strain							
	E2	Gh	Ke	Se	Is	Si	Th	Tw
E2	15/18	8/9	11/13	9/10	10/14	3/3	4/5	6/6
Gh	6/7	10/12	18/26	10/11	9/13	4/6	10/12	9/12
Ke	6/6	3/3	11/13	5/6	10/12	3/3	7/8	6/6
Se	5/6	13/17	13/17	6/10	5/10	4/4	6/8	3/4
Is	13/18	7/13	16/19	7/11	5/8	2/2	3/3	3/5
Si	7/10	4/5	19/24	5/7	11/14	6/8	5/7	10/11
Th	4/7	8/9	12/18	6/8	7/10	5/5	3/3	7/9
Tw	5/6	6/9	13/16	4/4	8/10	3/4	5/7	2/3
	E2	Gh	Ke	Se	Is	Si	Th	Tw
Total	66/78	67/77	51/146	55/67	56/91	67/35	52/51	46/56

Table 3. Numbers of Nile tilapia fingerlings stocked, mortality, tag loss and mean body we with standard deviations (SD) of males and females in each test environment.

Test eviron- ment	No. stocked		Mortality (%)	Tag loss (%)	Body weight at harves		
	Per strain comb.	Total			Males		
					Mean	SD	Mea
S1	40	2 587	13.7	2.0	39.7	11.1	29.9
S2	40	2 590	11.6	1.0	31.7	6.6	21.0
S3	40	2 597	85.1	0.0	6.2	2.4	5.3
P1	80	5 185	12.4	1.8	55.0	13.5	33.3
P2	80	5 182	14.6	6.8	82.6	18.6	50.8
C2	25	1 597	43.7	0.0	21.4	7.2	15.7
W2	31	1 992	29.6	10.4	83.5	17.2	55.
W4	31	2 009	18.0	10.8	57.2	12.5	36.
Total	367	23 739	17.5*	4.0*	53.0*		34.6

* Excluding test environment S3

Table 4. Analysis of variance of body weight at harvest in Nile tilapia according to model 1. Degrees of freedom (D.F.), marginal (Type III) sums of squares and marginal increase in the proportion of the total variance explained by the model (R^2) associated to each effect are shown.

Effect	D.F.	Marginal (Type III) sums of squares[1]	Marginal R^2 increase
Test environment	6	4 889.0	0.529[2]
Sex	1	7 037.5	0.131[2]
Batch	4	151.1	0.003[2]
Strain combination	63	1 178.2	0.018[2]
Test environment x Sex	6	2 382.2	0.038
Test environment x Batch	24	103.1	0.002
Test environment x Strain combination	378	1 105.1	0.018
Error	15 718	13 867.8	
Model	482	48 865.2	0.779

[1] All significant (p<0.0001)

[2] Adjusted for the marginal R^2 increase associated to the interaction terms involving the effect.

Table 5 Least square means of body weight at harvest (g) across test environments of the 64 strain combinations of Nile tilapia according to model 1.

Sire strain	Dam strain							
	E2	Gh	Ke	Se	Is	Si	Th	Tw
E2	46.1	43.8	54.8	52.7	51.1	45.0	48.7	52.2
Gh	42.5	37.3	42.3	46.1	39.8	39.4	43.2	44.2
Ke	49.5	41.4	48.5	44.2	48.8	42.5	46.4	49.1
Se	51.4	41.7	47.2	44.4	49.7	39.4	42.5	48.1
Is	45.4	39.6	45.3	42.4	41.8	41.9	43.3	42.8
Si	42.7	39.8	47.2	43.6	39.1	41.4	43.7	44.9
Th	47.8	45.1	48.5	46.2	47.1	41.0	42.1	42.6
Tw	45.8	42.5	47.2	43.3	45.8	43.8	43.5	46.7

Standard error of estimates were in the range of 0.80 - 1.08

Table 6. Pearson correlation coefficients between least square means for body weight at harvest according to model 1 of the 64 strain combinations of Nile tilapia in the different test environments and across all test environments.

	S2	P1	P2	C2	W2	W4	All
S1	0.51	0.54	0.43	0.61	0.51	0.55	0.73
S2		0.65	0.68	0.21[NS]	0.65	0.64	0.75
P1			0.83	0.27*	0.76	0.70	0.87
P2				0.11[NS]	0.78	0.62	0.84
C2					0.20[NS]	0.31*	0.45
W2						0.73	0.89
W4							0.85

NS : Non-significant ($p>0.05$)

* : $0.05>p>0.01$

All other correlation coefficients: $p<0.01$

Table 7. Additive genetic, reciprocal and general non-additive genetic deviations of body weight at harvest (in grams) for each strain of Nile tilapia (based on the b_A, b_R and b_D estimates according to model 2) from the least square means excluding non-additive genetic effects (LSM(A+R)) across and within test environments.

Strain	Across test env.	Within test environments						
		S1	S2	P1	P2	C2	W2	W4
Additive deviations								
E2	5.64^{a}	-0.66^{bcd}	0.62^{abcd}	6.17^{a}	10.87^{a}	2.21^{b}	11.40^{a}	8.78^{a}
Gh	-5.63^{d}	-3.25^{cd}	-3.29^{c}	-4.85^{cd}	-7.86^{c}	-3.12^{cd}	-9.74^{b}	-7.13^{cd}
Kc	3.94^{ab}	11.34^{a}	2.99^{a}	2.20^{ab}	4.04^{ab}	5.07^{a}	-2.53^{b}	3.11^{ab}
Sc	0.87^{bc}	3.60^{b}	-0.47^{bcde}	-0.60^{bc}	0.06^{bc}	2.40^{ab}	-0.47^{ab}	1.81^{ab}
Is	-4.35^{d}	-3.76^{cd}	-2.54^{de}	-5.12^{d}	-7.38^{c}	-1.78^{bcd}	-1.29^{b}	-8.40^{d}
Si	-1.30^{c}	-5.00^{d}	0.98^{abc}	0.18^{b}	-0.78^{bc}	-4.90^{d}	-0.73^{ab}	2.27^{ab}
Th	-0.53^{bc}	-2.28^{cd}	-1.00^{cde}	0.16^{b}	-0.20^{bc}	-1.44^{bcd}	1.09^{ab}	0.02^{b}
Tw	1.39^{b}	0.02^{bcd}	2.71^{ab}	1.84^{ab}	1.24^{b}	1.53^{ab}	2.27^{ab}	-0.47^{bc}
Reciprocal deviations								
E2	-2.95^{d}	1.51^{a}	-1.33^{c}	-2.97^{d}	-4.60^{d}	-1.02^{bc}	-6.75^{d}	-5.40^{d}
Gh	-0.51^{c}	-0.24^{ab}	-0.16^{bc}	0.07^{bc}	-0.70^{c}	0.37^{abc}	-1.79^{bcd}	-1.54^{c}
Kc	1.20^{b}	-1.39^{b}	0.25^{ab}	1.81^{ab}	-0.64^{bc}	0.90^{abc}	4.89^{a}	3.54^{ab}
Sc	-0.07^{c}	-1.21^{b}	0.24^{ab}	-0.45^{c}	0.47^{bc}	-0.40^{abc}	1.27^{abc}	-0.37^{c}
Is	2.50^{a}	0.90^{ab}	1.41^{a}	2.46^{a}	4.56^{a}	0.94^{ab}	2.43^{ab}	4.81^{a}
Si	-0.94^{c}	-1.00^{b}	-0.81^{bc}	-1.52^{cd}	-1.94^{cd}	-1.30^{c}	-1.19^{bc}	0.77^{bc}
Th	-0.89^{c}	0.10^{ab}	1.36^{a}	-1.48^{cd}	0.02^{bc}	-0.68^{abc}	-3.66^{cd}	-2.30^{cd}
Tw	1.66^{ab}	1.33^{a}	-0.96^{bc}	2.07^{a}	2.83^{ab}	1.20^{a}	4.79^{a}	0.48^{bc}
General non-additive deviations								
E2	3.49**	4.35**	2.45**	1.62*	3.39**	0.66	7.21**	4.73**
Gh	1.40	2.48*	0.56	-0.05	-0.80	2.83*	0.81	4.00**
Kc	1.09*	2.98**	-0.17	-0.56	-1.58	1.98*	3.28	1.69
Sc	1.77**	3.24**	0.01	1.74*	0.22	1.25	3.61	2.34
Is	1.69**	2.31*	0.62	2.55**	1.03	1.22	1.92	2.15
Si	0.02	2.40*	-0.14	-1.00	-1.61	3.45**	-0.81	-2.10
Th	2.01**	2.32*	1.12	1.39	1.77	1.92	4.01	1.56
Tw	0.53	2.35*	0.69	0.37	0.43	-0.66	-0.73	1.28
LSM(A+R)	43.66	33.11	25.50	44.44	67.96	18.49	69.76	46.98

a,b,c,d,e : Additive or reciprocal deviations within the same column sharing the same superscript letter do not differ significantly ($p<0.05$)

* : $p<0.05$

** : $p<0.01$

Table 8. Least square means of non-additive deviations of body weight at harvest (grams) from the mean of the parent strains across test environments (LSM b_D according to model 2) of the 28 strain crosses of Nile tilapia (reciprocals pooled together).

	Strain						
	Gh	Ke	Se	Is	Si	Th	Tw
E2	1.28	4.79**	6.68**	4.52**	0.38	4.16**	2.60**
Gh		-1.47	3.26**	0.41	0.47	4.58**	1.31
Ke			-0.69	1.91*	0.23	2.02*	0.83
Se				3.29**	-1.31	1.16	0.01
Is					-0.90	2.65**	-0.08
Si						0.88	0.43
Th							-1.36

* :p<0.05

** :p<0.01

Table 9. Mean percent heterosis (H%) on body weight at harvest across test environments in the 56 strain crosses of Nile tilapia based on the estimates of genetic components according to model 2.

Sire strain	Dam strain							
	E2	**Gh**	**Ke**	**Se**	**Is**	**Si**	**Th**	**Tw**
E2	-	3.8	9.9	11.6	9.3	3.1	9.5	5.7
Gh	4.0	-	-1.5	10.4	2.6	3.9	14.0	3.7
Ke	10.6	-1.5	-	-0.8	4.8	1.9	5.6	1.6
Se	12.3	10.3	-0.8	-	6.3	0.3	2.8	0.0
Is	10.4	2.8	4.9	6.7	-	0.6	7.0	0.4
Si	2.8	3.5	1.7	0.2	0.4	-	5.8	2.5
Th	10.0	13.7	5.4	2.7	6.5	6.0	-	-5.2
Tw	6.0	3.8	1.7	0.1	0.3	2.8	-5.8	-

Table 10. Pearson correlation coefficients (r_{AD}) between least squares means of additive (including reciprocal) genetic components and non-additive genetic components of body weight at harvest of the 56 strain crosses of Nile tilapia (LSM(A+R) and b_D according to model 2), and mean of percent heterosis (H%) within and across test environments.

Test environment	Mean body weight components (grams)		r_{AD}	mean heterosis (H%)
	additive	non additive		
S1	33.11	2.80**	0.18	8.40**
S2	25.50	0.64	-0.19	2.53
P1	44.44	0.76	-0.03	1.78
P2	67.96	0.36	0.22	-0.05
C2	18.49	1.59**	-0.46**	9.62**
W2	69.76	2.41	0.12	3.48
W4	46.98	1.96*	-0.20	4.35*
All	43.66	1.50**	0.08	4.30**

* : $p < 0{,}05$

** : $p < 0{,}01$

Table 11. Pearson correlation coefficients between additive (including reciprocal) genetic effects on body weight at harvest of the 64 strain combinations of Nile tilapia (LSM(A+R) according to model 2) in the different test environments and across all test environments.

	Test environment						
	S2	P1	P2	C2	W2	W4	All
S1	0.57	0.60	0.56	0.82	0.35	0.45	0.72
S2		0.64	0.69	0.54	0.54	0.69	0.76
P1			0.91	0.73	0.86	0.78	0.95
P2				0.67	0.83	0.72	0.92
C2					0.59	0.47	0.82
W2						0.74	0.87
W4							0.84

All correlations significant ($p<0.01$)

Table 12. Pearson correlation coefficients between non-additive genetic effects on body weight at harvest of the 28 strain crosses of Nile tilapia (b_D according to model 2) in the different test environments and across all test environments.

	Test environment						
	S2	**P1**	**P2**	**C2**	**W2**	**W4**	**All**
S1	0.34^{NS}	0.46*	0.49	0.17^{NS}	0.54	0.42*	0.70
S2		0.47*	0.60	-0.29^{NS}	0.62	0.64	0.71
P1			0.70	-0.14^{NS}	0.55	0.62	0.78
P2				-0.17^{NS}	0.61	0.56	0.81
C2					-0.11^{NS}	-0.16^{NS}	0.01^{NS}
W2						0.60	0.86
W4							0.79

NS : Non-significant (p>0.05)

* : 0.05>p>0.01

All other correlations significant (p<0.01)

1 **LEGEND TO FIGURES**
2
3 Fig. 1. Least squares means of body weight at harvest of Nile tilapia according to model 1 in
4 the different test environments. Standard errors are shown by vertical lines.
5
6 Fig. 2. Least squares means of body weight at harvest of the different batches (i.e. age groups
7 at stocking) of Nile tilapia across test environments according to model 1. Standard errors are
8 shown by vertical lines.
9
10 Fig. 3. Plot of least squares means (LSM) of body weight at harvest of the 64 strain
11 combinations of Nile tilapia across test environments according to model 1 and model 2.
12
13 Fig. 4. Least squares mean contributions to body weight at harvest (grams) of the progeny of
14 the eight strains of Nile tilapia when the strains were used as sires or as dams. The estimates
15 are based on additive genetic and reciprocal effects across test environments according to
16 model 2. The standard errors of the estimates were between 0.47 and 0.56 grams.
17
18 Fig. 5. Least squares means of body weight at harvest for the eight pure strains of Nile tilapia
19 (open bars) and of general heterosis of each strain in crosses with all other strains (striped
20 bars), across all test environments according to model 2. Standard errors are shown by
21 vertical lines.
22
23 Fig. 6. Distribution of least squares means (LSM) of body weight at harvest of the 64 strain
24 combinations across test environments according to model 2 compared to the LSM body
25 weight of the best performing pure strain (Kc).

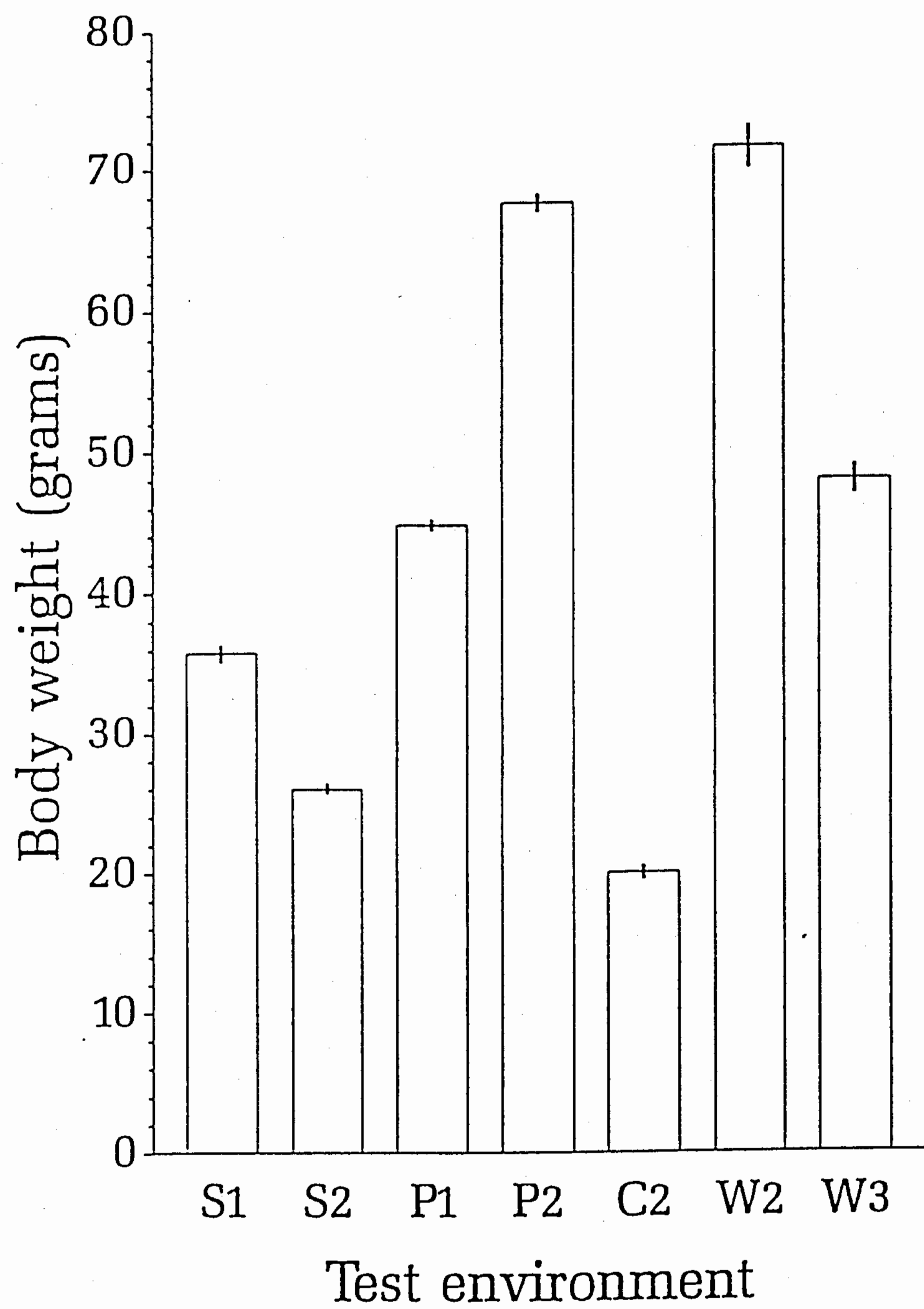

FIG. 1

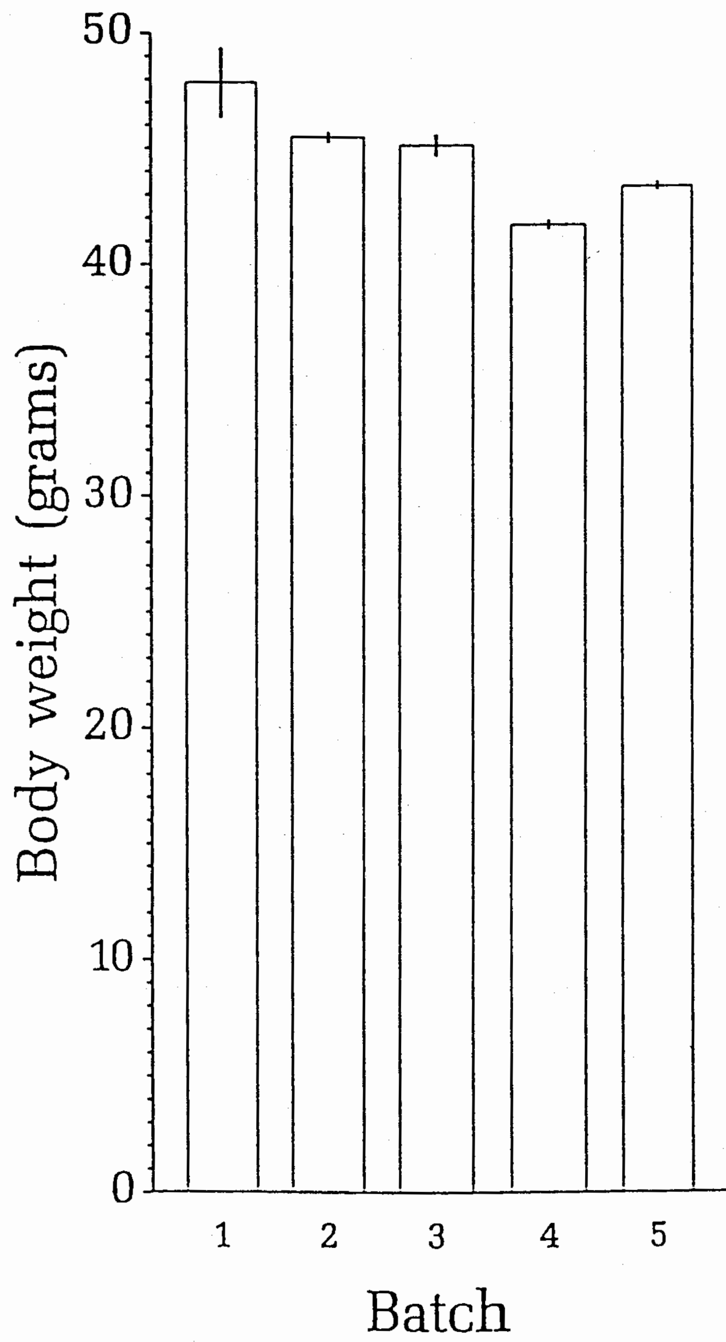

FIG. 2

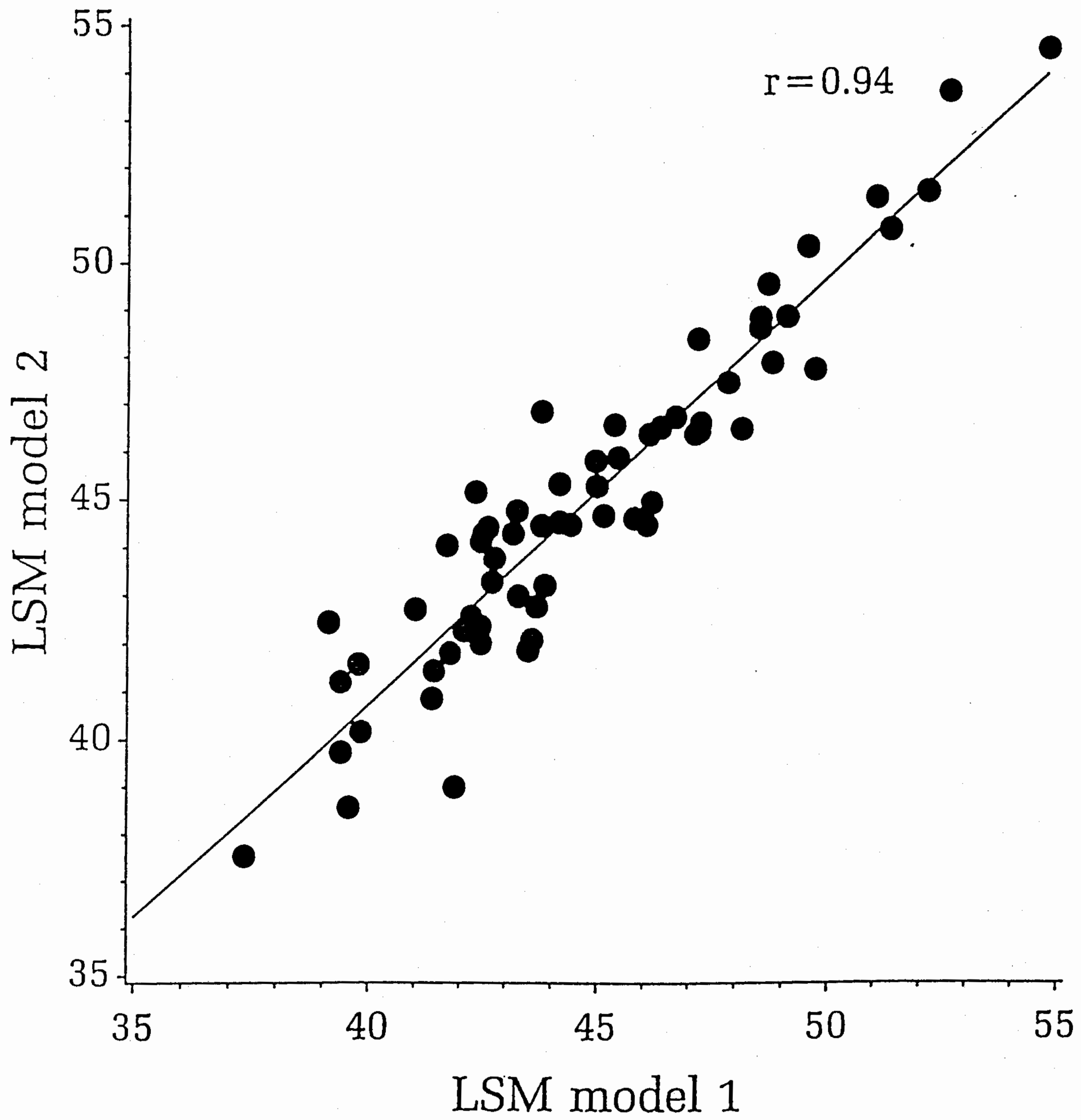

FIG. 3

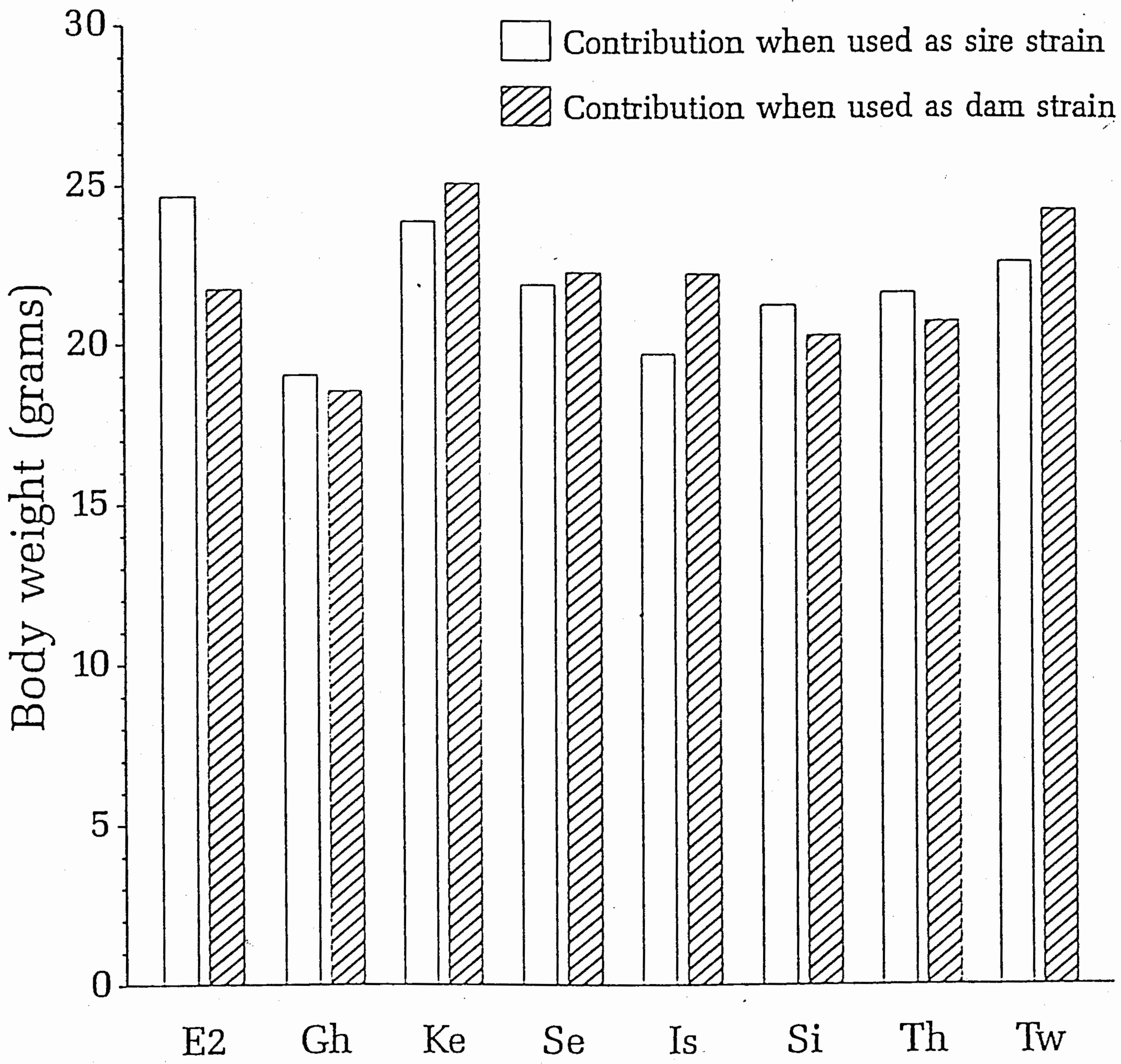

FIG. 4

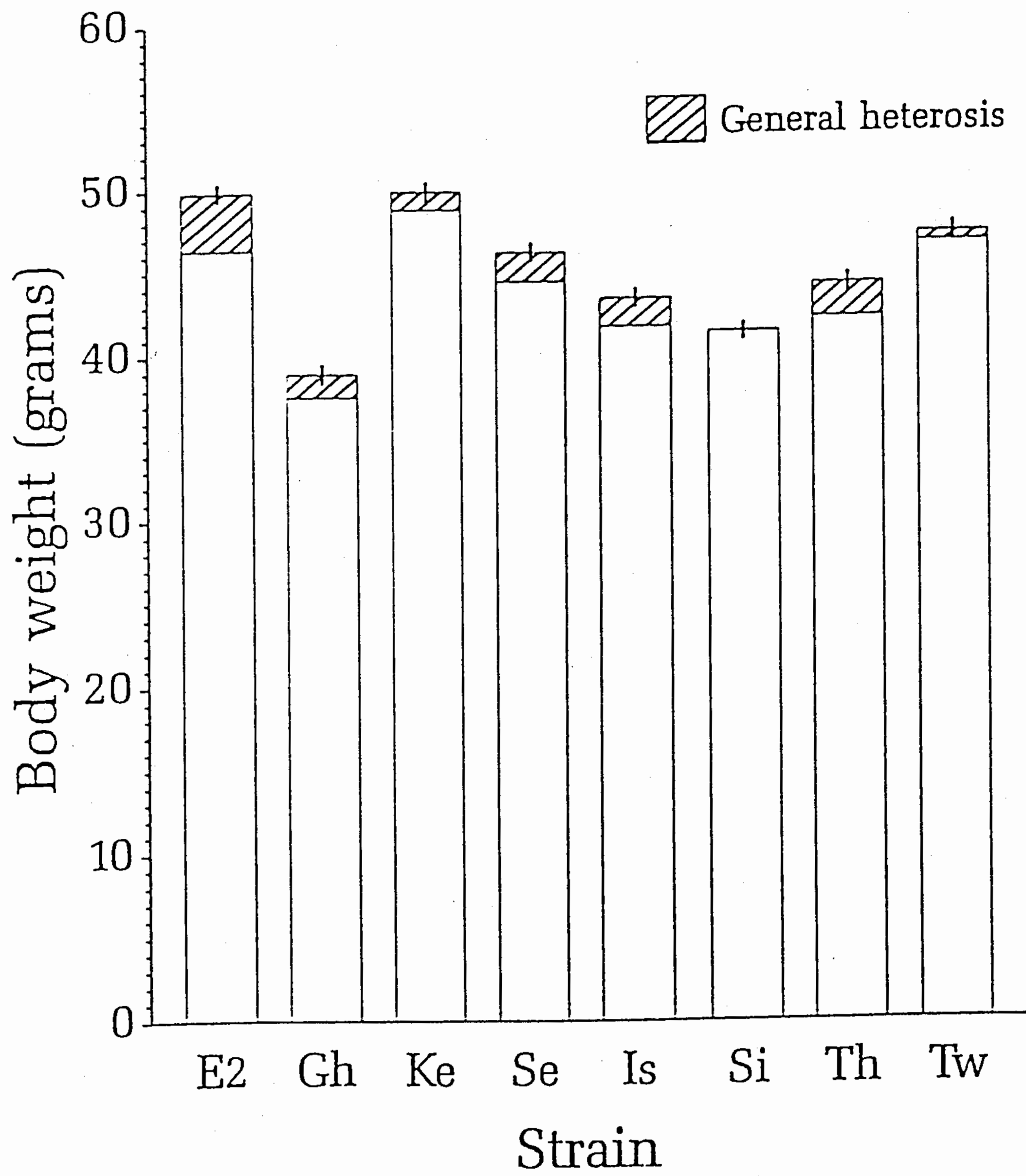

FIG. 5

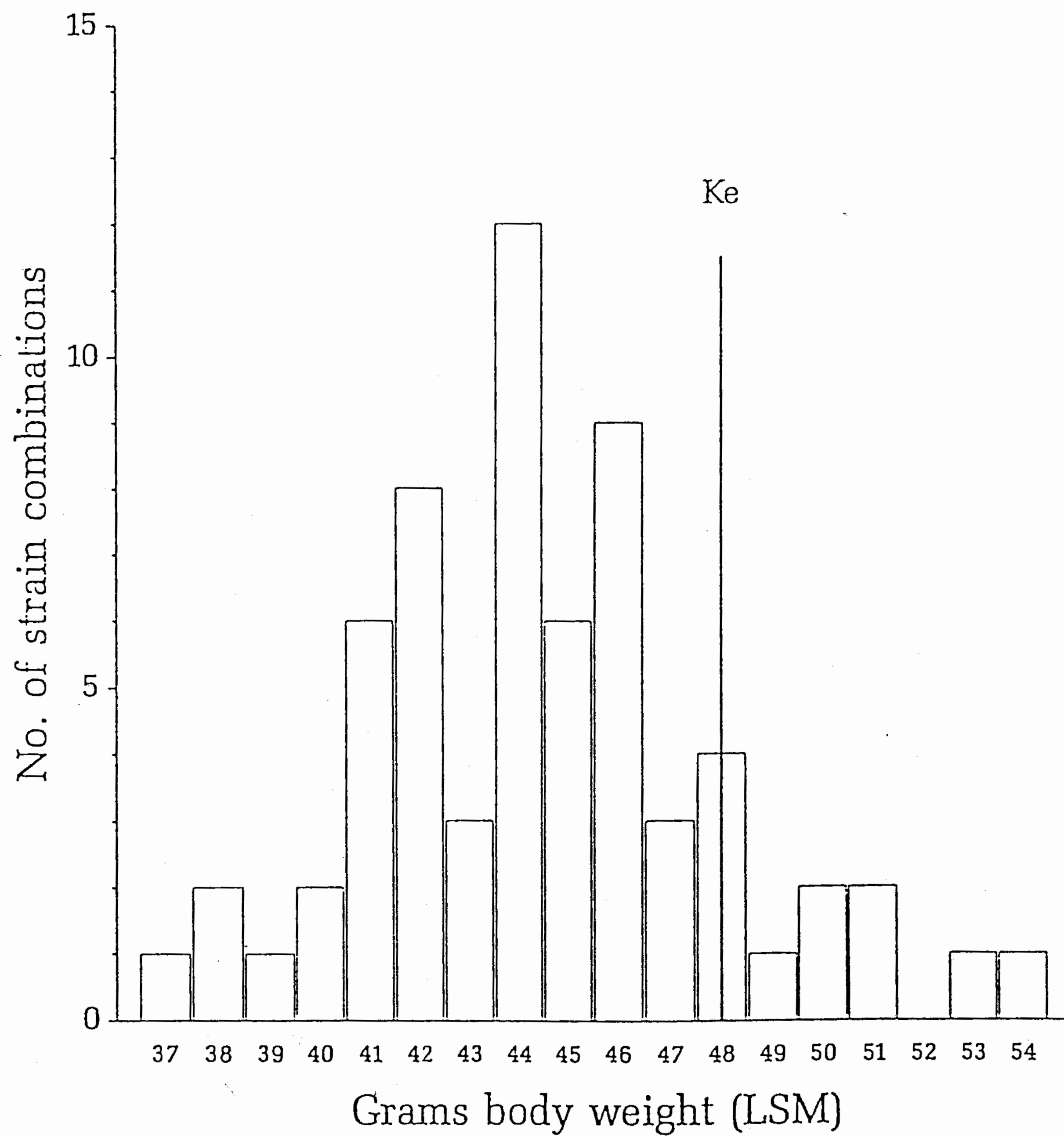

FIG. 6

Chou, L.M., Munro, A.D., Lam, T.J., Chen, T.W., Cheong, L.K.K., Ding, J.K., Hooi, K.K., Khoo, H.W., Phang, V.P.E, Shim, K.F. & Tan, C.H. (editors). 1994. The Third Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.

EARLY GROWTH AND SURVIVAL OF EIGHT STRAINS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*) AND THEIR CROSSES*

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*ICLARM Contribution No. 895

Abstract

A complete diallele crossing experiment was performed using eight strains of Nile tilapia (*Oreochromis niloticus*) for a comparative analysis of two traits: early growth and survival. The eight strains include four new strains recently imported to the Philippines from open waters in Egypt (E2), Ghana (Gh), Kenya (Ke) and Sénégal (Sé), and four Asian farmed strains, namely Israel (Is), Singapore (Si), Taiwan (Tw) and Thailand (Th). The progeny were reared under uniform conditions up to about 5 g body weight. A total of 5 batches and 216,915 fry were collected. The Generalized Linear Models procedure was used to estimate the effects of batch and cross on the two traits. Weight gain of all batches differed significantly ($p < 0.05$) except Batch 1 vs. Batch 2 and Batch 2 vs. Batch 3. Lower survival was observed in Batch 1 than other batches, while Batch 5 had lower survival than Batch 3. Ke showed significantly higher weight gain than Gh ($p < 0.01$) among purebreds. Significant reciprocal effects for early weight gain were found for E2 X Th ($p < 0.01$) and Sé X Si ($p < 0.05$). All other reciprocal effects (effects of switching sire and dam line in a cross) were not significant. Si gave lower survival than Sé and Tw ($p < 0.01$) and E2 and Gh ($p < 0.05$). The reciprocal effects on survival were also not significant. There were no significant heterotic effects on early weight gain and a moderate mean heterosis (7.3%). Significant heteroses for early survival were found in some crosses but the mean heterosis was still modest (10.2%).

Introduction

Improvement of farmed fish strains for specific traits has rarely been attempted but has great potential. Such traits include growth, age/size at first maturation and survival. Differences in growth performance have been observed in tilapia strain comparisons (Jayaprakas et al. 1988; Tave 1988; McAndrew et al. 1989; Eknath et al., in press). The measurements made in such studies are often body weights at harvest after 47 to 120 days. Smitherman et al. (1988) found significant differences among the early growth rates and survival of fry of three strains of *Oreochromis niloticus*.

The study reported here was undertaken as part of a collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT) which had the aim of developing a synthetic strain of *O. niloticus* by selection from a base population built from eight strains: Egypt, Ghana, Kenya, Sénégal, Israel, Singapore, Taiwan and Thailand. The work comprised a complete 8 X 8 dialelle crossing experiment, data on heterosis, guidelines for combining the strains to form the base population, and comparisons of the early growth and survival (from yolk-sac fry to 63 days) of purebreds and crossbreds.

Materials and Methods

Experimental design

A complete diallele crossing experiment was carried out among eight strains of *O. niloticus* to produce the possible purebreds (8) and crossbreds (56). The eight strains were E2, Gh, Ke, Sé, Is, Si, Tw and Th. Their origins were described by Eknath et al. (in press).

Prior to breeding, females were segregated from males and conditioned in 3 X 3 X 1 m hapas at 10 fish/m^3 for two weeks. Broodstock were fed twice daily with a mixture of 70% rice bran and 30% fish meal at 10% of the body weight. Males and females for each strain combination were stocked in 1-m^3 breeding hapas installed in a 0.3 ha earthen pond at a sex ratio of 1:2. Six to 12 replicate breeding hapas were used for each strain combination. The weights of the breeders were recorded before stocking and after spawning. Breeding was set during the early part of December 1989.

Fry collection and rearing

First inspection of the hapas was done seven days after stocking of breeders. Fish which spawned were removed from the hapa and fry were collected. Fry or yolk-sac fry collected within a seven-day period were referred to as a batch. The numbers and bulk weight of fry were recorded. The batches were then stocked in fine mesh hapas at densities of 200-250/m^3. They were fed with 70% rice bran and 30% fish meal twice daily. After 21 days, the batches were

transferred to bigger mesh size hapas at stocking density of $100/m^3$. The weights were again taken and the numbers that survived in the hapas were recorded.

Data analysis

The mean weight gain (g) of fry from yolk-sac fry to 3-5 grams (63 days post-hatching) and percent survival were analyzed separately using the Generalized Linear Models (GLM) procedure as follows:

$$Y_{ij} = a + C_i + B_j + e_{ij}$$

where:

Y_{ij} is the weight gain/survival of the jth batch of the ith cross

a - is a constant

C_i - is the fixed effect of the ith cross

B_j - is the fixed effect of the jth batch

e_{ij} - is the random error

The same model was used to estimate the least square means of weight gain and survival of genetic groups.

The heterosis effects were obtained by estimating and testing the differences between all batches of a given cross (including both reciprocals) and the mean or purebreds of both parent strains. Heterosis for growth was expressed as a percentage of the mean of the parental strains.

Predation by a goby (*Glossogobius* sp.) caused extremely high mortality in Batch 1 of the purebred Egypt (E2) and these were deleted from the dataset.

Results and Discussion

Fry Collection

Tables 1 and 2 show the number of fry collected by batch and genetic groups, respectively. A total of 216,915 fry were collected from the five batches. The highest number of fry was collected in Batch 4 and the lowest in Batch 3. Among the genetic groups, the African wild strains gave the highest production (63,238) compared with the Asian farmed strains (42,484).

Table 1. Collection period, number of fry stocked and least square means of early weight gain (g) and survival (%) in different batches of fry produced from matings between breeders from African wild strains of *Oreochromis niloticus* and Asian farmed strains.

Batch	Collection period	No. of fry stocked	Weight gain	Survival (%).
1	Dec. 27-28, 1989	40,015	5.08^{a}	66.1^{c}
2	Jan. 10-11, 1990	31,010	4.89^{ab}	78.8^{ab}
3	Jan. 23-24, 1990	5,296	4.20^{b}	86.3^{a}
4	Feb. 21-23, 1990	98,476	2.33^{d}	81.9^{ab}
5	Mar. 12-16, 1990	42,118	3.24^{c}	75.0^{b}

Weight gain or survival of batches with different superscirpt letters differs significantly ($p < 0.05$).

Table 2. Number of fry stocked and least square means of early weight gain (g) and survival (%) in offspring from matings between breeders from African wild strains of *Oreochromis niloticus*, Asian farmed strains and their crosses (sire x dam).

Groups	No. of fry stocked	Weight gain (g)	Survival (%)
AFR X AFR	63,238	3.82^{a}	81.94^{a}
AFR X PHI	56,421	4.09^{a}	79.03^{a}
PHI X AFR	54,772	4.03^{a}	75.88^{ab}
PHI X PHI	42,484	3.76^{a}	71.27^{b}

Weight gain or survival of groups with different superscirpt letters differs significantly ($p < 0.05$).

Early Growth and Survival in Batches and Genetic Groups

Weight gain of all batches differed significantly ($p < 0.05$) except Batch 1 vs. Batch 2 and Batch 2 vs. Batch 3. Batch 1 had a significantly lower survival than other batches, and Batch 5 had lower survival than Batch 3. All other differences were not significant ($p > 0.05$).

There were no significant differences in weight gain among the genetic groups but significantly lower survivals were observed when both parents came from Asian farmed strains than when both parents ($p < 0.01$) or the sire ($p < 0.05$) came from African wild strains.

Early Growth and Survival in Eight Strains and Reciprocal Crosses

The least square means of early weight gain in 8 strains of tilapia and the 56 reciprocal crosses between males and females from the 8 strains are given in Table 3. Among purebreds, Ke showed significantly higher weight gain than Gh ($p < 0.01$), all other differences were not significant. The observed faster growth of Ke was consistent with the findings of Eknath et al. (in press), Palada-de Vera and Eknath (in press) for later growth performance of this strain. Because of its fast growth potential at early age, the pure strain Ke mated to Si or E2 dams appear to be the better strains for good growth. Eknath et al. (in press) reported that these two strains gave better performance in over 90 days in various culture environments.

Significant reciprocal effects for early weight gain were found for the E2 X Th ($p < 0.01$) and Sé X Si crosses ($p < 0.05$). All other reciprocal effects (effects of switching sire and dam line in a cross) were not significant.

Significantly lower survival in Si was observed than in Sé and Tw ($p < 0.01$) and E2 and Gh ($p < 0.05$); all other differences were not significant. The reciprocal effects were also not significant. The Sé strain showed better survival than other purebreds, and when crossed with Ke attained the highest survival amongst the crosses.

Table 3. Least square means of early weight gain (g) in 8 strains and the 56 crosses of *Oreochromis niloticus* composed of Egypt (E2), Ghana (Gh), Kenya (Ke), Sénégal (Sé), Israel (Is), Singapore (Si), Taiwan (Tw) and Thailand (Th).

Strains (f) (m)	E2	Gh	Ke	Sé	Is	Si	Tw	Th
E2	3.30	3.15	3.57	3.63	3.06	3.12	3.75	5.19
Gh	3.10	2.47	4.06	3.63	3.05	3.69	3.37	3.52
Ke	5.47	4.19	4.84	4.46	4.73	5.63	4.36	4.71
Sé	3.70	4.33	4.87	3.39	3.33	6.39	3.74	4.27
Is	3.13	4.13	6.34	3.32	3.95	3.05	3.82	3.61
Si	3.92	3.67	4.72	4.05	3.71	4.26	4.31	4.17
Tw	3.48	4.31	5.91	3.40	3.69	3.66	3.13	3.06
Th	2.35	3.91	4.19	3.91	3.98	3.96	2.88	4.61

(m) - males (f) - females
Significance limits of pairwise comparisons:
$p < 0.05$: Differences exceeding 1.9 - 2.2 g
$p < 0.01$: Differences exceeding 2.3 - 2.6 g

Table 4. Least square means of early survival (%) in 8 strains and the 56 crosses of *Oreochromis niloticus* composed of Egypt (E2), Ghana (Gh), Kenya (Ke), Sénégal (Sé), Israel (Is), Singapore (Si), Taiwan (Tw) and Thailand (Th).

Strains (f) (m)	E2	Gh	Ke	Sé	Is	Si	Tw	Th
E2	78.7	85.0	78.8	95.2	86.6	75.9	72.2	74.0
Gh	79.3	76.1	70.7	75.5	74.8	74.3	64.9	68.3
Ke	92.4	85.7	65.4	96.9	88.3	95.8	86.9	80.2
Sé	86.6	79.2	86.1	88.2	82.8	86.8	81.3	83.8
Is	82.8	60.1	85.1	74.3	71.5	91.8	75.1	86.5
Si	89.7	73.7	77.1	56.5	69.5	44.1	59.8	54.7
Tw	89.1	77.2	63.4	82.7	84.9	91.3	83.3	72.0
Th	86.1	58.9	88.7	74.3	72.3	61.4	66.5	66.7

(m) - males (f) - females
Significance limits of pairwise comparisons:
$p < 0.05$: Differences exceeding 30-35%
$p < 0.01$: Differences exceeding 37-42%

Heterosis effects

Heterotic effects on early weight gain were not significant (Table 5). The overall mean heterosis across all groups was only moderate (7.2%). The mean heterosis for early weight gain of each strain in crosses with all other strains were E2, 3.7%; Gh, 17.0%; Ke, 15.1%; Sé, 12.7%; Is, -0.4%; Si, 4.4%; Tw, 10.6%; and Th, -4.9%. In general, the African strains seem to give higher heterosis for early weight gain (mean 12.1%) than the Asian strains (mean 1.4%). None of the heterosis effects were significantly different from zero.

Significant heterosis for early survival were found in the Ke X Si ($p < 0.01$) and Ke X Is and E2 Si ($p = 0.05$) crosses, but the mean heterosis was still modest (10.2%). Heterosis for early survival was mainly seen in crosses involving the Ke and the Si strains; mean heterosis in crosses with all other strains - Ke, 23.3%, Si, 25.3%. The other strains gave medium low heterosis: means - E2, 13.7%; Gh, 0.10%; Sé, 3.0%; Is, 10.5%; Tw, 1.10% and Th, 5.4%.

Table 5. Percent heterosis for early weight gain (weight gain of both reciprocal crosses relative to average of parental strains) for strains of *Oreochromis niloticus* composed of Egypt (E2), Ghana (Gh), Kenya (Ke), Sénégal (Sé), Israel (Is), Singapore (Si), Taiwan (Tw) and Thailand (Th).

Strains	Gh	Ke	Sé	Is	Si	Tw	Th
E2	8.6	15.8	9.4	-14.8	-5.4	11.5	0.7
Gh		12.7	33.4	16.2	9.3	34.1	4.8
Ke			13.3	24.0	13.8	29.2	-3.3
Sé				-9.8	29.4	9.8	3.4
Is					-14.6	5.7	-9.8
Si						6.3	-8.0
Tw							-22.2
Overall mean heterosis							7.3

None of the heterosis effects were significantly different from zero.

Table 6. Percent heterosis for early survival (survival of both reciprocal crosses relative to average of parental strains) for strains of *Oreochromis niloticus* composed of Egypt (E2), Ghana (Gh), Kenya (Ke), Sénégal (Sé), Israel (Is), Singapore (Si), Taiwan (Tw) and Thailand (Th).

Strains	Gh	Ke	Sé	Is	Si	Tw	Th
E2	6.7	20.8	7.8	12.2	36.6*	2.7	9.2
Gh		9.7	-6.3	-11.1	23.2	-12.9	-9.8
Ke			19.2	27.2*	58.2**	1.1	25.9
Sé				-1.6	2.9	-4.1	3.0
Is					29.6	5.0	12.4
Si						22.0	4.4
Tw							-7.2
Overall mean heterosis							10.2

* $p < 0.05$
** $p < 0.01$

Overall, there seems to be low to modest levels of heterosis for early weight gain and survival among the crosses made, and no significant reciprocal effects were observed for early weight gain and survival.

Acknowledgements

This work forms a part of the collaborative research project on the Genetic Improvement of Farmed Tilapias (GIFT), co-financed by the Asian Development Bank (RETA 5279) and the United Nations Development Program/Division for Global and Interregional Programmes (INT/88/019). We are grateful to all the GIFT Project staff for providing the technical support to this study. We also thank Roger S.V. Pullin and Mr. Jay L. Maclean for their comments on the manuscript.

References

Eknath, A.E., M.M. Tayamen, M.S. Palada-de Vera, J.C. Danting, R.A. Reyes, E.E. Dionisio, J.B. Capili, H.L. Bolivar, T.A. Abella, A.V. Circa, H.B. Bentsen, B. Gjedre, T. Gjedrem and R.S.V. Pullin. 1993. Genetic improvement of farmed tilapias: The growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. Aquaculture (in press).

Jayaprakas, V., D. Tave and R.O. Smitherman. 1988. Growth of two strains of *Oreochromis niloticus* and their F_1, F_2 and backross hybrids, pp. 197-201. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds.) The Second International Symposium on Tilapia in Aquaculture. ICLARM Conf. Proc. 15, 623 p.

McAndrew, B.J., and Malumdar, K.C. 1989. Growth studies on juvenile tilapia using pure species, hormone -treated and nine interspecific hybrids. Aquaculture and Fisheries Management 20:35-47.

Palada-de Vera, M.S. and A.E. Eknath. Predictability of individual growth rates in tilapia. Aquaculture (in press).

Smitherman, R.O., A.A. Khater, N.I. Cassel, and R.A. Dunham. 1988. Reproductive performance of three strains of *Oreochromis niloticus*. Aquaculture 70:29-37.

Tave, D. 1988. Genetics and breeding of tilapia: a review, pp. 285-293. *In* R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (eds) The Second International Symposium on Tilapia in Aquacuture. ICLARM Conf. Proc. 15, 623 p.

Genetic improvement of farmed tilapia: a complete diallele cross between four African and Asian strains of Nile tilapia (*Oreochromis niloticus*)

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The objective of this study was to determine the magnitude of non-additive (heterotic) genetic effects when four African and four Asian strains of Nile tilapia were crossed and reared under different Philippine tilapia farming systems. The purpose was to decide if a breeding program for farmed tilapia may be based on a simple purebreeding strategy from a mixed base population, or if a crossbreeding program should be established.

A total of 26000 individually tagged fingerlings representing the 64 possible different pure strain and reciprocal crosses were reared in eight different test environments for 90 days. Additive, reciprocal and heterotic effects were estimated. The heterotic effect for growth and survival ranged from −5% to 10%. The overall heterosis for growth and survival was negligible (2.3%). Thus, the results do not justify a crossbreeding program. Protocols for establishing a base population by selecting the purebred and crossbred test fish on their additive performance across test environments can be developed.

PROGENY SEX RATIO OF A COMPLETE DIALLEL CROSS WITH EIGHT STRAINS OF NILE TILAPIA (Oreochromis niloticus L.)

EDNA E. DIONISIO

INTRODUCTION

This study focuses on determining the progeny sex ratio in a complete diallel cross of eight strains of Nile tilapia (Oreochromis niloticus). It was envisioned as a benchmark study in intraspecific hybridization in Nile tilapia and also to provide directions for future research on genetic manipulation of sex ratios and for understanding the mechanisms of sex determination in Nile tilapia.

The systematic design and execution of this relatively large-scale experiment involving eight strains of Nile tilapia was envisioned as a benchmark study in intraspecific hybridization

OBJECTIVES OF THE STUDY

This study was conducted primarilly to determine if significantly high proportion of males could be produced through intraspecific (inter-strain) hybridization. It has used strains of known origins and genetic characteristics.

SUMMARY OF METHODS

Eight diverse strains of Nile tilapia were used to produced 64 strain combinations in a complete diallel cross. The study was conducted in two phases.

a) Phase I involved a complete diallel cross of eight strains was conducted as part of a major study under the auspices of the GIFT project. This study focused on determing the proportion of males at the end of 3 months grow-out period. The analysis was based on the tagged individuals recovered after 90 days across all environments

b) Phase II involve systematic repeat breeding of a certain strain combinations that had yielded relatively high proportion of males during Phase I to determine the repeatability of sex ratio.

Phase I was conducted from December 1989 to September 1991, at the following facilities and environments:

Breeding of test strains: BFAR/NFFTRC, Muñoz, Nueva Ecija.

Grow-out: BFAR/NFFTRC; FAC-CLSU;Freshwater Fisheries Demonstration Station, Bai, Laguna and Regional Fisheries Station, Salinungan, San Mateo Isabela

Phase II

This study was conducted from July 1994 to December 1994, at BFAR/NFFTRC, Muñoz, Nueva Ecija.

DATA ANALYSIS

Phase I

The association between male proportion in 64 strain combinations and the survival of the strain combinations from tagging until the end of the experiment was investigated by computing the correlation coefficients. The sources of variation such as batch, parent strains as sires (SST), parent strains as dams (DST) and the interaction effects of various strain combinations, on the proportion of males were analyzed across all environments together according to the generalized linear models (GLM) procedure using the Statistical Analyses System software (SAS, 1992).

To analyze the effects of crossing the two major groups of strains used in this study, namely, the African strains and the Asian farmed strains, the proportion of males in the 64 strain combinations were grouped into five categories: 1)purebreds (PU); 2) crossbreds between African strains (AA); 3) crossbreds between Philipine strains (PP); 4) crossbreds between African sire strains and Asian dam strains (AP); and 5) crossbreds between Asien sire strains and African dam strains (PA). General lineer models procedures was used .

Phase II

Chi square was used to test for the deviation of the observed proportion of males from the expected 50% proportion.

SUMMARY OF RESULTS

Phase I. The variation in propotion of males as a function of four independent factors namely; batch, the use of eight parent strains as sires (SST), the use of eight parent strains as dams (DST), and the interaction between SST*DST. Of these, only SST and DST significantrly affected the proportion of males, Table 1. The lack of significance of the SST*DST interaction effects suggests that the proportion of males is almost fully

explained by the additive genetic effects of the strains involved. A significantly higher proportion of males was observed when Thailand strain was used as sire strain ($p<0.05$) compared to all other strains. Using Senegal strain as a dam strain resulted in a significantly lower proportion of males ($P<0.05$) as compared to all other strains.

Table 1. Sources of variation in proportion of males: degrees of freedom (df), marginal mean square (MS), and percent contribution of different effects in General Linear Model 1. (R^2=0.45;n=183).

EFFECTS	df	MEAN SQUARE[a]	% CONTRIBUTION[b]	F-VALUE
Batch	4	0.109595	15.16	0.93
Parent strain as				
sires	7	0.0243437	33.67	2.06*
dams	7	0.0241076	33.36	2.04*
Sire x Dams	49	0.0128371	17.77	1.09
Error	115	0.0118167		

[a] Type III MS
[b] based on total MS for all independent variables in the model
* significant ($p<0.05$)

The correlation between the observed proportion of male at harvest by batch and strain combinationss and survival from the stage of swim up fry to tagging and stocking to different environments were not signficant ($p<0.05$) suggesting that the observed proportion of males was not affected by survival at early stage and at later stage of their life cycle.

Of the 64 strain combinations, 19 yielded significantly more males than females ($p<0.05$). The grand mean proprotion of males across all batches and strain combinations was 57.5 percent. Over all, the highest mean proportion of males were produced by Asian sire strains x Asian dam strains (63%), and the lowest by the crossbreds of African sires x African dam strains (53%). The mean proportion of males across the 12 African crossbreds (AA) and the 8 purebreds (PU) did not deviate significantly from 50 percent proportion of males ($p<0.05$), Table 2.

GROUP	PROPROTION OF MALES (LSM) (%)
AA	53.45[bc] (±0.035)
AP	59.36[ab] (±0.094)
PA	55.56[ab] (±0.056)
PP	63.39[a] (±0.134)
PU	54.80[bc] (±0.048)

Column means having common superscript letter do not differe significantly, ($p<0.05$).

The 19 strain combinations that produced significantly high proportion of males were bred again to determine the repeatability of sex ratio during Phase II. Based on the limited information provided by the present Phase II study, it seems that the high proportion of males produced by a certain strain combination in the main experiment (Phase I) may be repeated.

STATUS OF THE PAPER

1. The whole paper was submitted as masteral thesis leading to the degree of Master of Science in Aquaculture, Central Luzon State University, Muñoz, Nueva Ecija.

2. The Phase I of this paper was presented as poster paper during the 5th ISGA, Halifax, Nova Scotia, June 1994.

3. The review and editing of this paper for Publication is still in progress. Draft was given to Dr. A. E.Eknath and it is now with Hans Bentsen.

Attachment 6.

Estimated heritabilities within each environment, and across all environments, during each generation

G3

		TOTAL			*AGE0 (SEXF)* COVARIABLE NO.1		*SEXF* within environment *ENVR x SEXF* across environments FIXED EFFECT NO. 1				GENETIC GROUP FIXED EFFECT NO. 2				PARAMETER ESTIMATES WITH THEIR APPROX. S.E.				APP SAM COR
Generasjon	Resultatfil	N	MEAN	SDEV	LEVEL	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	h2	seh2	c2	sec2	
G3	bailbwf.res	1674	64,137	17,3964			1	890	54,3539326	-9,7833434					,294	,127	,073	,055	
							2	784	75,2433674	11,1060914									
G3	bailbwc.res	1674	64,137	17,3964	1	0,125792638	1	890	54,3539326	-9,8540256					,308	,132	,071	,055	
					2	-0,00479271	2	784	75,2433674	11,1030343									
G3	bfar1bwf.res	1743	83,372	25,7641			1	877	65,8491448	-17,5228					,247	,163	,186	,077	
							2	866	101,117321	17,7453761									
G3	bfar1bwc.res	1743	83,372	25,7641	1	0,135481942	1	877	65,8491448	-17,571714					,241	,155	,172	,073	
					2	0,352786193	2	866	101,117321	17,8743626									
G3	bfar2bwf.res	1772	70,412	22,2104			1	928	55,6492457	-14,762436					,207	,122	,123	,057	
							2	844	86,6433649	16,2316832									
G3	bfar2bwc.res	1772	70,412	22,2104	1	0,050016559	1	928	55,6492457	-14,802457					,210	,119	,120	,055	
					2	0,104067526	2	844	86,6433649	16,3232418									
G3	bfcgbwf.res	1016	16,911	5,3445			1	515	14,9328155	-1,9784049					,224	,205	,244	,099	
							2	501	18,9449102	2,03368971									
G3	bfcgbwc.res	1016	16,911	5,3445	1	0,102679936	1	515	14,9328155	-1,9091625					,284	,168	,163	,079	
					2	0,118711129	2	501	18,9449102	1,95139959									
G3	fac1bwf.res	1674	62,142	21,7796			1	876	48,7063927	-13,436021					,161	,114	,129	,055	
							2	798	76,8917293	14,7493159									
G3	fac1bwc.res	1674	62,142	21,7796	1	-0,020252445	1	876	48,7063927	-13,423837					,161	,110	,119	,053	
					2	0,285074691	2	798	76,8917293	14,937577									
G3	fac2bwf.res	1554	49,728	17,6885			1	805	38,2524224	-11,475248					,137	,131	,184	,067	
							2	749	62,0608812	12,3332107									
G3	fac2bwc.res	1554	49,728	17,6885	1	0,094961548	1	805	38,2524224	-11,531655					,153	,117	,155	,062	
					2	0,274431503	2	749	62,0608812	12,5084096									
G3	fwasbwf.res	1631	53,573	16,5863			1	887	42,757159	-10,815864					,145	,115	,113	,055	
							2	744	66,4677419	12,8947193									
G3	fwasbwc.res	1631	53,573	16,5863	1	-0,056285675	1	887	42,757159	-10,832741					,138	,112	,116	,054	
					2	-0,071500154	2	744	66,4677419	12,9202788									
G3	pncgbwf.res	768	59,506	25,8074			1	340	47,2411765	-12,265204					,678	,270	,019	,104	
							2	428	69,2497664	9,74338615									
G3	pncgbwc.res	768	59,506	25,8074	1	0,342580306	1	340	47,2411765	-12,313641					,564	,209	,000	,085	
					2	0,806911423	2	428	69,2497664	9,83401836									
G3	smalbwf.res	1722	61,242	14,1253			1	886	53,7732506	-7,4683871					,170	,112	,114	,053	
							2	836	69,1566986	7,91506093									
G3	smalbwc.res	1722	61,242	14,1253	1	0,166803688	1	886	53,7732506	-7,4795195					,148	,106	,116	,052	
					2	0,046538476	2	836	69,1566986	7,91835265									
G3	g3bwf.res	13554	60,091	24,9671			1	890	54,3539326	-5,7371254					,177	,087	,093	,038	
							2	784	75,2433674	15,1523094									
							3	877	65,8491448	5,75808682									
							4	866	101,117321	41,026263									
							5	928	55,6492457	-4,4418123									
							6	844	86,6433649	26,5523069									
							7	515	14,9328155	-45,158242									
							8	501	18,9449102	-41,146148									
							9	876	48,7063927	-11,384665									
							10	798	76,8917293	16,8006713									
							11	805	38,2524224	-21,838636									
							12	749	62,0608812	1,96982318									
							13	887	42,757159	-17,333899									
							14	744	66,4677419	6,37668395									
							15	340	47,2411765	-12,849882									
							16	428	69,2497664	9,15870836									
							17	886	53,7732506	-6,3178074									
							18	836	69,1566986	9,06564057									
G3	g3bwc.res	13554	60,091	24,9671	1	0,125792638	1	890	54,3539326	-6,0339684					,175	,083	,086	,036	
					2	-0,00479271	2	784	75,2433674	15,157869									
					3	0,135481942	3	877	65,8491448	6,92363935									
					4	0,352786193	4	866	101,117321	44,3176413									
					5	0,050016559	5	928	55,6492457	-4,098576									
					6	0,104067526	6	844	86,6433649	27,4412951									
					7	0,102679936	7	515	14,9328155	-45,956814									
					8	0,118711129	8	501	18,9449102	-42,231742									
					9	-0,020252445	9	876	48,7063927	-11,352935									
					10	0,285074691	10	798	76,8917293	16,7137925									
					11	0,094961548	11	805	38,2524224	-22,108075									
					12	0,274431503	12	749	62,0608812	1,52937493									
					13	-0,056285675	13	887	42,757159	-16,883797									
					14	-0,071500154	14	744	66,4677419	6,9954512									
					15	0,342580306	15	340	47,2411765	-17,808782									
					16	0,806911423	16	428	69,2497664	-2,3167312									
					17	0,166803688	17	886	53,7732506	-5,1630631									
					18	0,046538476	18	836	69,1566986	9,39421363									

		TOTAL			AGE0(SEXF) COVARIABLE NO.1		*SEXF* within environment *ENVR x SEXF* across environments FIXED EFFECT NO. 1				GENETIC GROUP 1=10, 2=21, 3=30, 4=32 FIXED EFFECT NO. 2				PARAMETER ESTIMATES WITH THEIR APPROX. S.E.				APPROXIMATE SAMPLING CORRELATIONS
Generasjon	Resultatfil	N	MEAN	SDEV	LEVEL	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	h2	seh2	c2	sec2	
G4	ballbwf.res	2380	32,385	11,6520			1	1290	28,8928682	-3,0939923	1	2278	32,7745	0,0000	,337	,141	,149	,053	-,897
							2	1090	36,5181651	4,52126423	2	79	25,65696	-7,3936					
											3	13	15,03077	-16,0177					
											4	10	19,4	-14,4803					
G4	ballbwa.res	2380	32,385	11,6520	1	0,172348904	1	1290	28,8928682	-3,1720786	1	2278	32,7745	0,0000	,331	,142	,127	,052	-,901
					2	0,297562203	2	1090	36,5181651	4,59584477	2	79	25,65696	-6,9218					
											3	13	15,03077	-14,8393					
											4	10	19,4	-13,0846					
G4	blar1bwf.res	2392	80,123	28,0674			1	1329	68,3266366	-11,106706	1	2288	80,85376	0,0000	,231	,116	,126	,043	-,862
							2	1063	94,8714958	15,7069846	2	76	65,76711	-15,9515					
											3	16	58,10625	-24,3180					
											4	12	61,09167	-28,0365					
G4	blar1bwa.res	2392	80,123	28,0674	1	0,11082603	1	1329	68,3266366	-11,204409	1	2288	80,85376	0,0000	,263	,124	,106	,044	-,877
					2	0,428579613	2	1063	94,8714958	15,8815727	2	76	65,76711	-15,1072					
											3	16	58,10625	-21,8440					
											4	12	61,09167	-25,0586					
G4	blar2bwf.res	2142	70,719	27,7361			1	1224	58,8661765	-11,308196	1	2054	71,32137	0,0000	,175	,102	,151	,040	-,812
							2	918	86,5223312	16,6834402	2	67	57	-14,5264					
											3	15	57,14	-19,0656					
											4	6	51,58333	-35,8189					
G4	blar2bwa.res	2142	70,719	27,7361	1	0,219319234	1	1224	58,8661765	-11,435235	1	2054	71,32137	0,0000	,185	,105	,123	,040	-,838
					2	0,624175393	2	918	86,5223312	16,845338	2	67	57	-13,2272					
											3	15	57,14	-15,6909					
											4	6	51,58333	-31,1958					
G4	blcgbwf.res	2602	17,753	8,8625			1	1607	16,5	-0,919853	1	2479	18,04102	0,0000	,200	,099	,229	,047	-,840
							2	995	19,7753769	2,17155344	2	86	13,64651	-3,6332					
											3	25	8,024	-9,1796					
											4	12	7,841667	-10,2791					
G4	blcgbwa.res	2602	17,753	8,8625	1	0,156441462	1	1607	16,5	-0,9281162	1	2479	18,04102	0,0000	,209	,101	,209	,046	-,842
					2	0,164089104	2	995	19,7753769	2,11523793	2	86	13,64651	-3,7741					
											3	25	8,024	-7,2356					
											4	12	7,841667	-9,1452					
G4	fac1bwf.res	1813	37,996	18,3499			1	1139	34,4363477	-3,3231091	1	1773	38,22775	0	0,408958	0,188572	0,0557896	0,0602542	-0,928
							2	674	44,0124629	6,21112215	2	33	28,31818	-10,1110					
											3	6	27,21667	-7,4566					
											4	1	11,8	-22,8733					
G4	fac1bwa.res	1813	37,996	18,3499	1	0,184612178	1	1139	34,4363477	-3,3534025	1	1773	38,22775	0,0000	,426	,176	,035	,054	-,922
					2	0,360063568	2	674	44,0124629	6,19294946	2	33	28,31818	-8,9678					
											3	6	27,21667	-6,2926					
											4	1	11,8	-19,5555					
G4	fac2bwf.res	2089	44,833	20,0290			1	1249	39,5280224	-4,9443428	1	2010	45,21313	0,0000	,422	,210	,081	,063	-,938
							2	840	52,7207143	8,2359861	2	59	37,15932	-6,7503					
											3	14	27,17857	-17,4172					
											4	6	34,1	-16,7722					
G4	fac2bwa.res	2089	44,833	20,0290	1	0,176553211	1	1249	39,5280224	-5,0248767	1	2010	45,21313	0,0000	,393	,146	,072	,046	-,889
					2	0,424010577	2	840	52,7207143	8,32613732	2	59	37,15932	-5,6443					
											3	14	27,17857	-14,5599					
											4	6	34,1	-22,0444					
G4	fwasbwf.res	937	66,862	19,8074			1	561	58,9754011	-7,6388462	1	909	67,11859	0,0000	,211	,101	,052	,041	-,652
							2	376	78,6279255	11,9713599	2	28	58,51786	-7,7085					
G4	fwasbwa.res	937	66,862	19,8074	1	0,120798739	1	561	58,9754011	-7,6866023	1	909	67,11859	0,0000	,215	,114	,047	,044	-,726
					2	0,277555395	2	376	78,6279255	12,0635612	2	28	58,51786	-7,1850					
G4	smatbwf.res	1639	37,378	11,9064			1	946	33,209408	-3,8633654	1	1562	37,70173	0,0000	,407	,179	,106	,061	-,912
							2	693	43,0672439	6,12296013	2	54	32,50185	-6,4506					
											3	16	24,03125	-14,4760					
											4	7	33,14286	-8,9310					
G4	smatbwa.res	1639	37,378	11,9064	1	0,1603939	1	946	33,209408	-3,8716572	1	1562	37,70173	0,0000	,357	,143	,111	,062	-,876
					2	0,210606756	2	693	43,0672439	6,09211386	2	54	32,50185	-5,4875					
											3	16	24,03125	-13,2024					
											4	7	33,14286	-7,5032					
G4	g4bwf.res	15994	47,071	28,8884			1	1290	28,8928682	-17,720298	1	15353	47,51049	0,0000	,234	,097	,094	,034	-,901
							2	1090	36,5181651	-10,085161	2	482	38,4612	-9,0158					
							3	1329	68,3266366	21,6625763	3	105	29,62952	-15,5835					
							4	1063	94,8714958	48,380424	4	54	32,9463	-18,6075					
							5	1224	58,8661765	12,151243									
							6	918	86,5223312	40,0103153									
							7	1607	16,5	-29,895332									
							8	995	19,7753769	-26,992256									
							9	1139	34,4363477	-12,378138									
							10	674	44,0124629	-2,8848655									
							11	1249	39,5280224	-7,1200594									
							12	840	52,7207143	8,04620369									
							13	561	58,9754011	12,1934523									
							14	376	78,6279255	31,7964826									
							15	946	33,209408	-13,42445									
							16	693	43,0672439	-3,350747									
G4	g4bwa.res	15994	47,071	28,8884	1	0,172191725	1	1290	28,8928682	-17,848426	1	15353	47,51049	0,0000	,253	,095	,075	,031	-,899
					2	0,294511733	2	1090	36,5181651	-9,7426888	2	482	38,4612	-8,3708					
					3	0,118865632	3	1329	68,3266366	22,2726394	3	105	29,62952	-13,4966					
					4	0,44410149	4	1063	94,8714958	51,1985363	4	54	32,9463	-17,2508					
					5	0,222503559	5	1224	58,8661765	13,1377592									
					6	0,635175226	6	918	86,5223312	43,3372849									
					7	0,140549576	7	1607	16,5	-31,189648									
					8	0,158563962	8	995	19,7753769	-28,47614									
					9	0,183582603	9	1139	34,4363477	-12,061863									
					10	0,360286632	10	674	44,0124629	-2,2635461									
					11	0,171982703	11	1249	39,5280224	-7,1308916									
					12	0,420607998	12	840	52,7207143	6,2418441									
					13	0,12024253	13	561	58,9754011	10,7090181									
					14	0,275798	14	376	78,6279255	28,5980962									
					15	0,163697396	15	946	33,209408	-13,081083									
					16	0,199491576	16	693	43,0672439	-2,9505899									

G5

		TOTAL			AGE0 (SEXF) COVARIABLE NO.1		*SEXF* within environment *ENVR x SEXF* across environments FIXED EFFECT NO. 1				GENETIC GROUP 1=10, 2=20, 3=21, 4=30 FIXED EFFECT NO. 2				PARAMETER ESTIM	
Generasjon	Resultatfil	N	MEAN	SDEV	LEVEL	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	h2	seh2
G5	bfarbwf.res	5076	107,974	39,2041			1	2841	90,3399155	-16,978525	1	4834	108,5748	0,0000	,221	0,0
							2	2235	130,389709	23,3910374	2	84	94,32857	-15,4100		
											3	79	92,74177	-21,2492		
											4	79	100,9595	-13,5424		
G5	bfarbwa.res	5076	107,974	39,2041	1	-0,091047187	1	2841	90,3399155	-17,019628	1	4834	108,5748	0,0000	,205	
					2	0,260692384	2	2235	130,389709	23,1931661	2	84	94,32857	-15,2814		
											3	79	92,74177	-21,9317		
											4	79	100,9595	-15,2689		
G5	facbwf.res	4036	82,065	31,6237			1	2226	66,9621743	-14,489493	1	3786	82,63185	0,0000	,180	
							2	1810	100,639061	19,5829398	2	80	73,1925	-8,0120		
											3	85	75,73529	-13,8871		
											4	85	71,49882	-16,1193		
G5	facbwa.res	4036	82,065	31,6237	1	-0,009677774	1	2226	66,9621743	-14,449733	1	3786	82,63185	0,0000	,172	
					2	0,18118276	2	1810	100,639061	19,564081	2	80	73,1925	-8,3105		
											3	85	75,73529	-14,5480		
											4	85	71,49882	-17,9327		
G5	g5bwf.res	9112	96,498	38,2709			1	2841	90,3399155	-5,589264	1	8620	97,18038	0,0000	,181	
							2	2235	130,389709	34,7556365	2	164	84,01829	-11,8110		
							3	2226	66,9621743	-28,811868	3	164	83,92744	-17,4527		
							4	1810	100,639061	5,29154619	4	164	85,69024	-14,8962		
G5	g5bwa.res	9112	96,498	38,2709	1	-0,091153393	1	2841	90,3399155	-5,876877	1	8620	97,18038	0,0000	,169	
					2	0,260948155	2	2235	130,389709	35,2781709	2	164	84,01829	-11,8909		
					3	-0,009877847	3	2226	66,9621743	-28,743252	3	164	83,92744	-18,1252		
					4	0,182028116	4	1810	100,639061	4,63935078	4	164	85,69024	-16,6717		

		TOTAL			AGE0 (SEXF) COVARIABLE NO.1		*SEXF* within environment *ENVR x SEXF* across environments FIXED EFFECT NO. 1				GENETIC GROUP 1=10, 2=20, 3=21, 4=30, 5=31 FIXED EFFECT NO. 2				PARAMETER ES	
Generasjon	Resultatfil	N	MEAN	SDEV	LEVEL	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	h2	seh2
G6	bfarbwf.res	3983	170,451	61,5040			1	2384	146,484815	-21,339929	1	3725	173,1013	0,0000	,052	
							2	1599	206,182364	38,757201	2	70	156,2514	-17,7568		
											3	63	130,9952	-49,5950		
											4	61	125,0213	-48,7195		
											5	64	113,8516	-58,7347		
G6	bfarbwa.res	3983	170,451	61,5040	1	0,8055	1	2384	146,4848	-20,9383	1	3725	173,1013	0,0000	,149	
					2	1,6175	2	1599	206,1824	37,8911	2	70	156,2514	-17,5269		
											3	63	130,9952	-47,4734		
											4	61	125,0213	-44,8832		
											5	64	113,8516	-69,7150		
G6	facbwf.res	3340	111,445	49,0724			1	1999	93,8303152	-15,316769	1	3073	113,6715	0,0000	,000	
							2	1341	137,703729	28,7627414	2	69	109,0478	-10,0788		
											3	61	85,37049	-33,1592		
											4	62	73,97742	-35,6594		
											5	75	74,62133	-40,314515		
G6	facbwa.res	3340	111,445	49,0724	1	0,587994094	1	1999	93,8303152	-15,118097	1	3073	113,6715	0,0000	,085	
					2	1,602905276	2	1341	137,703729	28,4555167	2	69	109,0478	-7,7302		
											3	61	85,37049	-30,8522		
											4	62	73,97742	-33,3962		
											5	75	74.621333	-50,6010		
G6	g6bwf.res	7323	143,539	63,3970			1	2384	146,484815	5,1465003	1	6798	146,2363	0,0000	,000	
							2	1599	206,182364	65,1789465	2	139	132,8194	-13,9942		
							3	1999	93,8303152	-46,894708	3	124	108,5508	-41,5498		
							4	1341	137,703729	-2,7344281	4	123	99,29187	-42,1843		
											5	139	92,68417	-48,8847		
G6	g6bwa.res	7323	143,539	63,3970	1	0,803651166	1	2384	146,484815	3,31339447	1	6798	146,2363	0,0000	,070	
					2	1,611209031	2	1599	206,182364	59,8498054	2	139	132,8194	-12,7096		
					3	0,587081683	3	1999	93,8303152	-44,752077	3	124	108,5508	-39,3434		
					4	1,612197847	4	1341	137,703729	2,29667369	4	123	99,29187	-39,1400		
											5	139	92,68417	-59,5030		

		TOTAL			AGE0 (SEXF) COVARIABLE NO.1		***SEXF*** within environment ***ENVR x SEXF*** across environments FIXED EFFECT NO. 1				GENETIC GROUP 1=10, 2=21 FIXED EFFECT NO. 2				PARAMETER ESTIMAT	
Generasjon	Resultatfil	N	MEAN	SDEV	LEVEL	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	h2	seh2
G7	bfarbwf.res	1269	120,301	34,6476			1	738	109,561382	-11,332418	1	1131	119,578	0,0000	,064	
							2	531	135,226365	14,2531936	2	138	126,2232	5,7600		
G7	bfarbwa.res	1269	120,301	34,6476	1	0,480713004	1	738	109,561382	-9,9364789	1	1131	119,578	0,0000	,018	
					2	0,743063247	2	531	135,226365	17,3775893	2	138	126,2232	-12,3565		
G7	facbwf.res	3837	120,933	44,6249			1	2413	104,506672	-15,473037	1	3444	121,6847	0,0000	,147	
							2	1424	148,766713	29,0523439	2	393	114,3417	-10,2649		
G7	facbwa.res	3837	120,933	44,6249	1	0,294070952	1	2413	104,506672	-14,002606	1	3444	121,6847	0,0000	,155	
					2	0,937240397	2	1424	148,766713	32,1836918	2	393	114,3417	-28,1233		
G7	pncgbwf.res	2022	117,493	57,0351			1	1163	102,695959	-15,957517	1	1797	115,9401	0,0000	,453	
							2	859	137,526077	18,5566467	2	225	129,8929	11,6375		
G7	pncgbwa.res	2022	117,493	57,0351	1	0,758638039	1	1163	102,695959	-13,100151	1	1797	115,9401	0,0000	,661	
					2	1,130339895	2	859	137,526077	21,5204661	2	225	129,8929	-14,9640		
G7	g7bwf.res	7128	119,844	47,0118			1	738	109,561382	-10,193806	1	6372	119,6907	0,0000	,106	
							2	531	135,226365	15,4831013	2	756	121,1389	-0,8654		
							3	2413	104,506672	-15,2573						
							4	1424	148,766713	29,02511						
							5	1163	102,695959	-17,062033						
							6	859	137,526077	17,7915796						
G7	g7bwa.res	7128	119,844	47,0118	1	0,512530941	1	738	109,561382	-4,0417762	1	6372	119,6907	0,0000	,129	
					2	0,778019169	2	531	135,226365	25,8296175	2	756	121,1389	-21,3139		
					3	0,271260053	3	2413	104,506672	-12,409253						
					4	0,907989888	4	1424	148,766713	36,1900808						
					5	0,779065022	5	1163	102,695959	-25,26899						
					6	1,154058804	6	859	137,526077	4,47696426						

		TOTAL			AGE0 (SEXF) COVARIABLE NO.1		***SEXF*** within environment ***ENVR x SEXF*** across environments FIXED EFFECT NO. 1				GENETIC GROUP 1=10, 2=20 FIXED EFFECT NO. 2				PARAMETER ESTIMATE	
Generasjon	Resultatfil	N	MEAN	SDEV	LEVEL	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	h2	seh2
G8	bfarbwf	3522	169,569	45,5954			1	1766	145,775311	-23,09195	1	2514	171,3597	0,0000	,258	,13
							2	1756	193,498349	24,4782361	2	1008	165,1032	-2,1859		
G8	bfarbwa.res	3522	169,569	45,5954	1	0,483306155	1	1766	145,775311	-22,212026	1	2514	171,3597	0,0000	,086	,07
					2	1,461848246	2	1756	193,498349	25,9497173	2	1008	165,1032	-5,5645		
G8	facbwf.res	2521	132,064	47,1904			1	1258	112,683784	-16,410385	1	1786	135,2022	0,0000	,199	,11
							2	1263	151,368171	22,0827098	2	735	124,4395	-9,8588		
G8	facbwa.res	2521	132,064	47,1904	1	0,691150434	1	1258	112,683784	-15,679172	1	1786	135,2022	0,0000	,203	,09
					2	1,587014807	2	1263	151,368171	24,4032325	2	735	124,4395	-13,9713		
G8	pncgbwf.res	1413	146,922	83,3267	1		1	656	118,668902	-26,624212	1	1047	148,8424	0,0000	,238	,16
					2		2	757	171,406341	25,9915645	2	366	141,4301	-6,0386		
G8	pncgbwa.res	1413	146,922	83,3267	1	1,234489891	1	656	118,668902	-24,062988	1	1047	148,8424	0,0000	,312	,13
					2	2,506924685	2	757	171,406341	27,3774685	2	366	141,4301	-14,1728		
G8	rfbwf.res	561	178,389	61,8724			1	281	135,278292	-42,876631	1	474	177,9924	0,0000	,000	,18
							2	280	221,654286	43,5413632	2	87	180,5517	-1,6465		
G8	rfbwa.res	561	178,389	61,8724	1	0,005717732	1	281	135,278292	-42,72261	1	474	177,9924	0,0000	,000	,32
					2	1,419286057	2	280	221,654286	44,6057034	2	87	180,5517	-2,7533058		
G8	g8bwf.res	8017	154,401	58,4112			1	1766	145,775311	-6,9084872	1	5821	156,7559	0,0000	,115	,08
							2	1756	193,498349	40,4405153	2	2196	148,1596	-5,3490		
							3	1258	112,683784	-40,105887						
							4	1263	151,368171	-1,5252884						
							5	656	118,668902	-34,289021						
							6	757	171,406341	18,3406487						
							7	281	135,278292	-18,361464						
							8	280	221,654286	68,1509749						
G8	g8bwa.res	8017	154,401	58,4112	1	0,505688348	1	1766	145,775311	-5,6114611	1	5821	156,7559	0,0000	,093	,05
					2	1,476206074	2	1756	193,498349	42,5171549	2	2196	148,1596	-9,6695		
					3	0,665020383	3	1258	112,683784	-34,449433						
					4	1,57468036	4	1263	151,368171	12,3133521						
					5	1,208674039	5	656	118,668902	-38,56758						
					6	2,490877616	6	757	171,406341	6,81924969						
					7	0,032660536	7	281	135,278292	-18,490918						
					8	1,426782506	8	280	221,654286	38,1174186						

		TOTAL			AGE0 (SEXF) COVARIABLE NO.1		***AAR * ENVR x SEXF*** across FIXED EFFECT NO. 1				GENETIC GROUP 1=10, 2=20 FIXED EFFECT NO. 2				PARAMETER ESTIMATES \	
Generasjon		N	MEAN	SDEV	LEVEL	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	LEVEL	NREC	MEAN	SOLUTION	h2	seh2
G3-8	TOTAL B	61128	91,445	58,6290			56 levels..								,458	,009
G3-8	TOTAL C	61128	91,445	58,6290			56 levels..								,360	,010
G3-8	TOTAL D	61128	91,445	58,6290			56 levels..								,122	,012
G3-8	TOTAL RES	61128	91,445	58,6290			56 levels..				18 levels..				,025	,011

B: Fitting fixed effect of aa*envr*sexf only
C: Fitting fixed effects of aar*envr*sexf and AGE0(aar*envr*sexf)
D: Fitting fixed effects of aar*envr*sexf and AGE0(aar*envr*sexf) and C2
RES: Fitting fixed effects of aar*envr*sexf and AGE0(aar*envr*sexf), C2 and Gentic group

Attachment 7.

Manuscript (accepted for publication) on "Response to bi-directional selection for frequency of early maturing females in Nile tilapia"

RESPONSE TO BI-DIRECTIONAL SELECTION FOR FREQUENCY OF EARLY MATURING FEMALES IN NILE TILAPIA*

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Abstract

Twenty tagged individuals were randomly sampled from each of 42 full-sib families within 21 randomly chosen half-sib families of Nile tilapia (*Oreochromis niloticus*) among the families representing the third generation of selection for improved growth performance in the GIFT project, and communally stocked in an earthen pond. The occurrence of sexual maturation in the females was recorded four weeks after the first swim-up fry was observed in the pond. Broodstock was selected from full-sib families with a high (> 75 percent) or a low (< 20 percent) frequency of mature females. After about four weeks of single pair stocking in breeding hapas, 16 pairs of breeders from families with a mean frequency of 83 percent early maturing females and 9 pairs of breeders, all from families with 0 percent early maturing females, had produced fry. The fry from each pair was reared separately until a size of 3-5 g, when totally 3 179 fingerlings equally representing the 25 progeny families were tagged and communally stocked in three replicated ponds for testing. Recording of sex, body weight and occurrence of sexual maturation in the females was carried out two, three or four weeks, respectively, after observing the first swim-up fry in the three ponds. The fish was restocked and reared until recording at harvest. The age corrected least square means across ponds and across families within selection groups for frequency of sexually mature females at intermediate recording was 57.0 percent and 33.6 percent in progeny of breeders from full-sib families with a high or a low frequency of early maturing females, respectively. The response to selection, measured as the difference between the two progeny groups, was highly significant (p=0.0002). The timing of the recording was appropriate in all three ponds. A significant correlated response in body weight of males at harvest (p=0.027) and a nearly significant correlated response in body weight of females at intermediate recording (p=0.052) was also observed. The age corrected least square means of body weight were higher in the progeny of breeders from full-sib families with a high frequency of early maturity in the females, suggesting an unfortunate genetic association between the two traits in Nile tilapia used in aquaculture. It is proposed to carry out combined selection for body weight and frequency of early maturing females in applied breeding programs for farmed Nile tilapia.

Key words: Nile tilapia, sexual maturation, selection, correlated response

1. Introduction

Nile tilapia, dubbed the 'aquatic chicken' (Maclean, 1984), is one of the most important cultured fish species in many parts of the Philippines, and is also an increasingly important food fish in many other developing countries (Pullin, 1997). In the Philippines, Nile tilapia contributes about 70 percent to the total fish production from freshwater and about 20 percent to the total aquaculture production. It has

1 been the mainstay of small-scale aquaculture for many resource poor farmers. Furthermore, Nile
2 tilapia is poised to become an international food commodity (Davlin, 1991). The world production of
3 tilapia in 1994 was about 600,000 tons with an estimated value of about 835 million US dollars (FAO,
4 1996). However, decades of reproduction in captivity since the introduction of Nile tilapia into Asian
5 aquaculture has not improved the performance of the species in a culture environment compared to
6 wild stocks brought directly into culture in the Philippines from it's native waters in Africa (Eknath et
7 al., 1993). To realize the huge potential of Nile tilapia culture, genetic improvement programs are
8 needed to develop adapted farm races of the species (Gjedrem, 1985a, Bentsen, 1990, Eknath et al.,
9 1991, Bentsen and Gjerde, 1994).
10
11 One of the major problems in tilapia culture is the tendency of the females to mature and reproduce
12 early and at small sizes. In pond culture, this results in stunted growth in the reproducing females
13 (males normally grow to 130 to 150 percent of the body weight of the females in most aquaculture
14 environments), overcrowding of the pond because of reproduction during the production cycle, feed
15 competition and poor growth performance in the entire stock, highly variable sizes when the pond is
16 harvested and consequently unpredictable yields and income to the fish farmers. To avoid the
17 problems of stunted growth in the females and overcrowding of the ponds, a number of investigations
18 have been directed towards the production of all-male populations of Nile tilapia for aquaculture
19 (Wohlfarth and Hulata, 1983, Guerrero, 1987). The technologies used to prevent early reproduction
20 include hormonal sex reversal, hybridization, intermittent harvesting, manual sexing, use of predators,
21 cage culture in large water bodies, high stocking density, sterilization, and the use of YY male
22 broodstock (Mair and Little, 1991).
23
24 In general, tilapias exhibit tremendous plasticity in growth and maturation. For example, in Lake
25 Albert, which is connected to the Nile river, Nile tilapia grows to very large sizes, and males and
26 females grow to and mature at about the same size. However, populations trapped in lagoons consist
27 of very small fish, the body weight at sexual maturation size is low, and the females are much smaller
28 than the males (Lowe-McConnell, 1982). In culture, the females that do not mature at an early stage
29 often seem to more or less follow the growth curves of males (Bolivar et al., 1993). The prospects of
30 delaying the onset of sexual maturation in Nile tilapia females by selection should consequently be
31 investigated. Genetic variation in the age or size of onset of sexual maturation in Nile tilapia in
32 captivity has been reported by several authors (Kronert et al., 1986, 1989, Lester et al., 1988,
33 Uraiwan, 1988, Odorf et al., 1989, Bolivar et al., 1993, Eknath, 1996). Response to selection for
34 delayed onset of sexual maturation has been demonstrated in rainbow trout (Donaldson and Olson,
35 1955, Gjedrem, 1985b), in chinook salmon (Donaldson and Menasveta, 1961) and in Atlantic salmon
36 (Gjerde, 1984, 1986, Gjedrem, 1985).

The present study was carried out to investigate a simple method for recording the occurrence of early maturing females in Nile tilapia at a fixed time after stocking (Longalong and Eknath, 1995, Eknath et al., 1997) and to estimate the response to bi-directional selection for this trait and a possible correlated response in growth performance. The study was a part of the project «Genetic Improvement of Farmed Tilapias» (GIFT) that was started in April 1988 (Pullin et al., 1991, Eknath et al., 1991, Eknath, 1993) to study the prospects of genetic improvement of farmed Nile tilapia by selection. The project has been executed by the International Center for Living Aquatic Resources Management (ICLARM) in co-operation with the National Freshwater Fisheries Technology Research Center of the Bureau of Fisheries and Aquatic Resources, Philippines (BFAR/NFFTRC), Freshwater Aquaculture Center of the Central Luzon State University, Philippines (FAC/CLSU) and the Institute of Aquaculture Research, Norway (AKVAFORSK).

2. Materials and methods

Selection of parents

Twenty individuals were sampled from each of 42 full-sib families within 21 half-sib families that were randomly drawn among the 153 full-sib families constituting the third generation of selection for improved growth performance in the GIFT project. The families had previously been reared in separate 1 m^3 hapas for 10-20 weeks until they reached an average body weight of 4-5 g in November 1993. The experimental fish were then tagged with modified Floy fingerling tags and communally stocked in a 1 200 m^2 earthen pond. About four weeks after the first occurrence of swim-up fry, the pond was drained and all females were scored for the occurrence external signs of sexual maturation. Females with whitish genital papilla, flat genital pore and no signs of swelling or compression of the abdomen were scored as non-matures. Females that had spawned (reddish genital papilla, shrunken and compressed abdomen), that were ready to spawn (protruding and pinkish to reddish genital papilla, distended abdomen) or under maturation (yellowish to pinkish genital papilla, slightly opened genital pore, slightly distended abdomen) were all scored as sexually mature (Longalong and Eknath, 1995). After scoring, the fish was restocked in the pond for about one month before selection was carried out.

The frequency of early maturing females in each of the full sib families was determined, and broodstock was selected from the eight full-sib families with the highest frequency of maturation (HFM, > 70 percent mature females) and the eight full-sib families with the lowest frequency of

maturation (LFM, < 20 percent mature females) as shown in Fig 1. The selected broodstock was then stocked for conditioning in 1 m^3 cages for two weeks, each family and sex separately. Feeding was carried out daily with a mixture of 70 percent rice bran and 30 percent fish meal at 3 percent of body weight.

Production and rearing of progeny

A total of 50 pairs of conditioned breeders equally representing the HFM and the LFM selection groups were stocked separately in 1 m^3 fine mesh breeding hapas in an earthen pond. The age of the breeders was then about 8-10 months. The genital papilla of all females was examined to ensure that they were ready to spawn. Related individuals were not mated to avoid inbreeding. The hapas were inspected at regular intervals for the occurrence of swim-up fry. The first spawn was collected in May 1994, 10 days after stocking of the breeding pairs. About four weeks later, 16 HFM pairs and 9 LFM pairs had produced progeny. LFM pairs were more difficult to reproduce than HFM pairs, and tended to spawn later. The mean frequency of early maturing females in the full-sib groups contributing HFM breeders that succeeded to spawn was about 83 percent (range 75-100 percent) while all the successful LFM breeders came from full-sib groups with 0 percent occurrence of early female maturity. Three additional LFM pairs produced progeny about nine weeks after collecting the first spawn. The progeny of these late spawners were tested along with the other families, but were never able to catch up with the growth and development of those. The records from individuals from the three late spawns were consequently discarded during the analysis of the experiment.

The number and bulk weight of fry from each pair was recorded, and spawns that were collected in the yolk sac fry stage was artificially incubated until complete absorption of the yolk sac before transfer to nursery hapas. The progeny full-sib groups were then reared separately in 1 m^3 nursery hapas in a 4 500 m^2 pond at a density of about 150 fry per hapa. After 21 days of nursing, the fry was transferred to 1 m^3 net cages at a density of 100 fish per cage, still keeping the full-sib families separated. The pond was fertilized weekly with chicken manure and inorganic fertilizer (16-20-0) at a rate of 3 000 kg per ha per month and 100 kg per ha per month respectively. Supplementary feeding was carried out twice daily with a mixture of 70 percent rice bran and 30 percent fish meal at the rate of 50 percent and 30 percent of body weight, respectively.

Testing of progeny

At a mean age of about 91 days, when the progeny had reached a body weight of 3 to 5 g, individual tagging was carried out with modified Floy Fingerling tags attached by a nylon loop through the

1 dorso-interior musculature above the lateral line and ether between the fifth and sixth or between the
2 sixth and seventh dorsal spine. The fingerlings were anaesthetized with tricaine methanesulfonate
3 (MS 222) to minimize stress during tagging. Equal numbers of tagged fingerlings from each full-sib
4 family were then pooled together and conditioned in concrete tanks for 1-2 days before communal
5 stocking in earthen ponds.
6
7 From each full-sib family, 43 tagged fingerlings were communally stocked (all families together) in
8 each of 3 replicated 600 m^2 earthen ponds. The total number of fingerlings stocked was 3 179. The
9 mean body weight was 4.17 g and the standard deviation 1.92 g. The ponds were fertilized with
10 chicken manure and inorganic fertilizer (16-20-0) at a rate of 3 000 kg per ha per month and 100 kg
11 per ha per month respectively. In addition, the fish were fed daily with a mixture of 70 percent rice
12 bran and 30 percent fish meal at a rate of 15 percent of fish body weight during the first 4 weeks.
13
14 The ponds were inspected at regular intervals to record the first occurrence of swim-up fry. The
15 optimal timing for recording the frequency of early maturing females in the progeny full-sib families
16 would be when about half of the females in the pond has reached maturity, since this is expected to
17 result in maximum variation between families. To investigate the timing of the recording, Pond 1,
18 Pond 2 and Pond 3 were drained for intermediate recording two, three and four weeks respectively
19 after the first occurrence of swim-up fry in each pond (or 85, 98 and 95 days respectively after
20 stocking). Body weight and sex of all fish and the maturity stage of the females (see above) was
21 recorded. Fish that were not recovered because of mortality or because they had lost the tags were
22 also recorded. The fish was restocked in the ponds for grow-out until harvest 49, 56 and 65 days later
23 respectively, and a final recording of body weight and sex was carried out for all fish harvested. The
24 number of fish recorded at each occasion is shown in Table 1. The occurrence of early maturing
25 females, the body weight at stocking, the body weight for males and females at intermediate recording
26 and at harvest, and the mortality, the tag loss and the sex ratio among tagged fish at intermediate
27 recording and at harvest are shown in Table 2.
28
29 Statistical analysis
30
31 Because of the systematic trend of the HFM breeders spawning earlier than the LFM breeders, the
32 mean age of the two progeny groups differed considerably. The difference in mean age (excluding the
33 progeny of the the late spawning LFM pairs) was 7.2 days at stocking, 6.2 days among the individuals
34 recovered with tags at intermediate sampling and 6.2 days among the harvested individuals with tags.
35 However, the range of ages was similar in the two progeny groups (from 62 to 106 days at stocking).
36 To correct for the age differences, age was included as a covariate during the statistical analysis. The

1 occurrence of early maturing females (0 = not mature, 1 = mature, based on external evaluation as
2 described earlier) at intermediate recording, body weight at stocking and for each sex separately at
3 intermediate and final recording, the occurrence of missing fish (including individuals that had lost
4 their tags, see above) at intermediate and final recording (alive = 0, dead or without tag = 1), and
5 frequency of females among surviving, tagged individuals at intermediate and final recording (male =
6 0, female = 1) were analysed as dependent variables according to the following general linear model:
7
8 $$Y_{ijl} = a + G_i + P_j + b_1*A_{ijl} + e_{ijl} \qquad \text{(Model 1)}$$
9
10 where:
11 Y_{ijl} is the dependent variable for the *l*th individual
12 a is a constant
13 G_i is the effect of the *i*th progeny group (HFM or LFM)
14 P_j is the effect of the *j*th pond (1, 2 or 3)
15 b_1 is the regression coefficient of the dependent variable on the age at recording
16 A_{ijl} is the age at recording of the *l*th individual
17 e_{ijl} is a random error associated to the record of the *l*th individual
18
19 For each dependent variable, the significance of possible progeny group by pond interactions (G_i*P_j),
20 possible non-linear regression effects on age at recording ($b_2*A_{ijl}^2$), and possible heterogeneity of
21 regression coefficients on age at recording between progeny group by pond subcells ($b_{1ij}*A_{ijl}$ and
22 $b_{2ij}*A_{ijl}^2$) were checked simultaneously by including the effects in the model. Non-significant effects
23 were eliminated in a stepwise series of analyses.
24
25 Least square means and significance tests of the G_i effects according to Model 1 will show the
26 combined effect of selection and possible effects of random genetic drift and unequal representation
27 of the full-sib families among the recorded individuals. Without individual tagging and pedigree
28 records, this would be the only way to analyse and test the response to selection. In the present
29 experiment, the effects of genetic drift and unequal representation of families may be accounted for
30 by testing the effect of selection group (G_i) against the effect of families within selection group as the
31 error term, and to compute the least square means for the selection groups across families. However,
32 this was not possible in Model 1 because of extensive confounding between the age effect and the
33 effect of families within selection groups. A second step of analysis was consequently carried out for
34 all dependent variables that were significantly affected by the G_i effect according to Model 1. The
35 dependent variables were corrected for the effect of age, using the regression coefficients obtained

1 from Model 1. The corrected variables were then reanalysed according to the following general linear
2 model:
3
4 $$Y_{ijkl} = a + G_i + P_j + F_k(G_i) + e_{ijkl} \qquad \text{(Model 2)}$$
5
6 where a, G_i and P_j are as defined for Model 1, and:
7 Y_{ijkl} is the age corrected dependent variable for the *l*th individual
8 $F_k(G_i)$ is the effect of the *k*th family within the *i*th selection group
9 e_{ijkl} is a random error associated to the record of the *l*th individual
10
11 The proper testing of the G_i effect using the $F_k(G_i)$ effect as the error term was then carried out, and
12 least square means were estimated for the G_i effect across families within and across ponds.
13
14
15 3. Results
16
17 All the investigated dependent variables were significantly affected by age effects according to Model
18 1 (Table 3). However, no significant differences were observed between the least square means of the
19 LFM and the HFM progeny groups (the G_i effects), the test ponds (the P_j effects) or the interaction
20 between them according to Model 1 for the dependent variables proportion of fish lost (sum of
21 mortality and tag loss) and the proportion of females among the surviving, tagged fish, neither at
22 intermediate recording or at harvest. The proportion of the total variation explained by Model 1 (R^2)
23 was also modest (2-4 percent) for these dependent variables (Table 3). Selective loss of experimental
24 fish depending on progeny group, pond or sex is consequently not expected to affect the estimates of
25 response to selection. Because of the lack of significant differences between the progeny groups, these
26 variables were not investigated in the second step of analysis. Non-significant G_i effects according to
27 Model 1 are not expected to be significant according to Model 2.
28
29 Highly significant differences (mostly $p < 0.0001$) were found between least square means of the
30 LFM and the HFM progeny groups for frequency of early maturing females and body weight at all
31 stages according to Model 1, both across and within test ponds. The least square means of the test
32 ponds were also mostly highly significant and the interactions between progeny groups and test ponds
33 were non-significant. For all these dependent variables except body weight of females at intermediate
34 recording and at harvest, the models explained a substantial part of the total variation (R^2 = 12-34
35 percent, Table 3).
36

1 A common linear effect of age was detected across test ponds on frequency of early maturing females
2 (b_1 = 0.0112 or 1.12 percent per day) and body weight of females at intermediate recording (b_1 = 0.37
3 g per day). A common non-linear effect was detected for body weight at stocking (b_2 = 0.0005 g per
4 day^2) and body weight of males at harvest (b_2 = 0.0032 g per day^2). The age effect on body weight of
5 females at harvest differed significantly between progeny groups but not between test ponds (b_1 =
6 0.65 g and 0.12 g per day for the LFM and HFM females, respectively). The age effect on body
7 weight of males at intermediate recording differed significantly between progeny groups within test
8 ponds (b_1 = 1.23 g and 0.72 g per day in Pond 1, 1.46 g and 0.96 g per day in Pond 2 and 2.21 g and
9 1.72 g per day in Pond 3 for the LFM and HFM males, respectively).
10
11 The records of occurrence of early maturing females and body weight were corrected for the age
12 effects described above and reanalysed according to Model 2. Highly significant differences were
13 detected for the frequency of early maturing females in the LFM and HFM progeny groups, both
14 across and within test ponds (Table 4). The distribution of least square means of the full-sib families
15 for frequency of early maturing females across test ponds according to Model 2 is shown in Fig. 2.
16
17 The least square mean of the body weight traits according to Model 2 in the LFM and HFM progeny
18 groups and the significances of the differences between are shown in Table 5. The greatly reduced
19 significances of the differences according to Model 2 compared to Model 1 was partly due to
20 increased standard errors of the least square mean estimates when using the variation in body weight
21 between full-sib families within progeny groups as the error term, and partly due to reduced
22 differences between the least square means of the progeny groups because by Model 2 accounted for
23 uneven distributions of the records across families within progeny groups (overrepresentation of
24 individuals from heavy families within the HFM progeny and overrepresentation of individuals from
25 less heavy families within the LFM progeny at intermediate recording and at harvest). Still, the HFM
26 progeny was consistently heavier than the LFM progeny, but significantly heavier only in the males at
27 harvest and nearly significantly heavier in the females at intermediate recording.
28
29
30 4. Discussion and conclusions
31
32 The timing of the recording of early female sexual maturation within the time interval investigated in
33 the present study (two, three or four weeks after the first occurrence of swim-up fry in Pond 1, 2 and
34 3, respectively) did not appear to be crucial (Table 2). Other effects than time or body weight seemed
35 to influence the frequency of early maturing females at recording in the tree ponds, but no such effects
36 were identified in the experiment. The frequency at recording in each of the ponds was close enough

1 to the optimal frequency of 50 percent to secure that a possible variation between progeny groups and
2 full-sib families in frequency of early maturing females would be revealed.
3
4 As shown in Table 3, age differences at recording significantly affected all the investigated variables.
5 In some species, this problem may be solved by synchronized spawning or stripping. However, in
6 naturally reproducing Nile tilapia, statistical correction for age effects is the only alternative. This will
7 require a system for tracking the age of each individual, e.g. individual tagging as in the present
8 experiment.
9
10 The direct response to selection, measured as the difference between age corrected least square means
11 according to Model 2 for the frequency of early maturing females in the LFM and the HFM progeny
12 groups, was highly significant both across and within test ponds (Table 4). The bi-directional
13 response was 23.5 percent units across test ponds and 16.4, 28.7 and 24.7 percent units in Pond 1, 2
14 and 3, respectively. Since no unselected control was included in the experiment, the relative response
15 to selection in the two progeny groups may not be determined. Comparisons with the performance of
16 the parent stock is not possible because of random, environmental differences during testing of the
17 two generations (e.g. season effects). Nevertheless, the results showed that the frequency of early
18 maturation in Nile tilapia females, and consequently the age at first maturation, may be changed by
19 the family selection approach described in the present study. This is in accordance with the previously
20 cited reports from studies on genetic variation in age at first maturity in Nile tilapia and response to
21 selection for age at first maturity in salmonids
22
23 The correlated response of body weight traits to selection for frequency of early maturing females was
24 not entirely clear according to Model 2 (Table 5). This was partly due to a considerable random
25 genetic drift (a large variation in body weight between full-sib families within the two progeny
26 groups) that reduced the significance of a possible correlated response according to Model 2.
27 However, the least square means of body weight was consistently lower in the LFM progeny group
28 than in the HFM progeny group, both across and within test ponds. Uraiwan (1988) reported similar
29 results from a study with Nile tilapia. This suggests that selection for a lower frequency of early
30 maturing females in Nile tilapia may result in poorer growth performance and that selection for
31 improved growth rate may increase the frequency of early maturing females. The nearly significantly
32 higher body weight in the HFM females at intermediate recording may be explained by a mechanism
33 similar to the enhanced growth rate in maturing females observed in salmonids (see e.g. Rye and
34 Refstie, 1995). The significantly higher body weight in the HFM males at harvest is in accordance
35 with the positive genetic correlation coefficients estimated between body weight and occurrence of
36 early sexual maturity in Atlantic salmon (see e.g. Gjerde et al., 1994) and in a preliminary analysis of

data from Nile tilapia in the GIFT project (Eknath, 1996). Similar genetic associations have been reported in mosquito fish (Campton and Gall, 1987) and in platyfish (see e.g. Kallman, 1983)

If tag loss is assumed to occur randomly across progeny groups and full-sib families, the frequency of fish without records at intermediate recording and harvest may be considered as a rough estimate of mortality, and the sex ratio among recorded fish may be considered as a rough estimate of sex dependent mortality. No correlated response to selection was observed in any of these traits according to Model 1.

Based on the results from the present study, applied breeding programs for Nile tilapia should consider to include the frequency of early maturing females within sib groups as a trait for selection, to delay the onset of sexual maturation and reproduction in the improved stock. Because of the apparently unfortunate genetic association between this trait and body weight, a combined selection for the two traits should be carried out, applying appropriate relative economic weights to the traits when computing breeding values for the breeding candidates.

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6. References

Bentsen, H.B., 1990. Application of breeding and selection theory on farmed fish. Proc. 4th World Congress on Genetics Applied to Livestock Production, Edinburgh, Scotland, Vol. 16:149-158.

Bentsen, H.B. and Gjerde, B., 1994. Design of fish breeding programs. Proc. 5th World Congress on Genetics Applied to Livestock Production, Guelph, Canada, Vol. 19:353-359.

Bolivar R.B., Eknath, A.E., Bolivar, H.L. and Abella, T.A., 1993. Growth and reproduction of individually tagged Nile tilapia (*Oreochromis niloticus*) of different strains. Aquaculture, 111:159-160.

Campton, D.E. and Gall, G.A.E., 1987. Response to selection for body size and age at sexual maturity in the mosquito fish, (*Gambusio affinis*). Aquaculture, 68:221-241.

Davlin, A.Jr., 1991. The nineties: a booming decade for the aquaculture industry! The aquaculture industry. An analyst's report. Vol. III (1):1-6.

Donaldson L.R. and Menasveta D., 1961. Selective breeding of Chinook salmon. Trans. Am. Fish. Soc., 90:160-164.

Donaldson L.R. and Olson P.R., 1955. Development of rainbow trout broodstock by selective breeding. Trans. Am. Fish. Soc., 85:96-101.

Eknath A.E., 1992. Genetic improvement of farmed tilapias. Final report from the GIFT project 1988-1992 to UNDP-DGIP and ADB. International Center for Living Aquatic Resourses Management, Manila, Philippines.

Eknath A. E., 1996. GIFT-II annual report (1995). Report from the Genetic Impovement of Farmed Tilapias project to UNDP-STPD. International Center for Living Aquatic Resourses Management, Manila, Philippines.

Eknath, A.E., Bentsen, H.B., Gjerde, B., Tayamen, M.M., Abella, T.A., Gjedrem, T. and Pullin, R.S.V., 1991b. Approaches to national fish breeding programs: Pointers from a tilapia pilot study. NAGA, The ICLARM Quarterly, 14(2):10-12.

Eknath, A.E., Tayamen, M.M., Palada-de Vera, M.S., Danting, J.C., Reyes, R.A., Dionisio, E.E., Capili, J.B., Bolivar, H.L., Abella, T.A., Circa, A.V., Bentsen, H.B., Gjerde, B., Gjedrem, T. and Pullin, R.S.V., 1993. Genetic improvement of farmed tilapias: The growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. Aquaculture, 111:171-188.

Eknath, A.E., Capili, J.B., Palada-de Vera, M.S., Dionisio, E.E., Bolivar, H.L., Reyes, R.A. and Tayamen, M.M., 1997. A practical quantitative method to estimate relative reproductive activity in

Oreochromis niloticus. In: R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (Editors), The Third International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings 41, in press.

FAO, 1996. Aquaculture productions statistics. FAO Fisheries Circular No. 815, Revision 8, 189 pp.

Gjedrem, T., 1985a. Improvement of productivity through breeding scheemes. GeoJournal 10.3:233-241.

Gjedrem, T., 1985b. Genetic variation in age at sexual maturity and its relation to growth rate. In: R.N. Iwamoto and S. Sower (Editors), Salmonid Reproduction. Washington Sea Grant Program, University of Washington, Seattle, WA, pp. 52-61.

Gjerde, B., 1984. Response to individual selection for age at sexual maturity in Atlantic salmon. Aquaculture, 38:229-240.

Gjerde, B., 1986. Growth and reproduction in fish and shellfish. Aquaculture, 57:37-55.

Gjerde B., Simianer, H. and Refstie, T., 1994. Estimates of genetic and phenotypic parameters for body weight, growth rate and sexual maturity in Atlantic salmon. Livest. Prod. Sci., 38:133-143.

Guerrero, R.D., 1987. Tilapia farming in the Philippines. Tech. Res. Center, Manila, Philippines

Kallman, K.D., 1983. The sex determining mechanism of the poecilid fish, *Xiphophorus montezumae* and the genetic control of the sexual maturation process and adult size. Copeia, 1983:755-769.

Kronert U., Hörstgen-Schwark, G. and Langholz, H.J., 1986. Investigation on selection of tilapia for late maturity. In: K. Tiews (Editor), Selection, hybridization and genetic engineering in aquaculture. EIFAC/86/Symp., Bordeaux. Schriften der Bundesforschungsanstalt für Fischerei, Hamburg, Vol II?:346-352.

Kronert U., Hörstgen-Schwark, G. and Langholz, H.J., 1989. Prospects of selecting for late maturity in tilapia (*Oreochromis niloticus*). I. Family studies under laboratory conditions. Aquaculture, 77:113-121.

1
2 Lester, L.J., Abella, T.A., Palada, M.S. and Keus, H.J., 1988. Genetic variation in size and sexual
3 maturation of *Oreochromis niloticus* under hapa and cage culture conditions. In: R.S.V. Pullin, T.
4 Bhukaswan, K. Tonguthai and J.L. Maclean (Editors), The Second International Symposium on
5 Tilapia in Aquaculture, ICLARM Conference Proceedings 15. Department of Fisheries, Bangkok,
6 Thailand and International Center for Living Aquatic Resources Management, Manila, Philippines,
7 pp. 223-230.
8
9 Longalong, F.M. and Eknath A.E., 1995. Development of technique for synchronization of natural
10 spawning in Nile tilapia (*Oreochromis niloticus*). Poster abstract. Aquaculture, 137:284.
11
12 Lowe-McConnell R.H., 1982. Tilapias in communities. In: R.S.V. Pullin and R.H. Lowe-McConnell
13 (Editors), The biology and culture of tilapias. ICLARM Conference Proceedings 7. International
14 Center for Living Aquatic Resources Management, Manila, Philippines, pp. 83-113.
15
16 Maclean J.L., 1984. Tilapia - the aquatic chicken. ICLARM News 7(1):17
17
18 Mair G.C. and Little D.C., 1991. Population control in farmed tilapias., NAGA, The ICLARM
19 Quarterly 14 (3):8-13.
20
21 Odorf W., Kronert, U., Balarin J., Haller, R., Hörstgen-Schwark, G. and Langholz, H.J., 1989.
22 Prospects of selecting for late maturity in tilapia (*Oreochromis niloticus*). II. Strain comparisons
23 under laboratory and field conditions. Aquaculture 77:123 133.
24
25 Pullin, R.S.V., 1997. World tilapia culture and its future prospects. In: R.S.V. Pullin, J. Lazard, M.
26 Legendre, J.B. Amon Kothias and D. Pauly (Editors), The Third International Symposium on Tilapia
27 in Aquaculture, ICLARM Conference Proceedings 41, in press.
28
29 Pullin R.S.V., Eknath, A.E., Gjedrem, T., Tayamen, M.M., Macaranas, J.M., and Abella, T.A., 1991.
30 The genetic Improvement of farmed tilapias (GIFT) project: The story so far. NAGA, The ICLARM
31 Quarterly. 14(2):3-6.
32
33 Rye, M. and Refstie, T., 1995. Phenotypic and genetic parameters of body size traits in Atlantic
34 salmon *Salmo Salar* L. Aquaculture Research 26:875-885.
35

1 Uraiwan, S., 1988. Direct and indirect responses to selection for age at first maturation of
2 *Oreochromis niloticus*. In: R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean (Editors),
3 The Second International Symposium on Tilapia in Aquaculture, ICLARM Conference Proceedings
4 15. Department of Fisheries, Bangkok, Thailand and International Center for Living Aquatic
5 Resources Management, Manila, Philippines, pp.295-300.
6
7 Wohlfarth G.W. and Hulata G., 1983. Applied genetics of tilapias. ICLARM studies and reviews
8 (second revised edition), International Center for Living Aquatic Resources Management, Manila,
9 Philippines, pp. 6-26.

Table 1. The number of Nile tilapia progeny recorded at stocking, at intermediate recording and at harvest in each pond

Recording	Pond 1	Pond 2	Pond 3	Total
At stocking				
LFM progeny	370	386	385	1141
HFM progeny	673	685	680	2038
Intermediate recording				
LFM females	158	198	170	526
LFM males	99	104	140	343
HFM females	296	331	356	983
HFM males	164	185	179	558
Untagged individuals	165	100	150	415
Mortality since stocking	161	153	69	383
At harvest				
LFM females	100	168	100	368
LFM males	76	86	74	236
HFM females	153	260	253	666
HFM males	104	138	117	359
Untagged individuals	228	89	94	411
Mortality since stocking	382	330	427	1139

Table 2. The occurrence of early maturing females, the body weight at stocking, the body weight for males and females at intermediate recording and at harvest, and the mortality, the tag loss and the sex ratio among tagged fish at intermediate recording and at harvest of the Nile tilapia progeny in the three test ponds

Trait	Pond 1		Pond 2		Pond 3	
	Mean	sd	Mean	sd	Mean	sd
At stocking						
Body weight (g)	4.11	1.90	4.43	1.89	3.97	1.96
Intermediate recording						
Mature females (%)	52.6	-	36.7	-	57.0	-
Body weight, females (g)	113.2	27.0	117.6	24.0	134.8	32.3
Body weight, males (g)	128.3	35.6	154.5	39.3	173.4	43.7
Sex ratio (% females)	63.3	-	64.7	-	62.2	-
Mortality (%)	15.4	-	9.3	-	14.1	-
Untagged (%)	15.8	-	14.3	-	6.6	-
At harvest						
Body weight, females (g)	134.9	30.9	139.7	29.6	137.4	32.9
Body weight, males (g)	201.9	49.3	209.6	54.7	180.0	46.8
Sex ratio (% females)	58.4	-	65.6	-	64.9	-
Mortality (%)	36.6	-	30.8	-	40.1	-
Untagged (%)	21.9	-	8.3	-	8.8	-

Table 3. Proportion of the total variation (R^2) in Nile tilapia progeny explained by the extended Model 1 for each of the dependent variables, and the significance of the linear and non-linear regression of the dependent variables on age across (b_1 and b_2) or within (b_{1ij} and b_{2ij}) progeny group by pond subcells.

Dependent variable	R^2	Age effects			
		b_1	b_2	b_{1ij}	b_{2ij}
Frequency of early maturing females	0.115	< 0.0001	ns	ns	ns
Body weight, stocking	0.327	ns	< 0.0001	ns	ns
Female body weight, intermediate	0.149	< 0.0001	ns	ns	ns
Male body weight, intermediate	0.342	-	ns	< 0.0001	ns
Female body weight, harvest	0.037	-	ns	0.0002[1)]	ns
Males body weight, harvest	0.206	ns	< 0.0001	ns	ns
Proportion of females, intermediate	0.024	-	-	0.0006	0.0006
Proportion of females, harvest	0.037	-	-	0.0003	0.0003
Lost fish, intermediate	0.017	-	ns	0.0071	ns
Lost fish, harvest	0.043	-	-	0.0032	0.0026

1): Heterogeneous regression coefficients only between selection groups (b_i)
ns: Not significant

Table 4. Age corrected least square means (± standard errors) for frequency of early maturing females according to Model 2 within and across test ponds in progeny of Nile tilapia broodstock selected from full-sib families with a low (LFM) and a high (HFM) frequency of early maturing females.

Test pond	Progeny group		Significance level (p) [1]
	LFM	HFM	
Pond 1	35.58 ± 5.67	51.95 ± 4.16	0.0230
Pond 2	17.43 ± 5.15	46.13 ± 3.94	< 0.0001
Pond 3	48.23 ± 5.48	72.92 ± 3.78	0.0004
Pooled	33.57 ± 3.14	57.03 ± 4.31	0.0002

1) Using family variation within progeny groups as the error term

Table 5. Age corrected least square means (± standard errors) for body weight at stocking and for each sex separately at intermediate recording and at harvest according to Model 2 across test ponds in progeny of Nile tilapia broodstock selected from full-sib families with a low (LFM) and a high (HFM) frequency of early maturing females.

Body weight records	Progeny group		Significance level (p) [1)]
	LFM	HFM	
Stocking	4.06 ± 0.20	4.23 ± 0.15	ns
Intermediate recording			
Females	113.68 ± 4.81	125.82 ± 3.51	(0.0523)
Males	149.94 ± 6.47	151.62 ± 4.16	ns
Harvest			
Females	133.62 ± 4.61	139.41 ± 3.41	ns
Males	181.86 ± 7.75	204.94 ± 5.95	0.0269

1): Using family variation within progeny groups as the error term
ns: Not significant

Legends to figures

Fig. 1. Distribution of the ferquency of sexually mature Nile tilapia females in the 41 full-sib families constituting the parent generation, recorded four weeks after observing the first swim-up fry in the pond. The families used as parent broodstock to produce progeny groups with low (LFM) and high (HFM) frequncy of early maturing females are shown

Fig. 2. Distribution of age corrected least sqare means for frequency of early sexual maturation Nile tilapia females according to Model 2 in full-sib families that were progeny of brodstock selected from families with low (LFM) or high (HFM) frequncy of early maturing females

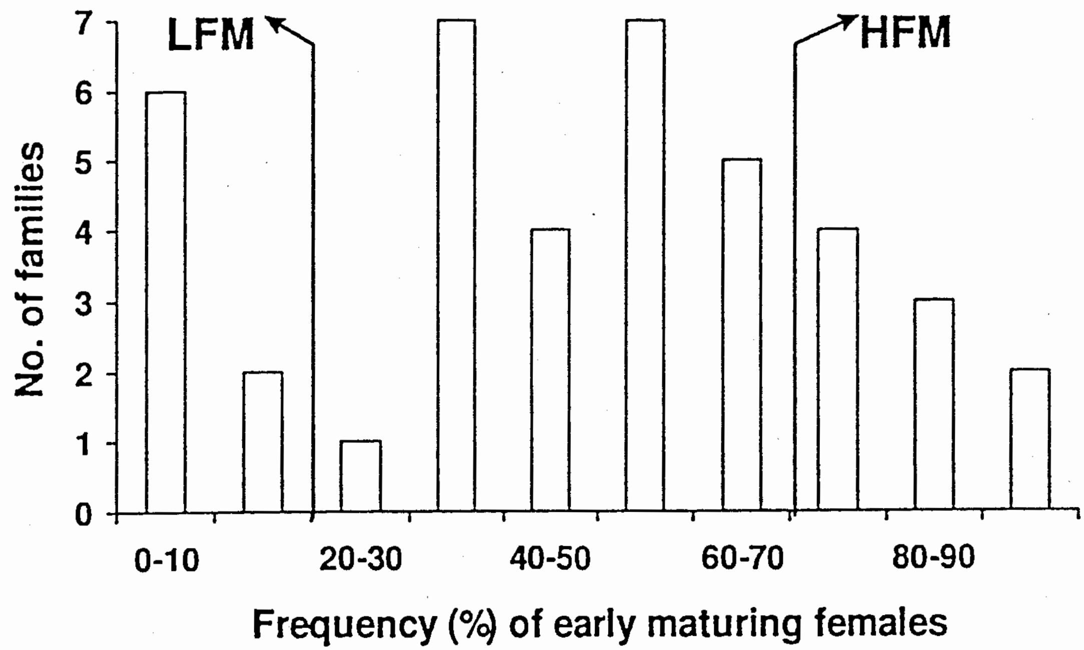

Figure 1

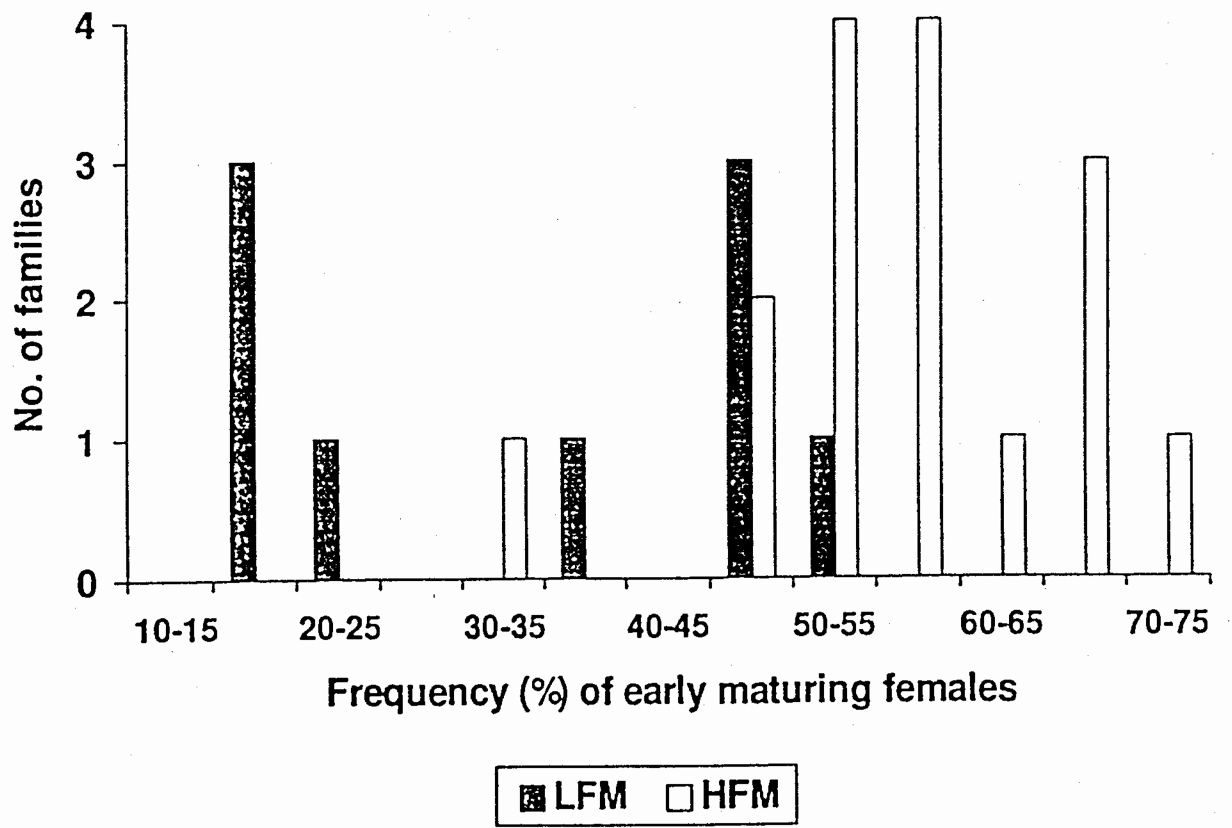

Figure 2

Attachment 8.

Manuscript on:

(a) Genetic variation in lysozyme and spontaneous haemolytic activities of blood serum from Nile tilapia;

(b) Genetic associations of serum lysozyme and serum spontaneous haemolytic activities with survival and body weight in Nile tilapia.

Genetic variation in lysozyme and spontaneous haemolytic activities of blood serum from Nile tilapia *

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Abstract

A study was carried out on lysozyme activity and spontaneous haemolytic (SH) activity of blood serum from 388 individuals of Nile tilapia (Oreochromis niloticus) coming from 42 full-sib groups within 21 paternal half-sib groups. The lysozyme activity was measured at both 15° C and 30° C incubation temperature, whereas SH activity was measured at 30° C incubation temperature. Significant variation in lysozyme activity was detected between half-sib groups at 30° C incubation temperature but not at 15° C incubation temperature and not for SH activity. Significant variation was found between full-sib groups for all parameters. The estimated heritabilities of lysozyme activity were relatively high (0.6 - 0.7) at 30° C incubation temperature and intermediate (about 0.3) at 15° C incubation temperature both when based on sire and dam components of variance or on variance components from an individual animal model. No other common effects than additive genetic effects were detected within the full-sib groups. The heritability estimate of SH activity was zero according to estimates based both on the sire component of variance and on variance components from an individual animal model, whereas the estimate based on the dam component of variance was about 0.7 and highly significant. Even if the common full-sib effect according to the individual animal model (0.16) was non-significant, this suggests the presence of non-additive genetic effects or common environmental effect for SH activity in the full-sib groups. The genetic correlation between lysozyme activity at 30° C and 15° C was 0.92-0.95. The genetic correlations between SH activity and lysozyme activity was not estimated in this study because of the lack of additive genetic variation in SH activity.

Keywords: Genetic variation; Lysozyme activity; Spontaneous haemolytic activity; Nile tilapia

1. Introduction

Disease is an important factor that causes problems in aquaculture production (Fjalestad et al., 1993). Disease control is often managed by vaccination or drug treatment. However, these methods are not always sustainable and have some disadvantages; vaccination may be used against specific disease only, and antibiotics or other drug treatments may cause problems because of residues that may affect the environment or human health (Branson, 1993).

Since significant genetic variation in disease resistance has been shown in various fish species (Gjedrem and Aulstad, 1974; McIntyre and Amend, 1978; Refstie, 1982; Standal and Gjerde, 1987; Gjedrem et al., 1991), genetic selection for increased disease resistance may be a promising alternative on a long term basis. Genetic resistance is a more persistent solution than treatments by antibiotics and other drugs. There are two methods of genetic selection for increased disease resistance: direct and indirect selection (Fjalestad et al., 1993). Direct selection may be practiced by selecting surviving individuals or individuals from families with a high survival rate after induced or natural outbreaks of diseases. However, since breeders that have been exposed to a disease agent may spread the disease in an applied breeding program, direct selection should only be practiced as family selection, using sibs that have not been exposed to the disease as breeders. Indirect selection may be practiced by selecting on traits (i.e. components of the immune system) that are genetically correlated to disease resistance. Correlated traits that may be rapidly measured in live animals will allow both family and individual selection to be practiced. Depending on the magnitude of the heritabilities of the direct and indirect traits and the genetic correlation between them; indirect selection based on a combination of individual and

family performance may increase the response to selection compared to direct selection based on family selection alone (Fjalestad et al., 1993). The traits used in indirect selection must show genetic variation and genetic correlation to disease resistance.

Immunological traits, either specific or non-specific immune response parameters, have been reported to show genetic variation. Non-specific immune response parameters, especially lysozyme and haemolytic activities of blood serum from fish, have been studied as genetic markers for selective breeding to increase disease resistance. Lysozyme is a mucolytic enzyme of leucocytic origin which exhibits antibacterial properties, and in higher vertebrates the enzyme may form part of a non-specific defence against parasitic, bacterial and viral infections (Ingram, 1980). Lysozyme is most likely of importance in fish immunity defence mechanism against infectious diseases (Fänge et al., 1976; Murray and Fletcher, 1976; Lundblad et al., 1979; Lindsay, 1986). In Atlantic salmon (Salmo salar), fish with symptoms of furunculosis as well as survivors after a disease outbreak had serum lysozyme levels significantly higher than non-infected control fish (Møyner et al., 1993). The increased lysozyme levels in response to the infection agree with a study in common carp (Cyprinus carpio) by Siwicki and Studnicka (1987). There was significant genetic variation in lysozyme activity in blood serum from Atlantic salmon (Røed et al., 1993a) and rainbow trout (Røed et al., 1993b). Moreover, serum lysozyme activity in Atlantic salmon was correlated to survival in challenge tests with Aeromonas salmonicida, Renibacterium salmoninarum and Vibrio salmonicida (Lund et al., 1995).

Two related mechanisms normally detected in blood serum from fish cause haemolysis of heterologous red blood cells. The mechanisms may be specific (antibody-dependent) or non-specific (natural) (Nonaka et al., 1981). Non-specific haemolytic activity is characterized by spontaneous haemolysis. The specific and non-specific haemolytic

activity of blood serum from fish probably arise via the classical and alternative pathways, respectively, of a complement system similar to that known in mammals (Giclas et al., 1981; Nonaka et al., 1981; Yano et al., 1984; Koppenheffer, 1987; Ingram, 1980, 1987, 1990; Røed et al., 1993a). The complement system plays a role in humoral and cellular immunity against pathogens and in inflammatory processes (Ingram, 1990). In salmonids, non-specific killing activity against A. salmonicida was proportional to the spontaneous haemolytic (SH) activity, indicating an association between SH activity and resistance against furunculosis (Sakai, 1983). Genetic variation in haemolytic activity of serum has been shown in Atlantic salmon (Røed et al., 1992), common carp (Wiegertjes et al., 1993) and rainbow trout (Hollebecq et al., 1995). Therefore, the lysozyme activity and SH activity may be candidates as genetic markers for selective breeding to increase disease resistance in fish.

The primary objective of the present investigation is to determine the genetic variation in lysozyme activity and SH activity in blood serum from a family material of Nile tilapia derived from an on-going selection program for increased growth performance in the Philippines (Eknath,1993). Nile tilapia has been chosen as a prominent model species for developing more productive and profitable aquaculture in developing countries by ICLARM's Aquaculture Program through the Genetic Improvement of Farmed Tilapias (GIFT) Project (Pullin et al., 1991; Eknath et al., 1991). The GIFT program has so far focused on selection for growth performance (body weight at the end of about 120 days grow-out period) in diverse tilapia farming system as its primary breeding goal. Investigations on genetic parameters of other economically important traits such as age at maturation, survival, and resistance to diseases are being actively persued for inclusion in a multi-trait selection program in the near future.

2. Materials and Methods

Fish

The fish were obtained from the third generation of Nile tilapia selected for increased body weight at harvest in the project Genetic Improvement of Farmed Tilapias (GIFT), carried out at the National Freshwater Fisheries Technology Research Center, Bureau of Fisheries and Aquatic Resources (NFFTRC/BFAR) and the Freshwater Aquaculture Center of the Central Luzon State University (FAC/CLSU) in Muñoz, Philippines (Pullin et al., 1991; Eknath, 1993).

The material consisted of 388 fish coming from 42 full-sib groups within 21 paternal half-sib groups. Male brood fish and female brood fish were conditioned separately in 3x3x1 m^3 hapas at a density of 10 fish per m^2 for two weeks before stocking for mating. Two female brood fish were then stocked with one male brood fish in 1 m^3 mating hapas. One week after stocking, all hapas were examined and swim-up fry were collected. Female brood fish which had spawned were removed from the hapas, and the fry were transferred to 1 m^3 fine mesh rearing hapas at a stocking density of 200-250 fry per m^3. The fry were reared in the fine mesh hapas for 21 days before being transferred to 1 m^3 larger mesh hapas, at a stocking density of 100 fish per m^3. The procedure was repeated with an interval of one week until a sufficient number of full-sib groups had been produced. The fingerlings were individually tagged with modified Floy fingerling tags, after rearing period of 10-20 weeks. On 18 November 1993, the tagged fingerlings were then communally stocked for grow-out in one 1200 m^2 earthen pond which was fertilized with inorganic fertilizer (46-0-0) at a rate of 10 kg per week. Supplementary feed was given daily with a mixture of 70% rice bran and 30% fish meal at a rate of 40% of the body weight.

During a three and a half months grow-out period, the water temperature varied between 17 and 28° C and pH 6 - 8.5.

During 7-8 March 1994, blood samples were collected from the fish. Body weight, sex and stage of sexual maturation were recorded. Blood samples were collected from the caudal vein of the anaesthetized fish. Vacutainer tubes without additives were used, and the blood was allowed to clot at room temperature for 3 hours. After centrifugation, the serum was removed from the clotted blood and frozen at -70° C until analysis.

Assays of lytic activity

Lysozyme activity was determined based on the Micrococcus lysoplate assay of Osserman and Lawlor (1966), and Lie et al. (1986), incorporating the modifications described by Røed et al. (1993b). Assay media was prepared by mixing 50 $\mu g\ ml^{-1}$ Micrococcus lysodeikticus with 1% agarose in 0.06 M NaH_2PO_4/Na_2HPO_4-buffer (pH = 6.0) at 56° C, and 15 ml of the mixture was poured on to 10x10 cm^2 defatted glass slide coated with agarose. Sample wells of 4 mm in diameter were punched and filled with 10 μl tilapia serum diluted with the phosphate buffer. All sera was analyzed at incubation temperature of both 15° C and 30° C with an incubation time for 20 h. The incubated plates were washed in distilled water for about half an hour, pressed under filter paper and completely air dried. For staining, the plates were placed in 1.25% methyl violet solution for 1 min, transferred to Lugol's solution (1% I_2, 2% KI) for 15 s, and then intensively destained with ethyl alcohol until stainless lysed zones appeared. The diameter of the areas cleared by lysis were measured using a measuring-viewer (Bering Institute). Because of the large number of serum samples, the analysis was carried out in three batches on different days. The day of analysis was recorded and corrected for during the statistical analysis.

Assay of the SH activity caused by untreated tilapia serum was determined according to the agarose plate technique of Lachman and Hobart (1979) and Lie et al. (1986), described by Røed et al. (1993a). Sheep red blood cell (SRBC) was used for determination of SH activity. Buffer for both washing SRBC and diluent in the assay was 0.094 M veronal/sucrose-veronal (SVB), pH 7.4, containing $3x10^{-4}$ M Ca^{2+} and $1x10^{-3}$ M Mg^{2+}. The SRBC was washed with SVB, and 0.25% packed SRBC was mixed at 56° C with 1% agarose in SVB. Fifteen ml of the mixture was poured on to $10x10\ cm^2$ defatted and agarose-coated prewarmed glass slide. Sample wells of 4 mm in diameter were punched and filled with 10 μl of the tilapia serum. The plates were incubated at 30° C for 20 h, fixed with formalin about 30 min and allowed to dry. The diameter of the lysed zones were measured by a Measuring-viewer as for the lysozyme assay.

The lytic activities of the individual test sera were expressed as the area of lysed zone.

Statistical analysis

The lytic activity data were analyzed with the GLM procedure of the SAS statistical package (SAS Institute Inc., 1988). The model used was

$$Y_{ijklm} = \mu + a_i + f_j + b_* w_{ijklm} + s_k + d_{kl} + e_{ijklm} \qquad \text{(Model 1)}$$

where

Y_{ijklm} = area of lysed zone (mm^2) caused by blood serum from the *m*th individual

μ = overall mean

a_i = fixed effect of the day of laboratory analysis ($i = 1, 2, 3$)

f_j = fixed effect of sex/sexual maturity group (j = 0, 1, 2, 3 where 0 = male, 1 = female, non-spawner, 2 = female, ready to spawn, 3 = female, recently spawned)

b = regression coefficient of the dependent variable on body weight

w_{ijklm} = body weight of the mth individual

s_k = random effect of kth sire (k = 1, 2,..., 21)

d_{kl} = random effect of lth dam (l = 1, 2) within the kth sire

e_{ijklm} = random residual effect.

Because the SH activity was analyzed within one day, the fixed effect of analyzing day (a_i) was deleted from the model.

The (co)variance components of both sire and dam within sire were used to estimate heritabilities, genetic and phenotypic correlations. The standard errors of the parameter estimates were estimated based on Becker (1992).

Heritabilities and the standard errors of the heritabilities were also estimated according to a mixed model using the computer program Derivative Free Restricted Maximum Likelihood (DFREML) version 2.0 described by Meyer (1989,1991). The following individual animal model was used for estimation of genetic parameters

$$Y_{ijk} = \mu + a_i + f_j + b_* w_{ijk} + \mu_{ijk} + c_{ijk} + e_{ijk} \qquad \text{(Model 2)}$$

where

Y_{ijk} , μ , a_i , f_j , b , w_{ijk} and e_{ijk} are as described in Model 1 and

μ_{ijk} = the random additive genetic effect of the kth fish

c_{ijk} = the random common full-sib effects (c-effect) for the kth fish due to other factors than additive genetic effects.

The relationship matrix used in the animal model contained the experimental fish, parents, grandparents, and grand-grandparents.

The least-squares means of the half-sib groups for the individual lytic activities were calculated from Model 1.

3. Results

The lysozyme activity of Nile tilapia at incubation temperature of 30° C and 15° C ranged from 120 to 344 mm^2 and 79 to 274 mm^2 with mean values of 224.56 and 152.61 mm^2 respectively (Table 1). The SH activity ranged from 0 to 59 mm^2 with mean value of 12.70 mm^2 (Table 1).

The GLM analysis revealed significant effects of day of analysis, body weight, sex and stage of sexual maturation and the family groups on the lytic activities (Table 2). The systematic analytical effects of day of analysis on lysozyme activity was significant at both 30° C and 15° C incubation temperatures. The estimated regression coefficient of SH activity on body weight was positive and significant ($b = 0.07 \pm 0.02$). No significant effect was found of body weight on lysozyme activity.

Sex and stage of sexual maturation of the fish showed a significant effect on lysozyme activity, whereas the SH activity was not affected by this trait. Males and female non-spawners showed significantly higher serum lysozyme activity than female spawners (females ready to spawn or that had recently spawned) (Table 3).

The effects of sire and dam within sire on lysozyme activity were found significant according to Model 1 (Table 2). The variation in the least-square mean values of lysozyme activity between half-sib groups was substantial (Fig.1). The estimated heritabilities for

lysozyme activity and standard errors are given in Table 4. When the samples were incubated at 30° C, the heritability estimates for lysozyme activity were high both when based on the sire (0.62) and the dam (0.74) components of variance. The heritability estimates were reduced considerably when based on the lysozyme activity at an incubation temperature of 15° C. In both cases, the heritability estimates based on the dam component of variance was quite similar to the heritability based on the sire component of variance.

For SH activity, the estimated heritability based on the sire component of variance according to Model 1 was close to zero, whereas the heritability based on the dam component of variance was high (0.7; Table 4).

The estimated heritabilities of lysozyme activity and SH activity according to an individual animal model (Model 2) were similar to the estimates based on the sire component of variance according to Model 1 (Table 4). The c-effect of lysozyme activity was zero at both incubation temperatures. The heritability of SH activity was zero and the c-effect (common effects within full-sib groups other than additive genetic effects) on lysozyme activity was moderate.

The phenotypic correlation between lysozyme activity at both incubation temperatures according to Model 1 was high (0.63±0.04), whereas the phenotypic correlations between SH activity and lysozyme activity at both incubation temperatures were low (0.12±0.06 and 0.00±0.06 with lysozyme activity at 30 and 15° C respectively). A highly significant genetic correlation was detected (0.92-0.95) between lysozyme activities at incubation temperatures of 30° C and 15° C. The genetic correlations between SH activity and lysozyme activity was not estimated, since SH activity was found to show no additive genetic variation.

4. Discussion

The estimated heritabilities of lysozyme activity at 30° C incubation temperature obtained from the sire and dam components of variance according to Model 1 were quite similar, and so were the estimated heritabilities of lysozyme activity at 15° C incubation temperature (Table 4). This suggests that the parameters were not affected by common full-sib effects due to other factors than additive genetic effects. The results agreed well with those obtained from the individual animal model (Model 2).

The lysozyme activity and the heritability of the parameter was much larger when determined at an incubation temperature of 30° C than that at 15° C. This could be due to the high natural ambient temperature of Nile tilapia. The genetic correlation between the lysozyme activity at the two incubation temperatures was very high (r = 0.95), indicating the same genetic mechanisms seems to be involved in the lysozyme activity at both incubation temperatures. However, the higher expression of lysozyme activity and the increased genetic variation of lysozyme activity at 30° C incubation temperature relative to the random environmental variation suggests that analysis of lysozyme activity in Nile tilapia should be carried out at an incubation temperature of 30° C rather than 15° C to increase the accuracy of the analysis.

In the present study, the estimated heritability of lysozyme activity at 30° C incubation temperature was considerable. Consequently lysozyme activity fulfills one of the requirements of a useful genetic marker for disease resistance in Nile tilapia. The genetic correlation between this marker trait and survival or disease resistance, which is a second requirement, will be investigated in a separate study.

For SH activity, the heritability based on the sire component of variance according to Model 1 was close to zero. This agreed well with the estimate obtained from the mixed model analysis (Model 2). The estimated heritability differed considerably when based on the sire and dam components of variance. The much higher estimate based on the dam component of variance may be attributable to non-additive genetic effects and/or common environmental effects on this trait. This is consistent with the common full-sib effect estimated by Model 2.

There have been reported moderate to relatively high heritabilities in serum haemolytic activities in rainbow trout (Røed et al., 1990) and low to moderate heritabilities in SH activity were found in Atlantic salmon (Røed et al., 1992, 1993a). The conclusion drawn by Røed et al. (1990, 1992, 1993a), that these may be suitable parameters for indirect selection to improve disease resistance, is in agreement with the study of Wiegertjes et al. (1993) in carp. The result of the present study does not confirm the above conclusions, but seems to agree more with the suggestions of Lund et al. (1995) and Hollebecq et al. (1995) that haemolytic activity is of limited interest as a candidate marker trait for indirect selection to improve additive genetic performance for disease resistance. The possibility that the significant variation between dam progeny groups could be caused by non-additive genetic effects should be investigated.

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6. References

Becker, W.A., 1992. Manual of quantitative genetics. 5th Edition. Academic Enterprises, Pullman, Washington. 189 pp.

Branson, E., 1993. Environmental Aspects of Aquaculture. In: L. Brown (Editor), Aquaculture for Veterinarians: Fish Husbandry and Medicine. Pergamon Press, Oxford, pp. 57-67.

Eknath, A.E., 1993. Genetic improvement of farmed tilapias. Final Report to ADB and UNDP from International Center for Living Aquatic Resources Management (ICLARM), Manila, Philippines.

Eknath, A.E., Bentsen, H.B., Gjerde, B., Tayamen, M.M., Abella, T.A., Gjedrem, T. and Pollin, R.S.V., 1991. Approaches to national fish breeding programs: Pointers from a tilapia pilot study. NAGA, The ICLARM Quarterly, pp 10-12.

Fjalestad, K.T., Gjedrem, T. and Gjerde, B., 1993. Genetic improvement of disease resistance in fish : an overview. Aquaculture, 111: 65-74.

Fänge, R., Lundblad, G. and Lind, J., 1976. Lysozyme and chitinase in blood and lymphomyeloid tissues of marine fish. Mar. Biol., 36: 277-282.

Giclas, P.C., Morrison, D.C., Curry, B.J., Laurs, R.M. and Ulevitch, R.J., 1981. The complement system of the albacore tuna, Thunnus alalunga. Dev. Comp. Immunol., 5: 437-447.

Gjedrem, T. and Aulstad, D.,1974. Differences in resistance to vibrio disease of salmon parr (Salmo salar). Aquaculture, 3: 51-59.

Gjedrem, T., Salte, R., and Gjøen, H.M., 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. Aquaculture, 97: 1-6.

Hollebecq, M.-G., Faivre, B., Bourmaud, C. and Michel, C., 1995. Spontaneous bactericidal and complement activities in serum of rainbow trout (Oncorhynchus mykiss) genetically selected for resistance or susceptibility to furunculosis. Fish & Shellfish Immunol., 5: 407-426.

Ingram, G.A., 1980. Substances involved in the natural resistance of fish to infection - A review. J. Fish Biol.,16: 23-60.

Ingram, G.A., 1987. Haemolytic activity in the serum of brown trout, Salmo trutta L. J. Fish Biol., 31: 9-17.

Ingram, G.A., 1990. Complement-fixation test. In: J.S. Stolen, T.C. Fletcher, D.P. Anderson, B.S. Robertson and W.B. van Muisewinkel (Editors), Techniques in Fish Immunology. SOS Publications, Fair Haven, USA. pp. 25-44.

Koppenheffer, T.L., 1987. Serum complement of ectothermic vertebrates. Dev. Comp. Immunol., 11: 279-286.

Lachman, P.J. and Hobart, M.J., 1979. Complement technology. In: D.M. Weir (Editor), Handbook of Experimental Immunology. Vol. 1 Immunochemistry. Blackwell Scientific Publications, Oxford. pp. 5A.1-5A.23.

Lie, Ø., Syed, M. and Solbu, H., 1986. Improved agar plate assays of bovine lysozyme and haemolytic complement activity. Acta Vet. Scan., 27: 23-32.

Lindsay, G.J.H., 1986. The significance of chitinolytic enzymes and lysozyme in rainbow trout (Salmo gairdneri) defence. Aquaculture, 51: 169-173.

Lund, T., Gjedrem, T., Bentsen, H.B., Eide, D.M., Larsen, H.J.S. and Røed, K.H., 1995. Genetic variation in immune parameters and associations to survival in Atlantic salmon. J. Fish Biol., 46: 748- 758.

Lundblad, G., Fänge, R., Slettengren, K. and Lind, J., 1979. Lysozyme, chitinase and exo-N-acetyl-β-D-glucosaminidase (Nagase) in lymphomyeloid tissue of marine fishes. Mar. Biol., 53: 311-315.

McIntyre, J.D. and Amend, D.F., 1978. Heritability of tolerance for infectious hematopoietic necrosis virus in sockeye salmon (Oncorhynchus nerka). Trans. Am. Fish. Soc., 107: 305-308.

Meyer, K., 1989. Restricted maximum likelihood to estimate variance components for animal models with several random effects using a derivative-free algorithm. Genet. Sel. Evol., 21: 317-340.

Meyer, K., 1991. DFREML. Programs to estimate variance components by Restricted Maximum Likelihood Using a Derivative - Free Algorithm. User notes. Version 2.0. 84 pp.

Murray, C.K. and Fletcher, T.C., 1976. The immunohistochemical localization of lysozyme in plaice (Pleuronectes platessa) tissue. J. Fish Biol., 9: 329-334.

Møyner, K., Røed, K.H., Sevatdal, S. and Heum, M., 1993. Changes in non-specific immune parameters in Atlantic salmon, Salmo salar L., induced by Aeromonas salmonicida infection. Fish & Shellfish Immunol., 3: 253-265.

Nonaka, M., Yamaguchi, N., Natsuume-Sakai, S. and Takakashi, M., 1981. The complement system of rainbow trout (Salmo gairdneri). J. Immunol., 126: 1489-1494.

Osserman, E.F. and Lawlor, D.P., 1966. Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia. J. Exp. Med., 124: 921-951.

Pullin, R.S.V., Eknath, A.E., Gjedrem, T., Tayamen, M.M., Macaranas, J.M. and Abella, T.A., 1991. The genetic improvement of farmed tilapias (GIFT) project: the story so far. NAGA, The ICLARM Quarterly (April). pp. 3-6.

Refstie, T., 1982. Preliminary results: differences between rainbow trout families in resistance against vibriosis and stress. Dev. Comp. Immunol., Suppl., 2: 205-209.

Røed, K.H., Brun, E., Larsen, H.J. and Refstie, T., 1990. The genetic influence on serum haemolytic activity in rainbow trout. Aquaculture, 85: 109-117.

Røed, K.H., Fjalestad, K., Larsen, H.J. and Midthjel, L., 1992. Genetic variation in haemolytic activity in Atlantic salmon (Salmo salar L.). J. Fish Biol., 40: 739-750.

Røed, K.H., Fjalestad, K.T. and Strømsheim, A., 1993a. Genetic variation in lysozyme activity and spontaneous haemolytic activity in Atlantic salmon (Salmo salar). Aquaculture, 114: 19-31.

Røed, K.H., Larsen, H.J.S., Linder, R.D. and Refstie, T., 1993b. Genetic variation in lysozyme activity in rainbow trout (Oncorhynchus mykiss). Aquaculture, 109: 237-244.

Sakai, D.K., 1983. Lytic and bactericidal properties of salmonid sera. J. Fish Biol., 23: 457-466.

SAS Institute Inc., 1988. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc., Cary, NC. 1028 pp.

Siwicki, A. and Studnicka, M., 1987. The phagocytic ability of neutrophils and serum lysozyme activity in experimentally infected carp, Cyprinus carpio L. J. Fish Biol., 31 (Suppl. A): 57-60.

Standal, M. and Gjerde, B., 1987. Genetic variation in survival of Atlantic salmon during the sea-rearing period. Aquaculture, 66: 197-207.

Wiegertjes, G.F., Yano, T. and van Muiswinkel, W.B., 1993. Estimation of the genetic variation in complement activity of common carp (Cyprinus carpio L.). Vet. Immunol. Immunopathol., 37: 309-319.

Yano, T., Ando, H. and Nakao, M., 1984. Optimum conditions for the assay of haemolytic complement titer of carp and seasonal variation of the titers. J. Fac. Agric., Kyushu Uni., 29: 91-101.

Table 1

Mean values and standard deviations for the traits measured.

Trait	Mean	Standard deviation
Body weight (g)	115.55	38.85
Lysozyme (30° C) (mm^2)	224.56	30.51
Lysozyme (15° C) (mm^2)	152.61	27.10
SH (30° C) (mm^2)	12.70	11.02

Table 2

Marginal (Type III) mean square values of the effects in Model 1 on lysozyme and spontaneous haemolytic activity of blood serum from Nile tilapia. The proportion of the variation explained by the model (R^2) are also shown.

Source	D.F.	Mean square values		
		Lysozyme (30 °C)	Lysozyme (15 °C)	SH
Analyzing day	2	14032.6***	7177.3***	-
Body weight	1	551.7	927.4	1009.4***
Sex/maturation	3	2810.8**	4217.4***	141.6
Sire[1)]	20	4034.3*	1832.9	249.4
Dam(sire)	21	1873.9***	919.6*	240.0***
Error	340	562.4	561.2	87.4
R^2		0.47	0.33	0.37

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

1) Sire components of variance are tested against dams within sire components of variance as error term.

Table 3

Least-squares means of serum lysozyme activity of Nile tilapia of different sexes and stages of sexual maturation.

	Lysozyme activity	
	30° C	15° C
Male	230.95 [a]	159.49 [a]
Female, non-spawner	227.49 [ab]	157.86 [a]
Female, ready to spawn	219.66 [bc]	145.58 [b]
Female, recently spawned	215.51 [c]	142.29 [b]

a, b, c: figures in the same column sharing the same superscript letter does not differ significantly ($p<0.05$).

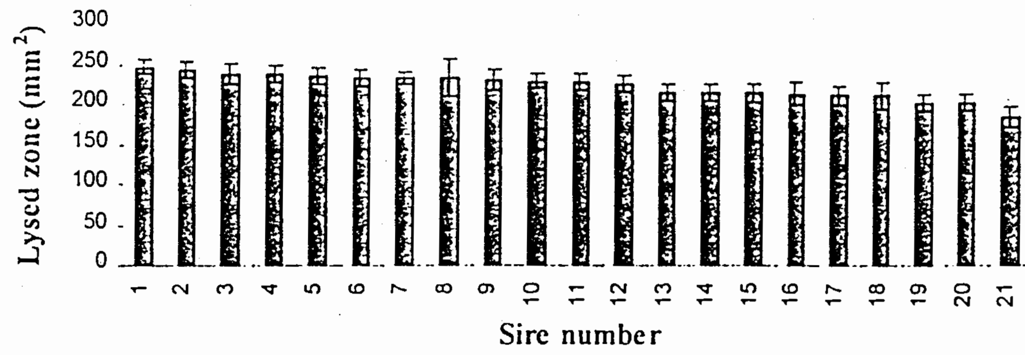
Table 4

Heritability estimates (h^2) and standard errors of lysozyme activity at 30° C and 15° C incubation temperatures and spontaneous haemolytic activity at 30° C incubation temperature of blood serum from Nile tilapia according to Model 1 and Model 2. Common full-sib effects other than additive genetic effects (c^2) according to Model 2 are also shown.

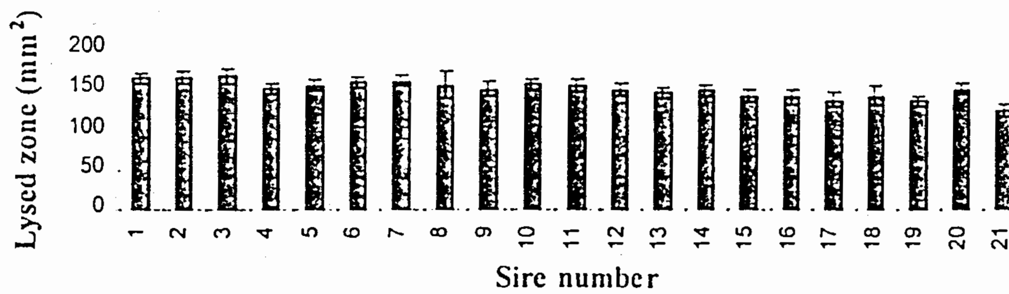
Lytic activity	Model 1			Model 2	
	h^2_{S+D}	h^2_S	h^2_D	h^2	c^2
Lysozyme, 30° C	0.68 ± 0.13	0.62 ± 0.35	0.74 ± 0.30	0.69 ± 0.29	0.00 ± 0.11
Lysozyme, 15° C	0.30 ± 0.10	0.34 ± 0.22	0.26 ± 0.20	0.28 ± 0.19	0.00 ± 0.08
SH, 30° C	0.37 ± 0.10	0.03 ± 0.24	0.71 ± 0.31	0.00 ± 0.30	0.16 ± 0.11

Figure captions

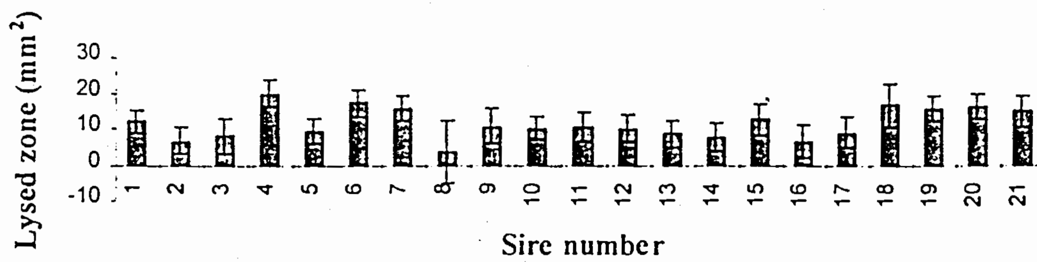
Fig. 1. The variation of least-squares means of half sib families of Nile tilapia. Standard errors are also shown. (a) Lysozyme activity at 30° C, (b) Lysozyme activity at 15° C and (c) SH activity at 30° C.



(a) Lysozyme activity at 30° C



(b) Lysozyme activity at 15° C



(c) SH activity at 30° C

Fig. 1 Chiayvareesajja et al.

Genetic associations of serum lysozyme and serum spontaneous haemolytic activities with survival and body weight in Nile tilapia *

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Abstract

The purpose of the study was to evaluate lysozyme activity and spontaneous haemolytic (SH) activity as possible indirect markers for selective breeding to improve the survival rate. Two parallel sets of families (Material I and Material II) from an on-going selection program for increased growth performance were evaluated for different traits. Growth and survival were recorded following 120 days grow-out period on individuals from 162 full-sib families within 91 paternal half-sibs (Material I). Individual from a random sample of the families in Material I (41 full-sib families within 20 paternal half-sib families; Material II) were evaluated for lysozyme activity and SH activity in blood serum. Significant negative correlations were found between least-squares means of the sib groups for lysozyme activity and survival rates for both half-sib groups (r = -0.53; p = 0.01) and full-sib groups (r = -0.32; p = 0.04). No significant correlations were found between SH activity and survival rate. The correlations between lytic activities and body weight at harvest were also not significant.

Keywords: Lysozyme activity; Spontaneous haemolytic activity; Genetic association; Indirect marker; Nile tilapia

1. Introduction

Survival rate is one of the economically important traits in aquaculture that has been considered to be included in breeding programs. A major source of mortality in aquaculture is infectious disease. The possibility to improve disease resistance by selective breeding is promising, since genetic variation has been shown in resistance to several diseases in various fish species (Gjedrem and Aulstad, 1974; McIntyre and Amend, 1978; Refstie, 1982; Standal and Gjerde, 1987; Gjedrem et al., 1991; Wolters and Johnson, 1994).

General survival rate, recorded as the proportion of surviving fish in sib groups in field tests, is a complex trait which may be affected by various pathogens as well as incidents not associated to diseases. Moreover, general survival rate shows low heritability (Gjedrem, 1985). Survival in relation to resistance against specific diseases may be measured in controlled challenge tests. This, however, requires sophisticated experimental facilities. Direct selection to improve disease resistance based on challenge testing will consequently be costly and laborious. Indirect selection based on more easily measured traits that are genetically correlated to disease resistance may then be an alternative. Important requirements for such correlated traits are that the heritability of the trait and the genetic correlation between the correlated trait and disease resistance are sufficiently high to obtain genetic progress in the survival rate. Recently, many possible candidate traits within the immune system, presumably related to disease resistance, have been studied (Fevolden et al., 1992; Fjalestad, 1993; Røed et al., 1992, 1993a, 1993b; Wiegertjes et al., 1995; Lund et al., 1995).

Non-specific immunological parameters such as serum lysozyme activity (Grinde et al., 1988; Røed et al., 1989; Lund et al.,1995; Chiayvareesajja et al.,1997) and serum spontaneous haemolytic (SH) activity (Røed et al., 1990, 1992, 1993a; Wiegertjes et al., 1993) have been shown to be genetically variable and to express some degree of associations with disease resistance.

Lysozyme is a lytic enzyme which has antibacterial activities by splitting the peptidoglycan component of the cell walls of micro-organisms, especially gram-positive bacteria, and therefore cause lysis of the cells (Chipman and Sharon, 1969). Several studies have confirmed the significant role of lysozyme action in the body's defence against infections in higher vertebrates (reviewed by Jollés and Jollés, 1984). In fish, indications of lysozyme as a defence mechanism against infectious diseases have been reported (Fänge et al., 1976; Murray and Fletcher, 1976; Lundblad et al., 1979; Lindsay, 1986; Grinde, 1989). At least one of two forms of lysozyme found in rainbow trout by Grinde (1989) has been shown to be able to lyse Vibrio anguillarum, Yersinia ruckeri and Aeromonas hydrophila which are important fish pathogens.

SH activity is one of the two related forms of haemolytic activity against heterologous red blood cells usually present in normal fish sera and being characterized by non-specific haemolytic activity (Nonaka et al., 1981). The other form is a specific (antibody-dependent) haemolytic activity. As in mammals, non-specific haemolytic activity of fish sera has been reported to occur as a result of the alternative pathway of the complement system (Ingram, 1980, 1987, 1990). The complement system is known to play a major role in bactericidal mechanisms and hence in host defence against pathogens.

Furthermore, both serum lysozyme activity and serum SH activity can be determined easily in blood samples drawn from live animals which make them potentially useful marker traits for indirect selection to improve disease resistance.

The present study is a part of an investigation to study the genetic variation for lysozyme activity and SH activity and to evaluate their potential use as genetic markers in indirect selection for the increased survival rate in Nile tilapia (Oreochromis niloticus). Nile tilapia is poised to become an internationally significant species for farming both in relatively small-scale aquaculture enterprises and in intensive aquaculture systems (Pullin, 1997). The

world production of tilapia in 1994 was about 600,000 tons with an estimated value of about 835 million US dollars (FAO, 1996). Nile tilapia has been chosen as a prominent model species for developing more productive and profitable aquaculture in developing countries by ICLARM's Aquaculture Program through the Genetic Improvement of Farmed Tilapias (GIFT) Project (Pullin et al., 1991; Eknath, 1993). The GIFT program has so far focused on selection for growth performance (body weight at the end of about 120 days grow-out period) in diverse tilapia farming systems as its primary breeding goal. Investigations on genetic parameters of other economically important traits such as age at maturation, survival, and resistance to diseases are being actively pursued for inclusion in a multi-trait selection program in the near future.

Chiayvareesajja et al. (1997) studied the genetic variation for lysozyme activity and SH activity based on the family material derived from the GIFT program. The estimated heritabilities of lysozyme activity at incubation temperature of 30 °C were high (0.7 based on both sire and dam components of variance). The heritabilities for SH activity were 0.03 and 0.71 based on sire and dam components of variance, respectively. The objective of the present study was to determine the genetic associations between lysozyme activity and SH activity and the survival rates in Nile tilapia. Since growth performance has been a primary focus in the GIFT program, the genetic association between lysozyme activity and SH activity and the growth performance were also investigated in this study.

2. Materials and Methods

Fish

The experimental fish came from the third generation of selection for increased growth performance under the auspices of the GIFT project being implemented at the research facilities of the National Freshwater Fisheries Technology Research Center, Bureau of Fisheries and Aquatic Resources (NFFTRC) and the adjacent Freshwater Aquaculture Center of the Central Luzon State University (FAC) in Muñoz, Philippines (Pullin et al., 1991; Eknath et al., 1991; Eknath, 1993).

Mating and rearing

A hierarchical mating design was used to produce the families with on average of two dams per sire. Before mating, male and female brood fish were conditioned separately in 3x3x1 m^3 hapas which were suspended in a fertilized earthen pond at a density of 10 fish per m^2 for two weeks. Mating was randomly made between one male brood fish and two female brood fish in 1 m^3 mating hapas installed together in 3000 m^2 fertilized earthen pond. One week after mating, all mating hapas were examined and swim-up fry were collected. Female brood fish which had spawned were removed from the hapas, and the different full-sib groups were kept in separate 1 m^3 fine mesh rearing hapas at a stocking density of 150 fry per m^3. The fry were reared in the fine mesh hapas for 21 days before being transferred to 1 m^3 larger mesh hapas, at a stocking density of 100 fish per m^3. The procedure was repeated at an interval of one week until 162 full-sib families within 91 half-sib families had been produced. After a rearing period of 10-20 weeks, the fingerlings were individually tagged with modified Floy fingerling tags.

Material I

After tagging, approximately equal numbers of individuals from each of the 162 full-sib families were communally stocked for grow-out testing in each of four different test environments. The fish in two of the test environments were lost because of flooding. The fish

in the third test environment were subjected to extensive tag loss (27 % of the 5716 fish stocked), while only 3.7 % of the fish died during the grow-out test. Since only tagged fish could be used to record the survival rate at harvest within the sib groups (i.e. tag loss was recorded as mortality), reliable estimates of survival could not be obtained from this environment due to excessive tag losses. Furthermore, the variation in mortality rate between sib groups in this test environment would be expected to be rather limited because of the low overall mortality rate. In the last test environment, an earthen pond at FAC, tag loss was about 10.9 % and mortality about 17.7% out of the 4689 fish stocked for grow-out testing. The relatively high mortality and the lower tag loss will increase the probability of detecting possible differences between the sib groups in survival rates. Tag loss will then have to be assumed randomly distributed across sib groups.

Consequently, only the records from the FAC test environment was used in the present study. During the culture period (13 October 1993 - 11 February 1994), the FAC pond was fertilized with chicken manure and inorganic fertilizer (16-20-0) at 62.5 kg and 6.25 kg respectively, on a weekly basis. In addition, the fish were fed commercial tilapia feed (30% crude protein) at 10% and 5% of the fish body weight on the first and second month respectively, and 3% on the third and fourth month. At harvest, survival rate within each family, individual body weight and sex were recorded.

Material II

In a separate experiment to determine lysozyme activity and SH activity, 388 individually tagged fish from full-sib representatives of about 25% of the families in Material I (41 full-sib groups within 20 paternal half-sib groups; selected randomly) were used. The fish were communally reared in a separate 1200 m² earthen pond at NFFTRC which was

fertilized with inorganic fertilizer (46-0-0) at a rate of 10 kg per week from November 1993. In March 1994, blood serum samples were collected from all 388 individuals for analysis of lysozyme activity and SH activity, and body weight, sex and stage of female sexual maturation were recorded. Blood samples were collected from the caudal vein of the anaesthetized fish using vacutainer tubes. Blood was allowed to clot at room temperature for 3 hours and the sera obtained by centrifugation were frozen at -70° C until analysis.

Assays of lytic activity

Serum lysozyme activity was assessed as described for rainbow trout by Roed et al. (1993b) using a Micrococcus lysoplate assay developed by Osserman and Lawlor (1966), and modified Gram-staining preservation technique developed by Lie et al. (1986). Micrococcus lysodeikticus 50 μg ml^{-1} in 1% agarose gel were used. Wells in the agarose gel were filled with the diluted blood sera from Nile tilapia and incubated at 30° C for 20 h (Chiayvareesajja et al., 1997). After the incubation, the gels were developed with methyl violet and Lugol's solutions, and the unstained lysed zones were measured.

The spontaneous haemolysis (SH) of sheep red blood cells (SRBC) caused by blood serum of Nile tilapia was assayed as described for Atlantic salmon by Roed et al.(1993a), using the agarose plate technique of Lachman and Hobart (1979). Nile tilapia blood sera were placed in sample wells in agarose gel containing 0.25% packed of SRBC and incubated at 30° C for 20 h. Further details are given by Chiayvareesajja et al. (1997).

The lysozyme and SH activities were measured as the diameter of the lysed zones and expressed as the area of the lysed zones.

Statistical analysis

Serum Lytic activities

Least-squares means of the serum lytic activities in each of the full- and half-sib families were obtained from Material II, analyzing with the standard general linear model (GLM) procedure of SAS statistical package (SAS Institute Inc., 1988), taking into account the day of analysis and sex/stage of sexual maturation as fixed effects and body weight at blood sampling as covariate. Further details about the statistical analysis is given by Chiayvareesajja et al. (1997).

Survival rate

Genetic correlations based on individual records from Material II of survival and serum lytic activities could not be obtained, since serum could only be collected from surviving fish. However, survival rates in parallel samples of the sib groups in Material II could be obtained from Material I as the ratio of number of fish with tags at harvest to number of fish at stocking in each sib group. The survival score for fish that were not recovered or had lost their tags at harvest was coded as 0, while surviving and tagged fish at harvest were coded as 1. The recorded survival rates were likely to be biased downwards because of untagged fish being scored as dead (coded as 0). However, tag loss was assumed to occur at random across sib groups.

A preliminary regression analysis revealed significant effect of age at stocking on survival rate (b = 0.0033). Therefore, the individual survival score was precorrected for age at stocking using the following formula:

$$\hat{Y} = Y + (\mu_a - a) \cdot b \qquad \text{Model I}$$

where

$\hat{Y}$ = the corrected survival score of individual fish

Y = survival score of individual fish

μ_a = grand mean of age at stocking

a = age at stocking of individual fish

b = regression coefficient of survival score on age at stocking

Body weight

Body weight at harvest in Material I was found to be significantly affected by sex and age at stocking. Mean body weight of males was 140.81 grams and of females was 95.73 grams, and the standard deviation within each sex was affected by the mean (56.75 grams and 32.70 grams respectively). Individual body weights were therefore precorrected for sex by multiplying with the ratio between the grand mean body weight and the mean body weight of each sex, to standardize both the mean and the standard deviation across sexes. The regression coefficient of sex corrected body weights on age at stocking was 1.01 grams. Precorrected body weights for sex and age at stocking were computed according to the following formula:

$$\dot{Y} = [Y \cdot (Y_g / Y_s)] + (\mu_a - a) \cdot b \qquad \text{Model II}$$

where

$\dot{Y}$ = the corrected body weight at harvest of individual fish

Y = body weight at harvest of individual fish

Y_g = grand mean of the body weight at harvest

Y_s = mean body weight at harvest of each sex (male and female)

μ_a = grand mean of age at stocking

a = age at stocking of individual fish

b = regression coefficient of sex corrected body weight at harvest on age at stocking.

Family least-squares means

The least-squares means of survival and body weight at harvest of each family were analyzed according to the GLM procedures by SAS statistical package (SAS Institute Inc., 1988). The following model was used.

$$Y_{ijk} = \mu + \text{sire}_i + \text{dam}_{ij} + e_{ijk} \qquad \text{Model III}$$

where

Y_{ijk}	=	precorrected survival score or body weight at harvest of the *k*th fish
μ	=	overall mean
sire_i	=	random effect of *i*th sire (i = 1, 2,..., 91)
dam_{ij}	=	random effect of *j*th dam (j = 1, 2) within *i*th sire
e_{ijk}	=	random residual effect.

The sources of variation of individual body weight at blood sampling were also investigated from Material II according to the GLM procedure of the SAS. It took into account sex and stage of female sexual maturation as fixed-effects and the random effects of sire and dam nested within sire. However, the sire and dam components of variance were not significant in this material, and genetic correlations between lytic activities and body weight based on individual observations from Material II should consequently not be estimated.

Pearson's correlation coefficients were estimated between the least-squares means of serum lysozyme activity or SH activity (Material II) and body weight at harvest or survival rates of parallel half- and full-sib groups in Material I. The correlation coefficients between least-squares means in parallel samples from the same set of sib groups may be biased compared to the true genetic correlation because of other sources of covariance than additive genetic effects, such as common environmental effects during rearing until tagging, maternal effects and non-additive genetic effects. Full-sib group correlations are expected to be much

more sensitive to such errors than half-sib group correlations. The least-squares means may also be affected by sampling errors compared to the true genetic values of the sib groups. The sample sizes in the present study (about 30 fish with survival records and 20 fish with body weight records per full-sib group in Material I and about 9 fish with records of serum lytic activities per full-sib group in Material II) were probably sufficient to prevent large sampling errors.

3. Results

The mean values of half-sib least-squares means of body weight at harvest and survival rates from Material I and serum lytic activities from Material II are presented in Table 1.

Correlation coefficients between least-squares means of half- and full-sib groups for serum lysozyme activity, serum SH activity, body weight at harvest and survival rate of the parallel half- and full-sib groups, are presented in Table 2. Significant negative correlations were estimated between serum lysozyme activity and survival rate in both half-sib groups ($r = -0.53$; $p = 0.01$) and full-sib groups ($r = -0.32$; $p = 0.04$). The correlations between SH activity and survival rate or body weight at harvest were found non-significant both for half-sib and full-sib groups. No significant correlations were found between survival rate and body weight at harvest of half-sib or full-sib groups.

4. Discussion

In a previous study (Chiayvareesajja et al., 1997), the heritability of serum lysozyme activity was estimated to about 0.6 at 30° C incubation temperature, based on the same fish as

Material II in the present study. The result suggests that there is adequate additive genetic variation in this trait to be utilized in selection. On the other hand, the estimated heritability of serum SH activity was not significantly different from zero.

In the present study, no significant correlations were found between least-squares means of serum SH activity and survival rate in two parallel sets of samples of sib groups, neither for half-sib nor full-sib families. This finding is in agreement with the observation of Lund et al. (1995), who reported no significant correlation between haemolytic activity and survival rate of Atlantic salmon in challenge tests with Aeromonas salmonicida (causing furunculosis), Renibacterium salmoninarum (causing Bacterial kidney disease, BKD) and Vibrio salmonicida (causing cold-water vibriosis).

Based on the present and previous results (zero heritability of serum SH activity and no significant genetic association with survival), serum SH activity will be a poor marker trait for indirect selection for increased survival rate in Nile tilapia.

Similar conclusions have been drawn from experiments with Atlantic salmon (Lund et al., 1995) and rainbow trout (Hollebecq et al., 1995). Roed et al. (1992, 1993a), however, reported significant additive genetic variation of complement activation in Atlantic salmon and evidence for a positive genetic association between serum SH activity and survival during a vibriosis infection. Chiayvareesajja et al. (1997) detected considerable variation in serum SH activity between full-sib groups of Nile tilapia suggesting a possible non-additive genetic variation in the trait. The possibility of genetic associations between serum SH activity and survival rate should consequently not yet be discarded.

The significant negative correlation coefficients between least-squares means of parallel sib groups for serum lysozyme activity and survival rate in Nile tilapia, as shown in Fig. 1, agrees well with previously reported results from Atlantic salmon (Lund et al., 1995). In the present study, the correlation coefficient based on least-squares means of full-sib

groups was moderate (r = -0.32, p = 0.04), and rather high when based on least-squares means of half-sib groups (r = -0.53, p = 0.01). Lund et al. (1995) detected a significant negative genetic association between serum lysozyme activity and survival of Atlantic salmon in challenge tests with A. salmonicida, R. salmoninarum and V. salmonicida, ranging from -0.31 to -0.25. Fevolden et al. (1994) reported negative genetic correlations between serum lysozyme activity in stressed Atlantic salmon and survival in challenge tests with furunculosis and BKD. A negative phenotypic correlation was also reported by Røed et al. (1993a) between serum lysozyme activity of Atlantic salmon and survival in a challenge test with vibriosis.

Fevolden et al. (1994) concluded that an apparent negative genetic correlation between post-stress lysozyme activity and disease resistance should not be interpreted as indicating superior immunological status of the fish with low serum lysozyme activity but rather that low stress tolerance may result in high serum lysozyme levels as well as increased susceptibility to disease. This conclusion is in accord with the implication of Lund et al. (1995), suggesting that the negative genetic association between survival rates and serum lysozyme activity in Atlantic salmon was probably due to a previous infection with furunculosis causing a prolonged stimulation of serum lysozyme activity in the families with a poorer ability to kill and eliminate the pathogens. Consequently, the families with high serum lysozyme activity were more susceptible to diseases. This is quite contrary to the most obvious biological hypothesis that high serum lysozyme activity would create a hostile environment for invading pathogens, and consequently result in reduced mortality rates.

In the present study, high serum lysozyme activity was also found to be genetically associated with low survival rate, though the fish were previously exposed to neither a controlled stress condition nor disease challenge. However, the increased serum lysozyme activity in the poor surviving sib groups may be caused by a prolonged exposure of lysozyme producing cells to invading pathogens in families with a poor second-line immune defence, as

suggested by Lund et al. (1995), or possibly by a poorer outer physical defence (skin, mucus, mucous membranes) resulting in an increased stimulation of serum lysozyme activity and a heavier infections load at the same time. No data were collected in the present study that might support either of these hypotheses.

A preliminary analysis of variance of individual body weight at blood sampling in Material II revealed no significant effect of the sire component of variance. However, significant effects were found of both the sire and the dam within sire component of variance on body weight at harvest in Material I. The estimated correlation between least-squares means of parallel sib groups of body weight at harvest and the serum lytic activities as well as survival rate were non-significant. According to this, selection for increased growth performance is not expected to result in correlated response in serum lysozyme activity or SH activity.

The high heritability of serum lysozyme activity estimated previously (Chiayvareesajja et al., 1997), together with the present significant negative genetic association between this trait and survival rate suggest that serum lysozyme activity may be a promising candidate trait for indirect selection to improve survival rate in Nile tilapia. According to the negative association between the trait and survival rate, broodstock should be selected among individuals and families with low serum lysozyme activity.

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6. References

Chiayvareesajja, J., Røed, K. H., Danting , J. C., De Vera , M. P., and Bentsen, H. B., 1997. Genetic variation in lysozyme and spontaneous haemolytic activities of blood serum from Nile tilapia. (Submitted to Aquaculture).

Chipman, D.M. and Sharon, N., 1969. Mechanism of lysozyme action. Science, 165: 454-465.

Eknath, A.E., 1993. Genetic improvement of farmed tilapias. Final Report to ADB and UNDP from International Center for Living Aquatic Resources Management (ICLARM), Manila, Philippines.

Eknath, A.E., Bentsen, H.B., Gjerde, B., Tayamen, M.M., Abella, T.A., Gjedrem, T. and Pullin, R.S.V., 1991. Approaches to national fish breeding programs: Pointers from a tilapia pilot study. NAGA, The ICLARM Quarterly, pp. 10-12.

FAO, 1996. Aquaculture production statistics. FAO Fisheries Circular No.815, Revision 8, Rome, Italy. 189 pp.

Fevolden, S. E., Refstie, T. and Røed, K. H., 1992. Disease resistance in rainbow trout (Oncorhynchus mykiss) selected for stress response. Aquaculture, 104: 19-29.

Fevolden, S. E., Røed, K. H. and Gjerde, B., 1994. Genetic components of post-stress cortisol and lysozyme activity in Atlantic salmon: correlations to disease resistance. Fish &Shellfish Immunol., 4: 507-519.

Fjalestad, K.T., 1993. Studies of variation in disease resistance and immune parameters in Atlantic salmon (Salmo salar). Doctor Scientiarum Thesis, Department of Animal Science, Agricultural University of Norway, Ås.

Fänge, R., Lundblad, G. and Lind, J., 1976. Lysozyme and chitinase in blood and lymphomyeloid tissues of marine fish. Mar. Biol., 36: 277-282.

Gjedrem, T., 1985. Improvement of productivity through breeding schemes. Geo. Journal, 10.3: 233-241.

Gjedrem, T. and Aulstad, D., 1974. Differences in resistance to vibrio disease of salmon parr (Salmo salar). Aquaculture, 3: 51-59.

Gjedrem, T., Salte, R. and Gjøen, H.M., 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. Aquaculture, 97: 1-6.

Grinde, B., 1989. Lysozyme from rainbow trout, Salmo gairdneri Richardson, as an antibacterial agent against fish pathogens. J. Fish Dis., 12: 95-104.

Grinde, B., Lie, Ø., Poppe, T.T. and Salte, R., 1988. Species and individual variation in lysozyme activity in fish of interest in aquaculture. Aquaculture, 68: 299-304.

Hollebecq, M.-G., Faivre, B., Bourmaud, C. and Michel, C., 1995. Spontaneous bactericidal and complement activities in serum of rainbow trout (Oncorhynchus mykiss) genetically selected for resistance or susceptibility to furunculosis. Fish & Shellfish Immunol., 5: 407-426.

Ingram, G.A., 1980. Substances involved in the natural resistance of fish to infection - A review. J. Fish Biol., 16: 23-60.

Ingram, G.A., 1987. Haemolytic activity in the serum of brown trout, Salmo trutta L. J. Fish Biol., 31: 9-17.

Ingram, G.A., 1990. Complement-fixation test. In: J.S. Stolen, T.C. Fletcher, D.P. Anderson, B.S. Roberson and W.B. van Muiswinkel (Editors), Techniques in Fish Immunology. SOS Publications, Fair Haven, USA, pp. 25-44.

Jollés, P. and Jollés, J., 1984. What's new in lysozyme research? Review. Mol. Cell. Biochem., 63: 165-189.

Lachman, P.J. and Hobart, M.J., 1979. Complement technology. In: D.M. Weir (Editor), Handbook of Experimental Immunology. Vol. 1 Immunochemistry. Blackwell Scientific Publications, Oxford. pp. 5A.1-5A.23.

Lie, Ø., Syed, M. and Solbu, H., 1986. Improved agar plate assays of bovine lysozyme and haemolytic complement activity. Acta Vet. Scan., 27: 23-32.

Lindsay, G.I.H., 1986. The significance of chitinolytic enzymes and lysozyme in rainbow trout (Salmo gairdneri) defence. Aquaculture, 51: 169-173.

Lund, T., Gjedrem, T., Bentsen, H.B., Eide, D.M., Larsen, H.J.S. and Røed, K.H., 1995. Genetic variation in immune parameters and associations to survival in Atlantic salmon. J. Fish Biol., 46: 748-758.

Lundblad, G., Fänge, R., Slettengren, K. and Lind, J., 1979. Lysozyme chitinase and exo-N-acetyl- β(beta)-D-glucosaminidase (NAGase) in lymphomyeloid tissue of marine fishes. Mar. Biol., 53: 311-315.

McIntyre, J.D. and Amend, D.F., 1978. Heritability of tolerance for infectious hematopoietic necrosis virus in sockeye salmon (Oncorhynchus nerka). Trans. Am. Fish. Soc., 107: 305-308.

Murray, C.K. and Fletcher, T.C., 1976. The immunohistochemical location of lysozyme in plaice (Pleuronectes platessa) tissue. J. Fish Biol., 9: 329-334.

Nonaka, M., Yamaguchi, N., Natsuume-Sakai, S. and Takakashi, M., 1981. The complement system of rainbow trout (Salmo gairdneri). J. Immunol., 126: 1489-1494.

Osserman, E.F. and Lawlor, D.P., 1966. Serum and urinary lysozyme (muramidase) in monocytic and monomyelocytic leukemia. J. Exp. Med., 124: 921-951.

Pullin, R.S.V., 1997. World tilapia culture and it's future prospects. In: R.S.V. Pullin, J. Lazard, M. Legendre, J.B. Amon Kothias and D. Pauly (Editors), The Third International

Pullin, R.S.V., Eknath, A.E., Gjedrem, T., Tayamen, M.M., Macaranas, J.M. and Abella, T.A., 1991. The genetic improvement of farmed tilapias (GIFT) project: the story so far. NAGA, The ICLARM Quarterly (April). pp. 3-6.

Refstie, T., 1982. Preliminary results: differences between rainbow trout families in resistance against vibriosis and stress. Dev. Comp. Immunol., Suppl., 2: 205-209.

Røed, K.H., Fjalestad, K.T. and Strømsheim, A., 1993a. Genetic variation in lysozyme activity and spontaneous haemolytic activity in Atlantic salmon (Salmo salar). Aquaculture, 114: 19-31.

Roed, K.H., Brun, E., Larsen, H.J. and Refstie, T., 1990. The genetic influence on serum haemolytic activity in rainbow trout. Aquaculture, 85: 109-117.

Roed, K.H., Fjalestad, K., Larsen, H.J. and Midthjel, L., 1992. Genetic variation in haemolytic activity in Atlantic salmon (Salmo salar L.). J. Fish Biol., 40: 739-750.

Roed, K.H., Larsen, H.J.S., Linder, R.D. and Refstie, T., 1989. The genetic influence on natural immunity in rainbow trout. Anim. Gen. (Suppl. 1): 54.

Roed, K.H., Larsen, H.J.S., Linder, R.D. and Refstie, T., 1993b. Genetic variation in lysozyme activity in rainbow trout (Oncorhynchus mykiss). Aquaculture, 109: 237-244.

SAS Institute Inc., 1988. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute Inc., Cary, NC. 1028 pp.

Standal, M. and Gjerde, B., 1987. Genetic variation in survival of Atlantic salmon during the sea-rearing period. Aquaculture, 66: 197-207.

Wiegertjes, G.F., Yano, T. and van Muiswinkel, W.B., 1993. Estimation of the genetic variation in complement activity of common carp (Cyprinus carpio L.). Vet. Immunol. Immunopathol., 37: 309-319.

Wiegertjes, G.F., Stet, R.J.M. and van Muiswinkel, W.B., 1995. Divergent selection for antibody production to produce standard carp (Cyprinus carpio L.) lines for the study of disease resistance in fish. Aquaculture, 137: 257-262.

Wolters, W.R. and Johnson, M.R., 1994. Enteric septicemia resistance in blue catfish and three channel catfish strains. J. Aquat. Anim. Health, 6: 329-334.

Table 1

Means and standard deviations of least-squares means of half-sib groups of Nile tilapia for serum lysozyme activity and serum SH activity (Material II), and body weight at harvest and survival rate (Material I).

Trait	Mean	Standard deviation	Minimum	Maximum
Lysozyme (mm^2)	223.40	15.59	184.74	246.29
SH (mm^2)	11.58	4.21	3.91	19.86
Body weight (g)	111.93	12.93	88.85	138.29
Survival rate	0.72	0.09	0.57	0.89

Table 2

Pearson's correlation coefficients between least-squares means of serum lysozyme activity, serum SH activity, body weight at harvest, and survival rate of parallel half-sib groups (above the diagonal) and full-sib groups (below the diagonal) of Nile tilapia.

	Lysozyme	SH	Body weight	Survival rate
Lysozyme		-0.23	-0.03	-0.53*
SH	-0.06		-0.05	0.34
Body weight	-0.02	-0.21		0.16
Survival rate	-0.32*	0.25	-0.03	

*$P < 0.05$

Figure captions

Fig. 1. Correlation between the least-squares means of parallel half-sib groups of Nile tilapia for serum lysozyme activity and survival rate.

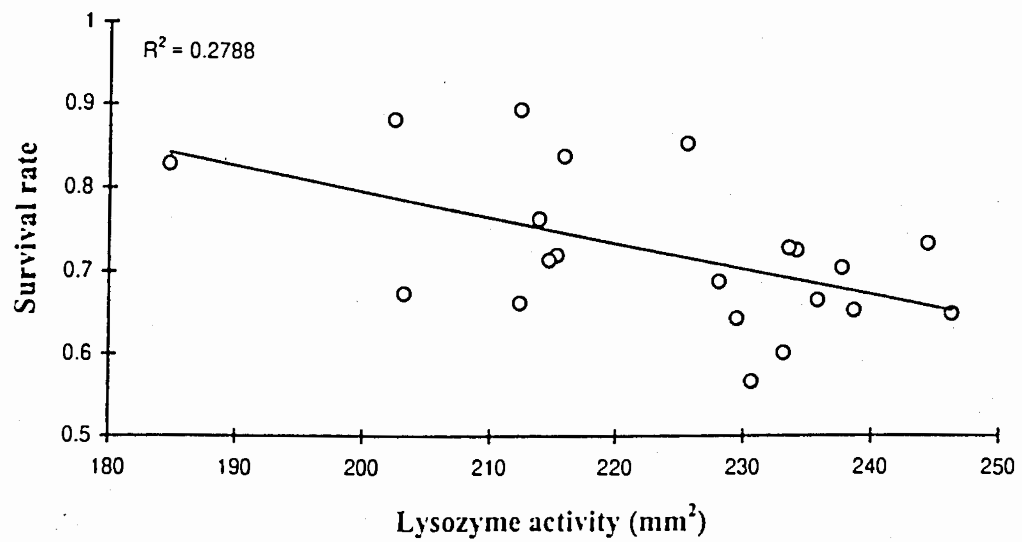

Fig. 1 Chiayvareesajja et al.

Attachment 9.

Draft manuscript on "Combined effects of genetic improvement through selection and sex reversal on growth and survival of Nile tilapia (*Oreochromis niloticus*)".

COMBINED EFFECTS OF GENETIC IMPROVEMENT THROUGH SELECTION AND SEX REVERSAL ON SURVIVAL AND GROWTH OF NILE TILAPIA *(Oreochromis niloticus)**

Ambekar Eknath
Felicisima Longalong

Introduction

To capitalize on the faster growth of males over females, progressive tilapia culture systems grow an all-male population. The most widely used methods include hormone sex reversal, hybridization and a combination of both.

The primary objective of this study was to determine the combined effects of genetic selection for improved growth and sex reversal in Nile tilapia by comparing the growth performance of a genetically improved strain with that of a commercially farmers strain. The secondary objective were to determine the efficiency of sex reversal and subsequent growth performance of the two strain treated with a 17-α methyltestosterone obtained from two different sources; and the method of hormone administration, either hapas-in-tanks or hapas-in-pond.

Summary of Methods

Two strains were used in this study: GIFT and Israel. The experiment was conducted in two consecutive phases.

In Phase I, the swim-up fry of GIFT and Israel strains were fed diets with hormone for 30 days. The amount of feeds were adjusted weekly. Feeds were given at the rate of 30%, 25% and 20% of the body weight during the first second third/fourth weeks, respectively. The control groups with each strain were give feeds without hormones. Altogether, there were twelve treatment groups (replicate 3x). For the GIFT strain, the different treatment groups and the codes are:

Controls: fry fed control diets in tanks (GCT) and in ponds (GCP),
Sex reversal in pond: fry fed MT from Argent (GAT) and Sigma (GST)
Sex reversal in ponds: fry fed MT from Argent (GAP) and Sigma (GSP).
Similarly, for the Israel strain the six groups were ICT, ICP, IAT, IST, IAP and ISP respectively.

Fry from triplicate hapas of each treatment group were pooled together at the end of 30 days and transferred to 1 m^3. Samples of about 50 individuals from each treatment were dissected for sex determination using the aceto-carmine gonad squash technique.

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* Papers presented during the Second International Workshop on Genetics in Aquaculture and Fisheries Management in Asia, Bangkok, Thailand, 7-11 November 1994.

Phase II

A total of 2400 tagged individuals were communally stocked in two 600 m^2 earthen ponds (100 individuals per group per pond). The ponds were fertilized weekly with NPK (16-2-0) and chicken manure at the rate of 100 kg/ha/mo, and 3000 kg/ha/mo, respectively. The fish were harvested after a grow-out period of 120 days and their individual body weights recorded.

Summary of Results:

Phase I

The gonad squash technique adopted in the experiment showed a sex-ratio (M:F) of 1:0, regardless of the source of 17-α MT and treatment conditions (hapas in ponds or hapas in tanks). The sex ratio in control group was 2:1 in both GIFT and Israel, respectively.

The survival across all treatment groups and strains was lower than expected (50%). Between fry treated in ponds and tanks, across strains, survival of fry was better in ponds (about 60%) than those treated in tanks (about 43%). Between strains and source of MT (MT-sigma) in tanks, the survival of IST was better than GST, while in ponds, the trend was reversed. The GIFT fry after 30 day treatment period were significantly ($p<0.01$) heavier than Israel fry.

Phase II

Survival and Sex ratio

The tag losses across all treatment groups and replicates was 26% (Table 1). Across treatments, the survival of GIFT strain was better than the Israel strain. The percentage of males in treated groups, across two strains, ranged from 65% to 99%. In the control groups, the mean percentages of males were similar.

General trends in Growth

The LSM of body weight of fish without tags was intermediate between LSM values across treatments for GIFT and Israel (Table 1). Among the treatment groups in the GIFT strain, the group GAT was significantly heavier ($p<0.05$) than the other five groups, which did not differ from each other. For the Israel strain, the IST group registered the highest LSM value followed by IAT<ISP and the rest. Between GIFT and Israel, each of the six treatment groups in GIFT were significantly heavier ($p<0.05$) than the six groups in Israel, except from the group IST, which showed the LSM value similar to GCT and GSP.

Effects of Source of MT on Growth

For the GIFT fry treated in pond, the difference (+8g) between LSM of body weights of GSP and GAP was not significant, while for Israel strain, the difference (-12g) between IST and IAT was highly significant ($p<0.05$). The differential effect of source of MT on strain performance was substantial ($p<0.05$) when sex reversal was done in tanks. The difference of LSM of body weight of the groups treated with MT-Argent and MT-Sigma, were +43g and -17g, respectively. Overall, this result indicates significant interaction between strain and sources of MT.

Effects of Genetic Improvement and Sex Reversal

The combined effects of genetic improvement and sex reversal, however, depend on the method of hormone treatment (in pond or in tanks) Table 2 In pond the effect of sex reversal was only marginal (69.5 -64=5.5g) while in tanks the effect was substantial (94.5-64=+30.5g). Within the GIFT strain, the difference in LSM body weights between group sex reversed was +35 g. Similar trends were observed in the Israel strain. The difference between LSM of body weights of group sex reversed in pond or in tanks, and the corresponding control groups were +11.5g and +40g, respectively.

Table 1. Phase II: Mean weights (g) at stocking (Wi), number of individuals harvested, sex ratio, percentage of males, percentage survival, least square means of body weights (GLM, model 2) at harvest (g) of different treatment groups. The standard errors of LSM ranged from 6.7 to 8.0.

	TREATMENT	**Wi**	**N**	**Males**	**Females**	**Male (%)**	**Survival (%)**	**LSM***
GIFT	GCP	3.4^{b}	115	83	32	72	58	294^{a}
	GCT	4.1^{ab}	116	83	33	72	58	283^{a}
	GAP	2.8^{b}	89	88	1	99	45	298^{a}
	GSP	3.7^{b}	103	100	3	97	52	289^{a}
	GAT	4.9^{a}	127	126	1	99	64	335^{b}
	GST	4.7^{a}	126	125	1	99	63	302^{a}
ISRAEL	ICP	3.6^{b}	94	74	20	79	47	223^{a}
	ICT	3.1^{b}	72	49	23	68	36	226^{a}
	IAP	3.0^{b}	71	68	3	96	36	230^{a}
	ISP	3.0^{b}	85	81	4	95	43	242^{ab}
	IAT	5.4^{a}	95	92	3	97	48	256^{a}
	IST	5.0^{a}	92	91	1	99	46	272^{c}
Total	GIFT		676				56	301
	ISRAEL		509				42	243
Fish with-out tags			425	381		90	-	284
Tag loss (%)	26							

* Columns with the same superscript within strains GIFT and ISRAEL are significantly different ($p<0.01$).

Table 2. Phase II: Matrix of differences in LSM of final body weights between corresponding treatment groups to determine the effects of genetic improvement; Effect of sex reversal either in pond and tanks; and the combined effects of genetic improvement and sex reversal. All non-zero values are significantly at <0.01.

		GIFT	Israel	GIFT + Sex Reversal		Israel + Sex Reversal	
		A	B	C1	C2	D1	D2
A	GIFT	-					
B	Israel	-64.0	-				
GIFT + Sex Reversal							
C1	a. Pond	0	+69.5	-			
C2	b. Tanks	+35.0	+94.5	-25.0			
Israel Sex Reversal							
D1	a. Pond	-53.0	+11.5	-59.0	-83.0		
D2	b. Tanks	-24.5	+40.0	-29.5	-54.5	-28.5	-

Attachment 10.

Complementary studies published under FAC/CLSU research component of GIFT

GROWTH PERFORMANCE OF GIFT STRAIN OF NILE TILAPIA (*Oreochromis niloticus*) IN RICE-FISH ENVIRONMENTS

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ABSTRACT

This paper presents growth performance trials for an improved strain of Nile tilapia (GIFT strain) in communal and separate rearing rice-fish environments from February 16, 1993 to January 08, 1997. The GIFT strain was developed from a genetically mixed-based population by combining the best performing genetic groups from eight diverse strains and their crosses. A combined-family selection program for growth performance using GIFT strain was initiated in the Philippines in 1990.

The growth trials compared the GIFT strain with the widely used commercial Nile tilapia strains in the Philippines and a recently imported strain from Sénégal. The Sénégal strain performed relatively better than 8 other test strains in rice-fish systems during an earlier study to estimate the magnitude of genotype x environment interaction in 11 different tilapia-farming systems.

Overall results showed that GIFT strain had significantly better growth performance compared with the Philippine strains of Nile tilapia. GIFT strain survival, rates improved (from 46% to more than 60%) over the years, and were comparable with if not significantly higher than the other test strains. During the early trials, the Sénégal strain had the best growth rate but had the lowest survival rate, In later trials however, the Sénégal strain had high survival rate (71% in 1995) but its growth was not comparable to the GIFT strain (63% survival). Results from these trials indicate that the GIFT strain is a promising strain for culture in rice-fish systems.

INTRODUCTION

There are about 47 million ha of irrigated rice fields in Asia (Dela Cruz et al., 1992) but only a very small portion of this area is used for rice-fish culture. Adoption of rice-fish culture technology has remained poor. Despite improvements in the culture techniques and availability of insecticides that do not harm the fish, fish productivity in rice fields has remained low. A major technical problem that constrains wider adoption of rice-fish culture is the small size of fish at harvest due mainly to short rearing period in paddies planted to high-yielding rice varieties. One promising way of extending the fish culture period in rice paddies is through the construction of a 'pond refuge' (Dela Cruz 1990). A pond refuge is a small earthen pond dug at one end of the rice paddies, usually about 10% of the paddy area. Results from extensive on-station and on-farm experiments (Dela Cruz and Sevilleja, 1990), however, have so far shown only marginal increase in fish production (Israel, et al., 1994). At present, rice-fish culture is almost non-existent in the Philippines.

Substantial increase in fish production in rice fields is possible if genetic selection works quite well in farmed species. Even if you obtain only a meager response to selection from each breeding cycle, this would mean sustained fish production in the future. Development of fish strain that can grow to acceptable market sizes within a short

period under normal rice-fish culture techniques, could lead to wider adoption of rice-fish culture technology. This paper focused on the evaluation on the comparative growth and yield potential of the GIFT strain of Nile tilapia under rice-fish farming system.

MATERIALS AND METHODS

Experimental design

Test strains

The *GIFT strain* was developed by combining the best performing genetic groups, based on their additive – genetic performance, from eight diverse Nile tilapia strains (including Sénégal and Israel) and their crosses. A combined family selection for growth performance using the GIFT strain was initiated in 1990. Progeny selected from the first generation selection were used in this study.

Sénégal strain

Sénégal strain: Progeny of founder stocks collected in 1988 from Sénégal.

Israel strain

Israel strain is one of the most widely used in the Philippines. Fingerlings were obtained from the Bureau of Fisheries and Aquatic Resources Tilapia Production Complex in Muñoz, Nueva Ecija, Philippines.

Thai strain

The *Thai strain* performed best from among Philippine strains in recent trials. Fingerlings were obtained from the Bureau of Fisheries and Aquatic Resources Tilapia Production Complex in Muñoz.

Field preparations, water quality analyses

We conducted all trials at the Rice-Fish Experiment area of the Freshwater Aquaculture Center of Central Luzon State University. Field preparations in all trials followed the standard procedures in Sevilleja (1992). Transplanting of rice seedlings adapted straight-line method, 20-25 cm spacing. Application of the recommended fertilizer (90-30-30 NPK) for the experimental site followed split applications. Half of the fertilizer was applied during the final harrowing or just after rice transplanting together with the granular insecticide (Furadan 3G) at 17 kg/ha, the other half as topdress fertilizer. In each trial, rice normally received one insecticide application throughout the culture period. We usually stock the experimental fish one week after rice transplanting for small paddies. For the large paddy, we stocked fish even before rice transplanting and they remained confined in the pond refuge during plowing and harrowing. Water level, dissolved oxygen (DO), and temperature were monitored daily while total ammonia-nitrogen (TAN) and pH, every two weeks (APHA 1980). Secchi disc visibility (SVD) and alkalinity were monitored every two weeks in 1996-1997 trials.

In 1993 trial, eighteen 200 m^2 rice paddies with pond refuge each were used, twelve paddies for separate rearing and six for communal rearing. Both communal and separate rearing trials used the 3 test strains. In separate rearing, 100 individuals of each strain were stocked in 4 replicated rice paddies laid-out in a Randomized Complete Block Design (Snedecor and Cochran 1976). In communal rearing, six 200 m^2 paddies were stocked with 33 individuals of each strain to bring the stocking density to 100 per paddy. Fish in three communal paddies were fed during the culture period with the

commercial tilapia diet (30% crude protein) at 7% of fish body weight/day. The fish were reared for 90 days (February to May 1993).

In 1995 trial, we included the Thai strain (performed best in recent trials) in the three 1993 test strain, and were tested in the same separate rearing units used in 1993 trial but without feeding. We stocked 100 fish in each 200 m^2 paddy on June 1, and harvested them on August 15, 1995.

In 1996, growth performance trials for 200 selected GIFT families were conducted in 6,000 m^2 rice paddies with 750 m^2 pond refuge. We stocked the family test fish on February 2 at $0.5/m^2$, and fed with commercial tilapia diet (30% CP) at 7% per day during the first half of the culture period and 5% during the later culture period. The communal rearing trial of GIFT and THAI strains in this paddy followed. Details of the family testing procedures and results are dealt with in a separate paper by the GIFT project staff. In this communal rearing trial, we stocked 3,000 each strain with 8.13g and 7.31g for the GIFT and THAI strains, respectively. The fish were stocked on August 27, 1996, and were given commercial tilapia feed twice daily with 27% crude protein at 5% of their body weight per day. During the second half of the culture period, feeding was at 3% of their body weight per day. We harvested the fish on January 08, 1997.

Data analysis:

Fish body weights at harvest for each environment was analyzed using the Generalized Linear Models Procedure of the Statistical Analysis System (SAS Institute Inc., 1990) for the following models:

Separate Rearing (model 1):

$$Y_{ijkl} = a + R_i + G_j + S_k + R_i*G_j + R_i*S_k + G_j*S_k + e_{ijkl}$$

Communal Rearing (model 2):

$$Y_{ijklm} = a + R_i + G_j + S_k + F_l + F_l*G_j + S_k*G_j + R_i*G_j + e_{ijklm}$$

Where:

Y_{ijkl} is the body weight at harvest of the 1st individual (model 1),
Y_{ijklm} is the body weight at harvest of the mth individual (model 2),
a is constant
R_i is the fixed effect on the ith replicate pond (i=1 to 3 in model 1; and 1 to 4 in model 2),
G_j is the fixed effect of the jth strain (j=Gift, Israel, Sénégal; Thai included in 1995 trial),
S_k ^{is} the fixed effect of the kth sex (Female or Male)
F_j is the fixed effect of the 1th culture management (with or without feeding, model 2),
e_{ijkl} is a random error for the 1th individual (model 1).
e_{ijklm} is a random error for the mth individual (model 2).

An asterisk (*) between two effects is interaction effect in each model. After the first run of each model in the GLM procedure, insignificant effects were removed from the model statement and a second run was performed for all significant effects in the model. The communal rearing in 1997 involved strain, sex and their interaction in the model, as only one pond was used in this trial.

RESULTS

Communal and separate rearing trails in 1993

Growth and survival

The marginal mean squares (MS) for all significant effects in models 1 and 2 are presented in Table 1. In separate rearing, block or pond replicate effect was high. This was due to significant differences in water depth between replicates. The magnitude of sex effects was about twice that of the strain effect. All other effects were low in magnitude. In communal rearing, feeding had the greatest effect (72% of the MS) followed by sex (20%). The effect of strain was low.

Least square means (LSM) of body weights at harvest (Table 2) of Sénégal and GIFT strains did not differ significantly across all environments. The poor survival of the Sénégal strain may have positive influence on its growth performance. The GIFT and Sénégal strains had better growth performance than Israel strain in both separate rearing and communal rearing with supplementary feeds.

The weight of fish at harvest in communal rearing with feeding were about twice more of those recorded in separate and communal rearing without feeding. Overall, the GIFT strain had the highest survival while the Sénégal strain had the poorest. In separate rearing, observed differences in survival among the strains were not significant, but it was significant in communal rearing in both paddies with and without feeding. The GIFT strain has significantly highest survival while the Sénégal had the lowest in both communal rearing environments. However, the survival of GIFT and Israel strains in paddies without feeding did not differ significantly ($P<0.05$).

Water quality and rice yield

All water quality variables were within the range for favorable growth (Table 3). Total ammonia-nitrogen in communal rearing paddies with feeding was relatively higher (0.6 to 1.2 mg/l) than paddies without feeding (0.57 to 0.6 mg/l). There was difficulty in maintaining a desired water level of about 10-20 cm in all paddies because of seepage. Mean water depth in paddies ranged from 5 to 6 cm. Water loss to seepage was worse in 2 to 4 replicates (about 2 cm) in separate rearing. Extrapolated rice yields ranged from 4.5 to 5.4 tons per hectare and there was no significant differences in rice yield between treatments.

Separate rearing trial in 1995

Growth and survival

The 1995 growth trial in separate rearing rice paddies revealed similar set of significant effects on fish body weight as that of the 1993 growth trial in the same environment. In Table 1a however, the pattern (ranking) of the proportion of the magnitude of effects has changed. From among the effects in the model, the strain effect ranked third in importance in the 1995 trial. In all the strains tested, males were significantly bigger than females, but the smallest difference of 14% was noted in the GIFT strain. In other strains, body weight difference between the male and female ranged from 24% to 39% ($P<0.01$). The block or replicate pond has the highest proportion of effect among the effects in the model (55% of the mean square).

The block corresponds to high and low water levels in the paddy. Mean body weight difference ($P<0.01$) between the two blocks was 43%. The ranking (no.1) of the GIFT strain was unaffected by water level, but this was not the case for the other strains. In rice paddies with high water level, the growth performance of the THAI strain was not

significantly different from that of the GIFT strain. However, in rice paddies with low water level, the growth performance of the GIFT strain was significantly different from the rest of the test strains. Overall, the GIFT strain had the best growth performance followed by the THAI, Sénégal, and ISRAEL strains. Survival of fish was highest (75%) in THAI strain and lowest in the GIFT strain (63%). The Sénégal strain had high survival rate (71%) in this trial, but its growth performance was not comparable to that of the GIFT strain as shown in the past trials. Rice yield ranged from 1.7 to 2.1 tons/ha. There were no significant differences among the experimental treatments ($P<0.05$).

Family testing and communal rearing in 1996

Growth and survival

Growth performance of the GIFT strain the aggregate performance of the whole GIFT population in the family testing rice-fish environment and communal rearing of the GIFT and THAI strains in the same paddy used for family testing. Details of the growth performance and ranking of different families, and the correlation of growth of families in rice-fish and ponds will be reported in a separate paper.

Results from this trial showed that the growth performance of the (8th generation) GIFT fish is comparable to those grown under normal pond conditions. From an initial average weight of 5.6 grams per fish (N=2,891), the fish grew to an individual average weight of 186.01 g (N=1,393) after 120 days of rearing. The fish received commercial tilapia diet and the pond refuge was applied with chemical fertilizer. Based on this growth performance trial, it is now possible to grow large size tilapia in a rice-fish environment with pond refuge of about 750 m^2.

Water quality and rice yield

Water quality were observed to be ideal for tilapia culture. From February to May, morning and afternoon water temperature, DO, and pH ranged from 24.4 -32°C; 2.8 – 11 mg/l; 7.4 – 8.86, respectively. Water depth in the pond refuge and paddy area SDV, TAN, and alkalinity fluctuated from values of 96.5-124 cm, and 5.1-11.5 cm; 23.6-29.2 cm; 0.06-0.35 mg/l; and 161-294 mg/l, respectively. Rice production in this study was low about 1,625 kg in 5,150 m^2 total area occupied by rice or roughly 3 tons/ha. The rice was attacked by tungro. The rice plants did not receive any application of pesticide during the culture period. Around CLSU, the potential of rice production in rice fields in about 4 tons/ha. Using this figure, rice yield lost due to total area of pond refuge for three paddies (750 m^2), was around 300 kg. Fish production in this trial with a recovery of 52%, was 250 kg. Results of the communal rearing trial in this paddy showed that GIFT strain had significantly higher ($P<0.001$) mean body weight (137.8 g ± 42.8SD) than the THAI strain (90.4 ± 34.0 SD). The variation in body weight was mainly strain (60% of MS) rather than sex effect (40%). Strain by sex interaction was not significant (P0.66).

DISCUSSION

The growth and survival of all the strains (1993 and 1995 trial) were much lower than those obtained in other culture systems such as cages and ponds. This was due to the smaller size of the pond refuge used and the low water level in the paddies throughout the culture period. Although the stocking density was maintained at 0.05/m^2 for almost all trials, large paddies gave high fish yields that are comparable to the pond systems. This is probably due to large pond refuge area or high water volume to fish ratio than small paddies. Since commercial feeds were given to fish in both small and

large paddies, it is likely that space and water circulation had effected fish growth significantly. This was revealed by the significant correlation between weight gain and water depth (even within each trial). Fluctuation in water level (or water loss) was higher in small paddies than in large paddies.

In any of the rice-fish test environments however, the GIFT strain showed consistently better growth and survival than any other test strains. It is interesting to note that the growth performance of Sénégal strain in early years of the trials was similar to the GIFT strain, but the survival of the Sénégal strain was discouragingly low. The low levels of survival recorded here was not consistent with the earlier results of Eknath et al. (1993). In 1995 trial however, the growth of the Sénégal strain was not comparable to that of the GIFT and it's survival was high. The THAI strain performed better than the Sénégal strain. With these results, the Sénégal strain has been showing inconsistent results. THAI and GIFT strains had comparable growth performance in this trial, but it was not true in later trials. The THAI strain should have grown better than the GIFT strain since it had significantly low survival. Whether its growth and survival are affected by the presence of GIFT, is not the domain of this paper.

Following the results of the rice-fish experiments since 1993, the growth performance of the GIFT strain has improved somehow. This can be roughly estimated from the difference between the mean body weights of the population in 1993 and 1996097 experiments. Over the years as shown in Table 2, the GIFT strain had consistent (better) performance, does it seem to indicate good progress in the on-going genetic selection program for tilapia? This question may find answer in the family testing paper that is being prepared.

Overall, the results indicate that the GIFT strain is a promising candidate for culture in rice-fish systems. The next step is to evaluate its growth performance in on-farm rice-fish trials before conclusions on its suitability for wider adoption by rice-fish farmers. Having a separate line (or strain) for rice-fish environment (other than the GIFT fish) does not seem to be warranted by the performance of other strains. The production potential of the GIFT fish remains to be seen. One thing certain is that we have the fish stocks, with a wide genetic base, that may transform the rice paddies into a more productive resource if the genetic selection program will continue. This will all depend on continuous research on fine-tuning of culture management practices, in line with the further improvement of the fish that we have at present.

LITERATURE CITED

APHA (American Public Health Association). 1980. Standard Methods for the examination of water and wastewater. 15th Edition. Washington DC, USA.

Dela Cruz, C.R. 1990. The Pond Refuge System in Rice-Fish Culture, Aquabyte, 3(2), International Center for Living Aquatic Resources Management, Manila.

Eknath, E.A., Tayamen, M.M., Palada-de Vera, M.M., Danting, J.C., Reyes, R.A., Dionisio, E.E., Capili, J.B., Bolivar, H.L., Abella, T.A., Circa, A.V., Bentsen, H.B., Gjerde, B., Gjedrem, T. and Pullin, R.S.V. 1993. Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments. Aquaculture, 111:171-188.

Dela Cruz, C.R., C. Lightfoot, B.A. Costa-Pierce, V.R. Carangal and M.P. Bimbao (Editors), 1992. Rice-fish research and development in Asia. ICLARM Conference proceedings 24, 457p.

Dela Cruz, C.R. and R.C. Sevilleja, 1990. Final Report: Rice-Fish Farming systems Research Project August 1987-February 1990. Freshwater Aquaculture Center, Central Luzon State University, Muñoz, Nueva Ecija, Philippines. 83p.

Israel, D.C., R.C. Sevilleja, A.V. Circa and R.D. Cocio. 1994. Rice-Fish Culture in the Philippines: An output risk programming analysis. Asian Fisheries Social Science Research Network (AFSSRN) Report Series No. 3-1. 89p.

SAS Institute, Inc. 1990. SAS/STAT User's Guide Version 6, 5th edition, Vol. 2 Cary, NC, USA.

Sevilleja, R.C. 1992. Rice-fish farming developments in the Philippines: past, present and future, In: C.R. Dela Cruz, C. Lightfoot, B.A. Costa-Pierce, V.R. Carangal and M.P. Bimbao (Editors). Rice-fish research and development in Asia. ICLARM Conference Proceedings 24, 77-89.

Snedecor, G.W. and W.G. Cochran. 1976. Statistical method 6th edition. The IOWA State University press, Ames, Iowa, USA. p.593.

Table 1. Degrees of freedom, marginal mean squares, F-Values and percent contribution of significant effects mode 1 (Separate rearing) and model 2 (Communal rearing) for 1993 trial. (F-Value with * is sig P<0.01).

Effects	Separate Rearing				Communal		
	Degrees of Freedom	Mean Square (Type III)	Contribution of Effects (%)	F-Value	Degrees of Freedom	Mean Square (Type III)	C
Block or Replicate Pond	3	13,125.0	30.4	252.4 ***	2	8,080.7	
Feed input	---	---	---	---	1	89,946.0	
Strain	2	6,589.4	15.3	126.7***	2	1,060.6	
Sex	1	17,434.4	40.4	335.3***	1	25,160.3	
Strain*Feed	---	---	---	---	2	1,113.2	
Strain*Sex	2	1,328.5	3.1	664.3***	---	---	
Strain*Block	6	4,713.3	10.9	785.6***	---	---	
Error	468	52.0			260	291.9	
R^2 (%)	**74**				**69**		
Overall Mean (g)	**35**				**55**		
C.V (%)	**20**				**31**		

Table 1a. Degrees of freedom, marginal mean squares, F-Values, and percent contribution of significant effects on fish body weight in 1995 separate rearing trial. (F-Value with *** is highly significant, ($P<0.01$).

Effects	Degrees of Freedom	Mean Square (Type III)	Contribution of Effects (%)	F-Value
Block or Replicate Pond	1	45,841.3	55.3	210.6***
Strain	3	9,522.3	44.5	126.7***
Sex	1	20,447.9	24.7	93.9***
Strain*Block	3	7,082.6	8.54	32.5***
Error	538	217.7		
R^2 = 49%				
Overall Mean = 52g				
C.V = 28%				

Table 2. Least square means (LSM) of final body weights (mixed-sex) and survival of the GIFT, Israel, separate and communal rearing rice paddies. In 1995, the Thai strain was included in the separate re

Strain	Separate Rearing		Communal Rearing					
	Without Feeding		Without Feeding		With Feeding			
	Body Weight (g)	Survival (%)	Body Weight (g)	Survival (%)	Body Weight (g)	Survival (%)	Pooled Body Weight (g)	
1993 Trial								
GIFT	36.4a	46a	29.4a	53a	80.7a	62a	55.1a	
ISRAEL	26.4b	44a	29.4a	35b	68.6b	69a	49.0b	
SÉNÉGAL	39.5c	31b	34.6a	15c	74.9ab	25b	54.7c	
1995 Trial								
GIFT	62.5a	63a						
ISRAEL	43.5b*	67ab			***NO COMMUNAL REARING***			
SÉNÉGAL	47.3c*	71bc						
THAI	57.1d	75c						
1996 Trial								
GIFT	***no separate rearing***			136.9a	58a			
THAI				96.3b	21b			
GIFT Family testing				186.01	52			

* Body weights were significantly different at (P<0.05)
° Trench system; Stocking density = 0.5/m^2; Rainy season 1989
Mean body weights within each column with different letter are significantly different (P<0.01).

Table 3. Means (standard errors) of water quality parameters in rice paddies and rice yield from the separate and communal rearing rice-fish environments in 1993 trial. Differences between values were not significant ($P<0.05$).

Rice-fish Environment STRAIN	No. of Paddies	Water Temperature (°C)	Dissolved Oxygen (mg/l)	pH	NH_3-N (mg/l)	Water Level (cm)	Rice Yield (ton/ha)
Separate rearing							
GIFT	4	22.2(0.6)	4.36(0.2)	7.35(0.1)	---	5.7(0.8)	6.1(0.1)
ISRAEL	4	22.4(0.2)	4.35(0.2)	7.35(0.1)	---	5.4(0.8)	5.9(0.7)
SÉNÉGAL	4	22.4(0.8)	4.25(0.5)	7.35(0.1)	---	5.7(1.8)	5.2(0.7)
Communal rearing							
With Feeding	3	22.5(0.4)	3.5(0.4)	7.23(0.06)	0.95(0.3)	6.9(0.7)	5.6(0.2)
Without Feeding	3	22.3(0.2)	3.9(0.4)	7.37(0.06)	0.59(0.0)	6.2(0.9)	5.6(0.9)

Study Title: GROWTH EVALUATION OF SEVEN STRAINS OF *Oreochromis niloticus* (L.) IN PONDS FERTILIZED WITH ON-FARM CROP RESIDUES

Lead Persons: Antonio V. Circa, Study Leader
Hernando L. Bolivar, FAC/GIFT Research Assistant 1989

Abstract

The study evaluated the growth performance of three African (Egypt, Ghana, Senegal) and four Asian (ppularly known in the Philippines as 'Israel', 'Singapore', 'Taiwan', 'Thailand' strains of (*Oreochromis niloticus*) earthen ponds fertilized with two types of on-farm crop residues: leaves and vines of sweet potato (*Ipomea batatas*) and ipil-ipil leaves (Leucaena leococephala). Ponds fertilized with chicken manure served as controls.

Growth performance among strains and fertilizer treatments was highly significantly different (P<0.01). Strains*fertilizer treatment effects, however, were not significant. Egypt strain registered the highest mean body weight gain followed by Taiwan, Thailand, Israel, Senegal, Ghana, and Singapore strains, respectively. Sweet potato leaves and vines gave the highest yields followed by chicken manure and ipil-ipil leaves. A multiple regression analysis of gowth and physico-chemical variables indicated significant effects of dissolved oxygen, temperature at dawn, and total ammonia-nitrogen.

INTRODUCTION

Evaluation of the growth performance of different strains of *Oreochromis niloticus* in waste loaded ponds must be carried out to determine how they respond to different types of on-farm agricultural wastes. The magnitude of interaction between strains and wastes is very important in identifying and developing most promising strains that have predictable and consistent performance for the particular on-farm residues.

Studies on organic waste recycling in aquaculture have focused on animal and human wastes (Edwards, 1980). Most small-scale Asian farmers grow ipil-ipil trees for firewood and vegetables in their farm for family consumption and as source of cash flow while waiting for rice harvest. Residues from vegetables and other crops are usually burned as a means of disposal. Using these residues as organic fertilizers in ponds may be more attractive to most resource-poor farmers. Crop residues (green manure) have been used in China but mostly involved macrophyte feeding fish (Edwards, 1987; Little and Muir, 1987), Therefore, the study also aimed to determine the fertilizing effect of novel on-farm crop residues compared to chicken manure which has a long history of use as organic pond fertilizer.

MATERIALS AND METHODS

Experimental design

The experiment involved three African strains (Egypt, Ghana, Senegal) and four Asian (popularly known in th· Phili·pines as 'Israel', 'Singapore', 'Taiwan' and 'Thailand') strains of Nile tilapia (*Ore· hrom·s niloticus*) and three on-farm agricultural wastes:

leaves and vines of sweet potato (*Ipomea batatas*), ipil-ipil leaves (*Leucaena leucocephala*), and chicken manure (as Control). To estimate growth performance and the magnitude of the wastes and genotypes interaction, the General Linear Model (GLM) Procedure of the SAS statistical software was adopted (SAS, 1985). The trial was conducted in six 5m x 20 m (100 m2) 1.0 m deep earthen ponds located in a side by side east-west direction. The ponds were devided into two blocks (replicates) and the three wastes were randomly assigned in each block.

Fish stocking, and Pond fertilization

The seven strains (each strain with 14 individual fish per pond) of *Oreochromis niloticus* was communally stocked at a density of 1/m2. Fish sampling of about 30% of the population in each pond was carried out every 21 days. Fertilization began one week after stocking at the rate of 100 kg dry matter/ha/day) for each waste. Fertilization rate was based on equal dry matter loading (rather than isonitrogenous) because waste with lower nitrogen will have higher organic loading which will demand more oxygen fo its decomposition than waste with lower loading. Fertilization was carried out twice or once weekly until one month before fish harvest. There was no inorganic fertilizer supplementation. Ipil-ipil leaves (dry matter, DM=28.4%±0.8, n=6; nitrogen,N=1.2%±0.3, n=3) were collected at the experimental site and applied in ponds fresh. Sweet potato leaves and vines (M=15.1%±1.2, n=6; N=1.4%±0.3, n=3) were collected from nearby farm and some were grown on the pheriphery of the ponds. There were no pre-treatment applied to the crop residues except chopping into 2-3 cm of fresh leaves and vines of sweet potato before their application in the ponds. The chicken manure applied contained 33.3%±3.2, dry matter and 2.2±0.3, n=6; nitrogen, n=3.

Water quality analysis

Water quality parameters were monitored every two weeks. Dissolved oxygen (DO) and temperature were taken at 0600 and 1400 hrs while total ammonia-nitrogen, and pH were monitored at 1400 hrs only. DO was determined by the azide modification of the iodometric (Winkler) titration method (APHA, 1980). Water temperature was measured using a mercury thermometer. Total ammonia-nitrogen was determined by the indophenol blue method (Boyd, 1979). Water pH measurements were done using Beckman Model 72.

RESULTS AND DISCUSSION

Table 1 shows the mean body weight gains of the seven strains in each waste. In the control, the strain with the highest mean body weight gain was obtained by Egypt strain followed by Thailand, Taiwan, Israel, Senegal, Singapore, and Ghana strains, respectively. In Ipil-ipil, the strain with the highest mean body wet gain was obtained by Taiwan followed by Israel, Thailand, Egypt, Ghana, Senegal, and Singapore strains, respectively. In Sweet potato, the strain with the highest mean body weight gain was obtained by Egypt followed by Thailand, Taiwan, Senegal, Israel, Singapore, and Ghana strains, respectively.

Among the strains tested, Egypt strain registered the highest mean body weight gain in the three wastes, while Singapore strain obtained the lowest weight gain. Among the wastes evaluated, Sweet potato gave the highest mean body weight gain followed by the Control and Ipil-ipil, respectively.

The poorest growth of the seven strains of O. niloticus tested was in Ipil-ipil. Fish growth in the Control and in the Sweet potato fertilized ponds were almost similar as indicated by their body weight gains. However, wider growth variation within the former was observed than the latter. Egypt strain performed best in all wastes tested, while Ghana on the other hand, had the poorest growth ($P<0.01$).

The wider growth variation within the Control than the other two treatments may be attributed to seepage in one pond of the Control that could have reduced algal productivity due to nutrient loss. When new water was added to the ponds to top up water levels, one pond of the Control required almost two to three times more than the rest of the ponds. Differences in the mean body weight gains of the seven strains in each and among the three wastes were significant ($P<0.01$).

Table 2 show that there was no apparent deterioration in water quality in all ponds fertililized with the wastes. Mean DO at 0600 hr in all treatments did not reach level below 2 mg/l. Average DO at 1400 hr in all wastes was more than 10 mg/l throughout the culture period. Water temperature at 0600 and 100 hrs ranged from 22-29°C to 34-35°C. A more drastic decline in the morning water temperature of about 4°C was noted between the last week of November and first week of December 1989. Mean water pH in the Control was always higher by a mean of about pH 0.7 throughout the culture period than the other two treatments. Total ammonia-nitrogen concentrations which were almost similar in all treatments did reach more than 1.0 mg/l. Multiple regresion analysis revealed that fish growth were significantly affected by the combined effect of early morning water temperature, dissolved oxygen, and total ammonia-nitogen.

The experiment revealed that Egypt strain performed best in all the wastes tested while the least was Ghana strain ($P<0.01$). The ranking of the seven strains of *O. niloticus* on the basis of decreasing absolute growth rate were: Egypt; Taiwan; Thailand; Israel; Senegal; Ghana; and Singapore strains, respectively. In each waste it appeared that the best four strains were Egypt, Taiwan, Thailand, and Israel strains, respectively.

The culture performance of the test strains was consistent across the three organic fertilizers. The best strain (Egypt) in one waste was also the best in the other two wastes.

Table 1. Mean weight gains (g) of seven strains of *Oreochromis niloticus* in 5 m X 20 m (100 m^2) 0.7-m deep earthen ponds loaded with three different types of waste from 7 September 1989 to 19 January 1990

Strain	Chicken Manure (Control)	Ipil-ipil Leaves	Sweet Potato leaves & vines	Strain Average	SD
EGYPT	32.43	20.26	28.74	26.35	5.10
	8.40	2.50	8.52	5.45	2.81
GHANA	18.05	20.24	17.11	19.14	1.31
	6.11	5.01	3.52	5.56	1.06
SENEGAL	22.44	16.81	24.35	19.62	3.20
	5.48	1.63	5.11	3.56	1.73
ISRAEL	23.03	21.25	23.04	22.14	0.84
	7.04	4.15	3.28	5.60	1.61
SINGAPORE	20.23	14.26	21.87	17.25	3.27
	6.05	0.71	3.18	3.38	2.18
TAIWAN	25.16	22.09	26.52	23.63	1.85
	7.06	3.81	5.97	5.44	1.35
THAILAND	25.53	20.84	27.27	23.18	2.72
	8.91	0.48	4.61	4.69	3.44
Waste Avg	23.84	19.39	24.13		
SD	4.27	2.60	3.64		

Table 2. Mean(SD)water quality parameters in each 5 m X 20 m (100 m^2) 0.7-m deep earthen ponds across the culture period (21 September to 30 December 1990). The ponds were fertilized with three types of wastes and stocked with seven strains of *Oreochromis niloticus*.

Treatment	Pond Number	Temperature °C 6AM	Temperature °C 2PM	D. O.(mg/l) 6AM	D. O.(mg/l) 2PM	pH 2PM	NH_3-N 2PM
Chicken	2	26.4	31.0	4.1	16.2	9.2	0.6
Manure	6	26.4	31.1	3.6	14.4	8.9	0.8
(Control)	Mean	26.4	31.1	3.8	15.3	9.0	0.7
	SD	0.0	0.1	0.2	0.9	0.1	0.1
	1	26.5	30.9	3.8	9.5	8.1	0.6
Ipil-ipil	4	26.5	31.0	4.1	10.8	8.3	0.7
Leaves &	Mean	26.5	30.9	4.0	10.2	8.2	0.6
Vines	SD	0.0	0.1	0.1	0.7	0.1	0.0
	3	26.5	31.0	3.9	10.4	8.3	0.6
Sweet Potato	5	26.4	30.8	3.7	10.0	8.3	0.8
Leaves & Vine	Mean	26.4	30.9	3.8	10.2	8.3	0.7
	SD	0.1	0.1	0.1	0.2	0.0	0.1

Attachment 11.

Studies and Reviews on "Genetics of Parasites and Diseases" by Tryve Gjedrem.

DISEASES AND PARASITES IN FISH. GENETIC IMPROVEMENT OF RESISTANCE.

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ABSTRACT

Gjedrem, T. 1996. Diseases and parasites in fish. Genetic improvement of resistance. ICLARM Studies and Reviews xx, xx p. International Center for Living Aquatic Resources Management, Manila, Philippines.

Diseases create a lot of problems in aquaculture, the major ones being losses, down grading at slaughtering and pollution through the use of antibiotics. Thus, diseases reduce the profitability of fish farming. It is, therefore, a major task to improve disease resistance of the fish. In the literature, estimates of genetic variation in survival have been reported for differnt diseases and parasites in some species. Selection experiments have successfully been carried out for resistance to bacterial diseases in common carp, rainbow trout and brook trout and for viral disease in rainbow trout. Estimates of heritabilities for survival rate are low when based on records from shorter or longer growout periods. By applying challenge tests to one disease at a time under controlled environmental conditions, the heritability estimates are in general quite high. Several attempts have been made to find correlated traits to serve as markers for selection to improve disease resistance, and immune parameters have in particular been candidates for such investigations. So far, no single parameter

with sufficiently high genetic correlation to disease resistance has been identified; interestingly, growth rate show positive genetic correlation to disease resistance for some bacterial diseases.

The additive genetic variance seems to be dominating disease resistance but in some species considerable heterosis effect have been found. This implies that selection should be practised in a breeding program to improve disease resistance. Family selection is advocated because survival is an all or none trait, and survivors from challenge tests cannot be used as broodstock because of the risk of infections. If heterosis is found to be considerable selection should be combined with crossbreeding. According to current knowledge the possibility for improvement of resistance to diseases and parasites in fish is good and looks to be better than for traditional farm animals.

INTRODUCTION

According to FAO statistics aquaculture production is expanding on all continents. Fish production has had a yearly growth of 12 % during the last 8 years. In 1992, the production reached 9.4 million metric tonnes. The possibility for further expansion is good and the expansion is expected to take place particularly in the marine environment. Available water and land area will limit expansion of fresh water aquaculture.

There are several problems associated with aquaculture production, and one is the occurrence of disease. In some cases, mortalities can be dramatic, threatening large industries to go bankrupt. In the late seventies and early eighties, cold water vibriosis caused large losses of farmed Atlantic salmon (Salmo salar) in Norway, and costs were estimated to 30 million US$ in one year (Poppe et al. 1985). However, an efficient vaccine was developed and brought the disease under control. It has been estimated that in 15 Asian countries, with a farm value of over US$ 22.7 billion in 1990, total loss in revenue due to diseases alone was at least US$ 1.36 billion or about 6% of total production (ADB/NACA,1991). According to FAO (1995) this is a very conservative estimate. In Taiwan, the shrimp production was approximately 80 000 tons in 1987 and was reduced to 20 000 tons the following year due to a viral disease (ADB/NACA 1991). Losses of fish not only reduces production but may also lead to the deterioration of the environment. Antibiotics and other chemicals used to reduce mortality are usually given through the feed. Eventually, the compounds will reach the water and affect water quality. Unconsumed pellets may be eaten by wild fish outside cages or net pens, and feed may build up in ponds or under cages and lead to the development of resistant bacteria.

Disease is not simply the result of contact between the fish and the pathogen but it is the result of a complex interaction between the fish, the pathogen and the environment. Changes in environmental conditions and in particular when changes are extreme, will stress the fish and make them susceptible to the pathogens. Numerous environmental factors during the whole lifespan of fish will effect their health status.

To control disease in aquaculture the following strategies are traditionally considered:

1) Use of antibacterial compounds.
2) Develop effective vaccine against the disease.
3) Improve the hygienic standards in hatcheries and growout systems to avoid introduction of pathogens in the future.

A fourth strategy for disease control, is selection of fish that are resistant to diseases. This stratigy has so far, been given little attention. The possibility, and application of breeding and selection as a means of disease control, will be discussed in this paper. These topics have partly been reviewed before by Price (1985), Chevassus and Dorson (1990) and Fjalestad et al. (1993).

I will, however, not deal with resistance to natural abiotic factors like temperature and pH, and to pollutants like heavy metals and chemicals or ionizing agents, nor with malformations.

The absolute requirements to improve disease resistance through breeding and selection are:

1) Variation in disease resistance must be possible to observe and record.
2) Some of the variation in disease resistance must be genetic.

MAJOR DISEASES AND PARASITES AFFECTING AQUACULTURE PRODUCTION

Fish diseases and parasites are different in tropical aquaculture compared to subtropical and cold water. Table 1 lists the most serious diseases and parasites in some of the important fish species used in aquaculture.

According to Pillay (1990) relatively few diseases have been reported in tilapia. This is in agreement with Roberts and Sommerville (1982) and Michel (1989) who report that tilapia are remarkably hardy fish that do not normally suffer high mortalities from parasitic infections. This do not mean that parasites do not attach to the fish. Natividad et al. (1986) found 7 species of parasites in a sample of fish, mostly protozoa and monogena. Landsberg (1989) found myxosporean parasites on tilapia but they apparently caused no problem. Lightner et al. (1988) found on the other hand, a number of diseases that adversely affected tilapia reared in high density recirculating systems: Skin and gill parasites were associated with mortalities. In freshwater, Aeromonas hydrophila and Edwardsiella tarda affected fish as did Vibrio sp. and Aeromonas sp. in brackish water.

As shown in Table 1, tilapia may be affected by several bacteria, parasites and fungi, but there is no report in the literature that any of these pathogens have caused mortalities (Michel, 1989) which may apply under extensive production systems. But Hubbert (1989) refer outbreak of Streptoccosis under intensive farming conditions and sometimes causing heavy losses (30-40 % of stocks, Eldar et al., 1995). Effords are made to develop vaccines to cope with this bacterial disease (Eldar et al., 1994; Bercovier et al., 1995)

LITERATURE REVIEW OF GENETIC VARIATION IN DISEASE RESISTANCE

Bacterial diseases

Variation between species

Salmonid fish are known to be susceptible to Areomonas salmonicida causing furunculosis infection but there is variation in the level of susceptibility between salmon species. Cipriano and Heartwell (1986) found that mortality from enzootic furunculosis are most severe among brown trout (Samo trutta), intermediate in brook trout (Salvelinius fontinalis) and virtually none existent in rainbow trout (Oncorhynchus mykis) cultured at the same fish farm. Brown trout was found to be more susceptible to furunculosis than rainbow trout (Ferguson and Rice, 1980; Cipriano and Hertwell, 1986). Brook trout and Atlantic salmon are more susceptible to furunculosis than rainbow trout according to Cipriano (1983). Ehlinger (1977) found higher survival in brook trout than in brown trout exposed to furunculosis. According to Novotny (1975) pink salmon (Oncorhynchus gorbuscha) is very susceptible to furunculosis, vibriosis and BKD while hybrids of chinook (Oncorhynchus tshawytscha) and pink had a higher survival rate. Beacham and Evelyn (1992 b) found that chum salmon (Oncorhynchus keta) was more susceptible to furunculosis than coho (Oncorhynchus kisutch), and chinook had a lower mortality than coho, while Beacham and Evelyn (1992c) could not find significant differences in mortality between these species of pacific salmon challenged with furunculosis.

Fingerlings of five species were tested to determine their susceptibility to the bacterium, E. ictauri. Channel catfish (Ictalurus punctatus) demonstrated high susceptibility indeed as 100 % of the fish died after injection with the bacteria. Tilapia was slightly susceptible to the pathogen while golden shiner, bighead carp (Aristichthys nobilis) and largemouth bass (Micropterus salmoides) were not susceptible (Plumb and Sanchez, 1983).

Llewellyn (1980) found that rainbow trout were more susceptible to a bacterium similar to redmouth bacteria infection than brook trout and landlocked salmon.

Common carp (Cyprinus carpio) and channel catfish are known to be very susceptible to bacterial haemorrhagic septicaemia or A. hydrophilia, Fijan (1972) and Thune et al. (1982), respectively. However, two strains of common carp were found to be very resistant when challenged by intraperitoneal injection with Aeromonas hydrophila while giant goramy (??) were more susceptible and walking catfish (Clarias batrachus) were most susceptible (Supriyadi, 1986).

Variation between populations

Gjedrem and Aulstad (1974) found significant variation between Norwegian Atlantic salmon stocks and the Swedish stock Luleå from the Baltic sea in susceptibility to vibriosis.

In a test involving 5 inbred strains and 9 crossbred strains of the common carp, one inbred strain was found to be infected with epidermal epithelioma disease while a second inbred strain was susceptible to a swim bladder inflammation. Crossbreds between these 2 strains, as well as between them and other strains did not show a single fish that were infected by either disease. It was concluded that susceptibility to both diseases is controlled by recessive genetic factors (Hines et al., 1974). Winter et al. (1980) demonstrated differences between stocks of coho salmon as well as between strains of steelhead (Oncorhynchus mykis) in resistance to BKD and vibriosis. The importance of transferrin genotype of coho salmon in resistance to BKD was stock specific; in stocks that showed differential resistance of genotypes, the AA was the most susceptible. No difference in resistance to vibriosis were observed among transferrin genotypes. Beacham and Evelyn (1992a) found that chinook from Kitimat river had the lowest mortality rates compared with two other populations for all pathogens examined (vibriosis, furunculosis and BKD).

Kirpichnikov et al. (1993) reported genetic variation among strains of common carp in resistance to dropsy disease. Significant differences in survival between strains of brook trout could be attributed to mortality caused by ulcer disease and furunculosis. Comparisons were made between two strains of common carp, a Polish and a Hungarian, and a significant variation was found in resistance to furunculosis (Houghton et al. 1991). Snieszko et al. (1959) found significant difference in resistance to ulcer disease and furunculosis between strains of brook trout. Differences in susceptibility to furunculosis were also found between genetic groups of common carp (Sovenyi et al., 1988).

Chevassus and Dorson (1990) summarised results from crossbreeding of fish strains (Hines et al., 1974 ; Pojoga,1972; Sovenyi et al., 1988 in common carp; and Plumb et al., 1975 in American catfish) and reported them to be rather generally "recessive" in susceptibility to diseases.

Genetic parameters

Heritability estimates for unspecified mortality under farming conditions indicate a significant but rather low additive genetic variance as summarised in Table 2.

Applying experimental challenge tests, i.e. infecting the fish with furunculosis by means of cohabitation, Gjedrem et al. (1991) found high genetic variation in survival of Atlantic salmon parr, the estimated heritability being as high as 0.48±0.17. The variation in survival between 50 full- and 25 half-sib families is illustrated in Fig 1. When 81 full- and 31 half-sib families of Atlantic salmon parr were challenged in a similar experiment, estimated heritabilities were 0.16±0.12, 0.23±0.10 and 0.13±0.08 for furunculosis, BKD and cold water vibriosis, respectively (Gjedrem and Gjøen, 1995). Correspondingly, Bailey (1986) reported a heritability of 0.32±0.06 based on full-sibs for mortality due to furunculosis in Atlantic salmon smolts.

Beacham and Evelyn (1992a) found no significant genetic variation in chinook salmon for susceptibility to vibriosis, furunculosis and BKD. The low and non significant heritability estimates in the pacific salmonids were found in populations which had been exposed to furunculosis for many generations, while the high estimates reported by Gjedrem et al. (1991) in Atlantic salmon, were from populations not previously exposed to A. salmonicida.

Refstie (1982) found significant variation between progeny from different sires of rainbow trout in resistance to vibriosis. Ten families of Atlantic salmon showed variation in survival after being subjected to furunculosis bacteria (Olivier et al., 1988). Smoker (1986) found significant variation between progeny from different sires of chum salmon in disease resistance after infection with V. anguillarum. Ayles (1974) reported high heritability for resistance to blue sac disease in young splake hybrids.

Response to selection

Already in 1925, Embody and Hayford selected surviving brook trout from a population with endemic furunculosis and increased the survival rate from 2 % in the initial population to 69 % after three generations of selection. Later work has shown considerable genetic variation in mortality due to furunculosis; Ehlinger (1977) obtained reduced mortality rates due to furunculosis in brown trout and brook trout after selection. Cipriano and Heartwell (1986) obtained after one generation of indirect divergent selection of brown trout for immunoprecipitation test of mucus extracts and obtained a difference in survival of 2 % in the selected group and 48 % in the none selected group with respect to furunculosis.

Wolf (1954) concluded that selection offers a great possibility to increase disease resistance in fish and that it should be possible to eliminate the use of chemotherapeutic methods to control disease.

Kirpichnikov et al. (1976), Ilyassov (1987) and Kirpichnikov et al (1993) reported on a selection program against dropsy disease in common carp which started in 1965. Three stocks were involved and the response to mass selection was moderate. The goals for future selection are to use family selection which should be more efficient for diseases, and to study further heterosis in crosses. The selection of common carp by Schaperclaus (1962) in Germany (reviewed by Schaperclaus, 1961) showed an average mortality rate of 11.5 % in 65 ponds stocked with progeny of selected fish vs.57 % in 76 ponds stocked with progeny of non-selected fish.

In review articles, Price (1985) and Kinghorn (1983) advocate selection to improve disease resistance in fish because additive genetic variation is present and that crossbreeding should be applied where heterosis effect has been shown.

Viral diseases

Variation between species

Salmonid species show large differences in susceptibility to viral diseases. Chevassus and Dorson (1990) have reviewed the findings and these are summarised and the results are shown in Table 3. There are also differences in susceptibility between serotypes of the same virus. According to these authors some crosses between species may have advantages regarding their resistance to virus infections. For salmonid species it has been demonstrated that, despite the existence of a gene assortment in favour of the susceptible species, triploid hybrids in some cases keep the paternal trait of resistance to viral diseases (Chevassus and Dorson, 1990). Dorson et al. (1994) found that rainbow trout was susceptible to VHS while rainbow trout x brook trout triploid hybrids showd high degree of resistance. Silim et al.(1982) demonstrated that various species of trout had different susceptibility to IPNV as indicated by mortality rate following infection with the virus. Brook trout had the highest mortality, followed by rainbow trout, while the least susceptible was lake trout.

Variation between populations

Large variation were shown to exist among strains of brook trout in susceptibility to IPNV, with mortalities ranging from 31-72 % (Silim et al., 1982). Okamoto et al. (1993) reported on an IPN resistant strain of rainbow trout. The strain was tested 20 times and the average mortality was 4.3 % compared to 96.1 % in a highly sensitive strain.

Genetic parameters
McIntyre and Amend (1978) estimated heritability for tolerance to infectious haematopoietic necrosis virus (IHN) to be h^2=0.32 while Yamamoto et al. (1991) gave estimates of heritability of 0.05 - 0.51. Amend and Nelson (1977) reported significant variation between full-sib families of sockeye salmon (Oncorhynchus nerka) in resistance to IHN virus. Chevassus and Dorson (1990) found high heritability (h^2= 0.69-0.25) for resistance to VHS

Fungal diseases

Variation between populations
When saddleback aureas (heterozygote for a dominant gene) were challenged by saprolegnia they were far more susceptible to infection than normal fish (Tave et al. 1983).

Genetic parameters
Nilsson (1992) recorded mortalities during outbreaks of fungal infections (Saprolegnia) in 92 families of Arctic char (Salvelinus alpinus). Heritability was estimated to be 0.34±0.14. A positive genetic correlation of 0.50 was found between growth rate and resistance to fungal infections. Singhal et al. (1987) presented several methods to experimentally transmit fungi to fish. Such methods could be used in a breeding program to evaluate family groups for their resistance to fungal infections.

Parasites

Parasites are quite abundant in fish. For example, Bondad-Reantaso and Arthur (1990) report that 21 species (10 protozoa, 6 Monogenea, 3 Digenea, 1 Copepoda and 1 Mullusca) appeared on tilapia (Oreochromis niloticus) in The Philippines. The most serious external parasites in salmon farming is costia (Ichthyobodo necator) and sea lice (Lepeophtheirus salmonis) while the, by far, most costly internal parasite is the helminth parasite Eubothrium crassum.

Not much is known about the mechanism of natural resistance to parasites in fish. In the few studies where this was examined more closely, the alternative pathway of complement activation was the protective mechanism (Woo, 1992).

Variation between species
The myxosporidian Ceratomyxa shasta (protozoa) is one of the few parasites causing mortalities in wild fish as well as large scale losses on fish farms. Juvenile rainbow trout and cutthroat trout (Salmo clarki) in hatcheries are particularly susceptibile (Sanders et al.,1970). Zinn et al. (1977) reported high mortality due to C. shasta in rainbow trout, cutthroat trout and brook trout while it was almost non-existent in Atlantic- and chum salmon. In a study where Pacific trout and salmon stocks were exposed to the infectious stage of C. shasta, considerable variation was found between species (Ching and Parker, 1989).

Two fish species, Goodeid fish (Ameca splendens) and platyfish (Xiphophorus variatus) were exposed to the parasite Ichthyophthirius multifiliis and a significant difference was found between them, the goodeid fish being the least susceptible. To account for different body areas and shape the authors proposed to standardize exposure and response levels (Clayton and Price, 1987).

Gyrodactylus salaris is a parasite reproducing on Salmo, Salvelinus, Thymallus and Onchorhynchus species, but not on the non-salmonids (Bakke, 1991). The salmonid host species tested could generally be arranged along a continuum from resistant to highly susceptible species: from brown trout which is very resistant, Neva salmon stock, brook trout, rainbow trout, through Arctic char, to Norwegian Atlantic salmon which is very susceptible (Bakke, 1991).

Variation between populations
Atlantic salmon is, in general, highly susceptible and the parasite has caused a total depletion of about 30 Norwegian river stocks (Johnsen and Jensen, 1986). Bakke et al. (1990) studied the susceptibility of different strains of Atlantic salmon to G. salaris and found that the hatchery reared Baltic Neva stock demonstrated both an innate and an acquired resistance to G. salaris, in contrast to the highly susceptible, Norwegian Alta and Lone salmon stocks. Native Scottish stocks from Shin and Conon rivers were susceptible to G. salaris (Bakke and MacKenzie, 1993) and likewise was the Swedish stock from Indalsriver susceptible (Bakke et al. 1992).

Various hatchery strains of chinook salmon were tested for resistance to the myxozporidian Ceratomyxa shasta and mortality ranging from 0 to 100 % was observed; the most resistant strains came from zones where the disease is endemic (Zinn et al., 1977). These results were later confirmed by Buchanan and Sanders (1983) who found that strains of rainbow trout from the columbia river where C. shasta is endemic, were resistant, while strains of rainbow trout from Siletz river where the parasite does not occur were susceptible. Hemmingsen et al. (1986) compared three strains of coho salmon and their crosses. Susceptibility of crossbred progeny nearly always was intermediate between the susceptibility of fish from parental stocks.

Ibarra et al.(1992) reported a significant difference in mortality and time to death after challenge with C. shasta between a susceptible and a resistant strains of rainbow trout and their reciprocal crosses, with the reciprocal cross being intermediate. In another experiment, Ibarra et al. (1994), studied mortality in six genetic groups, a high susceptible strain, a low susceptible strain and their F1, F2, and backcrosses. After exposing these strains to C. shasta continuously for 7 days, no simple Mendelian model of inheritance fit the observed mortality data. Segregation was not detectable in the distribution of time to death, indicating that the genetic control for these two traits involves more than one locus.

Woo (1992) stated that there is genetic variation in susceptibility to parasites and proposed two practical approaches to reduce parasite problems:

- (a) to breed those individuals that have innate or natural resistance to a specific pathogen in an otherwise susceptible fish species, and
- (b) to immunize susceptible fish with a vaccine.

To test fish for susceptibility to parasites Woo and Shariff (1990) uses challenge tests and measure what time it takes before the parasites are rejected by the fish.

The mechanism behind parasite resistance is not well understood. It is, however, known that fish mucus contain immunoglobulins and other immunological active components

Traits correlated to disease resistance

In a selection program, it may be desirable to include correlated traits as genetic markers in order to increase genetic gain in traits that are of economic importance (Gjedrem, 1967). To be of interest, there must be a high genetic correlation with the economic trait selected for and in addition the trait must show genetic variation (Fjalestad et al. 1993). Immunological and physiological parameters could be candidates for such indirect selection for increased disease resistance. Estimates of heritabilities for some immunological and physiological traits are listed in Table 4. Most of the estimates are quite low. For most of these traits the phenotypic variation was substantial.

The advantage of using immunological and physiological traits is that they can be recorded on the breeding candidates as well as their full- and half-sibs without risking to infect the commercial progeny. Since blood samples can be taken prior to selection of broodstock an individual selection can be practised in addition to family selection.

Correlations between some immunological and physiological parameters using family averages and survival measured in a challenge test are given in Table 5.. As shown in Table 5, none of these traits were highly correlated to survival. It should be pointed out that these estimates are based on a limited

number of genetic groups. However, by combining several genetic markers the correlation with disease resistance may be increased (Lund et al., 1995).

Wild fish and shellfish, as well as animals under farming conditions, will be influenced by natural selection which will adapt the population to its environment. In captivity, natural selection will probably favour individuals with a lower stress response level. Selection experiments for high and low stress response, expressed as blood cortisol level, have been carried out in both Atlantic salmon and rainbow trout (Fevolden et al., 1991). When challenged with furunculosis, the low stress response line showed a higher survival rate than did the high stress response line (Fevolden et al. 1992; 1993).

Direct selection for resistance to furunculosis by applying challenge tests will, according to the results given in Table 6, yield an increase in resistance to the bacterial diseases BKD, vibriosis and coldwater vibriosis but a decrease in resistance to the viral disease ISA (Infectious salmon anemia). This is in agreement with Beacham and Evelyn (1992b) who found that family mortality rates in challenge with Vibrio species (V. anguillarum and V. ordalii) and A. salmonicida tended to be positively correlated, but not as a result of additive genetic variation. On the contrary, Beacham and Evelyn (1992a) did not find correlations between mortality rates of families in chinook for the different pathogens studied (vibriosis, furunculosis and BKD).

Increased growth rate is usually one of the breeding goals in fish. It is therefore of interest to know what influence selection for increased growth rate will have on disease resistance. In Table 7 some estimates of genetic correlation between growth rate and survival rate is given. Standal and Gjerde (1987) estimated a positive genetic correlation of 0.18 between survival after an outbreak of cold water vibriosis during growout period and growth rate. Rye et al. (1990) reported a genetic correlation of 0.37 and 0.23 between survival and growth rate in the fresh water period for Atlantic salmon and rainbow trout, respectively. In brook trout, Robison and Luempert (1984) estimated a low positive genetic correlation of 144-day weight with survival and a negative correlation between 243-day weight and survival. Gjedrem et al. (1991) studied survival after a challenge test with furunculosis and estimated the genetic correlation between survival and growth rate to be 0.3. Beacham and Evelyn (1992b) did not find consistent genetic correlations between family weight and observed mortality rates and time to death. A positive genetic correlation will lead to an increase in survival when selecting for increased growth rate.

The refered genetic correlations (some based on family averages) are all from salmonid data, some are from survival during a shorter or longer period and others are from challenge test for a particular disease. It is therefore not possible to draw general conclusion.

Conclusion

Some conclusions which can be made from the literature review are as follows:

* Mortality can be considerable in most species. Hence, aquaculture is still a rather risky industry, a fact which stresses the importance of reducing mortality.

* Survival/mortality seems to be the best measure of disease resistance in fish and shellfish. When applying challenge test time to death may be an alternative measure.

* Significant genetic variation has been found for mortalities caused by bacteria, virus, fungi and parasites.

* Selection experiments have demonstrated genetic gain for resistance to bacterial and viral diseases.

* Heritability for survival over a given period of time under farming conditions is, in general, low. However, when challenge tests for specific diseases or parasites are applied in a controlled environment, the heritability is usually higher, as is sometimes the genetic variation.

* There is indication that the genetic variation for a disease is higher, for a species living in an environment where the pathogen is endemic compared with species not previously exposed to the pathogen.

* Low positive correlations among family averages have been found between suceptability to bacterial diseases and negative between bacterial- and virus (ISA) diseases.

* There seems to be a low, positive genetic correlation between growth rate and resistance to bacterial diseases.

* So far, no sufficiently high genetic correlations have been found between disease resistance and immunological or physiological characters.

APPROACHES TO GENETIC IMPROVEMENT OF DISEASE RESISTANCE THROUGH BREEDING

Recording disease resistance

In fish farming, diseases are observed as change in behaviour, reduced appetite, lesions and finally dead fish. In practise it is not possible to identify and record infected survivours. Our records will, therefore, be a list of identified dead and surviving animals. This is called an all or none trait with two classes, dead or alive.

Infectious diseases may be caused by a number of different pathogens. The prevalence of survivors may also depend on many other factors unrelated to resistance to diseases, such as accidents and management problems. In addition, the effects of the various pathogens and environmental factors differ between years and between farms and the level of mortality may be very different from one farm to an other or from one year to the next. These circumstances make observations on survival variable and inconsistent. Usually, the genetic variation and heritability for the trait survival is low as shown in Table 1. Records of field data of survival rates are therefore not a good measure of disease resistance in fish. In a breeding program, a more systematic approach to the testing of fish for their resistance to common diseases is needed.

A standardized way to test fish for resistance is to challenge them directly with a pathogen under controlled conditions. Challenge methods have to be described for each pathogen and frequently for each specie particularly taking into account water temperature and length of exposure. In general pathogen should be introduced into the water because the pathogen normally has to penetrate the barrers of the skin in natural infection. Because there may be strict fish disease regulations, it may be necessary to establish research facilities specifically for challenge tests, where the effluent can be sterilized in order to avoid discimmination of the disease. For genetic studies, small fish are used because they need little space, and are easy to handle. The results obtained may not be quite representative for larger fish during the growout period, but large fish will need more space and will be very expensive to test. Since the environmental conditions during challenge tests differ from those in commercial farms, the validity of the challenge test data may be reduced. In any case, the success of selection in the field, will depend on the genetic correlation between survival after a challenge test and resistance in a commercial farm environment.

The efficacy of a challenge test is highest when the mortality is around 50 % (Fjalestad et al. 1993). Survivors from the challenge test would be of interest to use as broodstock but because they may be carriers of the disease they should not be allowed for breeding. By testing families, the result from the challenge test can be applied to the uninfected siblings, which are maintained at the breeding station.

Breeding methods

In breeding programs for fish, there are two methods of particular interest, namely cross-breeding and pure-breeding combined with selection. Pure-breeding is the mating of animals within a population, while crossbreeding is the crossing of strains or inbred lines. Choice of breeding method depends primarily on the type of genetic variation present in the trait of interest. If the non-additive genetic variation is considerable, crossbreeding should be used, while pure-breeding with selection should be used to exploit additive genetic variation (Gjedrem 1985).

Interspecific hybrids has been studied in many experiments and Chevassus (1979) conclude that for survival rate the hybrids among salmonids often being semilar, or even superior, to the most hardy species which is in agreement with Refstie (1983). Chevassus and Dorson (1990) pointed out the practical problem that due to low viability of such hybrids they were of little interest for commercial farming. Crossing different strains has shown relativly high heterosis effect for survival in carp (Hines et al., 1974; Pojoga, 1974; Suzuki and Yamaguchi, 1980; Sovenyi et al., 1988) and in catfish (Plumb et al., 1975) . Interstrain crosses in salmonids have, on the other hand, given inconsistent results with respect to heterosis. Ayles and Baker (1983) found significant hybrid vigor while Gjerde and Refstie (1984) found significant but low degree of heterosis for survival rate.

If heterosis is considerable It would be of interest to develop specialized lines for crossbreeding purposes. There may exist populations beeing resistant to certain diseases and crossbreeding may be used to introduce such genes into the farmed population. A suceptible strain used in production may be crossed with another strain that has low susceptibility if the resulting overall performance is increased. However, as pointed out by Gjedrem (1985) a breeding program applying crossbreeding should be combined with contineous improvement through selection. The heterotic results of such breeding strategies must be large enough to balance the extra costs of developing and maintaining the specialized strains/lines.

Methods of selection

The most widely used method of selection in fish is individual selection (mass selection). It is easy to practise, inexpensive to run, and does not extend the generation interval. However, individual selection has its limitations. The efficacy is low for traits with low heritability, and it can not be used for all or none traits like survival. Moreover, it cannot be used for carcass quality traits. In fish breeding this usually limits individual selection to selection for growth rate.

Family selection is relatively more efficient than individual selection for traits with low heritabilities, it can be applied to all or none traits, meat quality traits, and it does not extend the generation interval. Family selection is of particular interest in fish because of their high fecundity, and because it is possible to produce large numbers of half- and full-sib groups. Since it is impossible to mark newly hatched larvae or fry, each family must be reared in separate tanks for the first months of their life. This type of rearing will, however, introduce some environmental effects common only to family members. Generally, combination of individual and family selection will be the most efficient (Falconer, 1961).

The high fecundity in fish and shellfish make it possible to apply a strong selection which is important in order to achive large genetic gain. Further the high fecundity make it possible to disseminate the gain to the industry rapidly. Indirectly this mean that a breeding system can be consentrated and the cost of broodstock production will be low for the industry.

Direct selection for disease resistance

Direct selection means to select animals which are superior for one or more traits.
In order to increase disease resistance family selection should be applied. Mortality in growout and results from challenge tests should be used as selection criteria. Since the families must be reared in separate tanks before tagging the tankeffects may be considerable and heritability estimates for survival in this period are low. To keep the tankeffect low the fish should be tagged as early as possible. The families can then be mixed and reared communally, and challenge test of a subsample

from each family can be applied in an efficient way. Mortalities should be recorded during the challenge test and possible diagnosis should be made.

It is of greatest interest to select for natural resistance, in order to improve disease resistance and to make vaccination redundant. The fish should therefore be challenged before vaccination. Another argument for testing fish before vaccination is that new technology may change vaccine antigens and therefore over time challenge tests may not be readily comparable even for the same pathogen.

In the Norwegian National Breeding Programme for Atlantic salmon, all families (around 240 each year) are challenged with the two most serious diseases, furunculosis and ISA (Infectious Salmon Anaemia). This work started in 1990 and results of the selection have not yet been estimated. The expected genetic gain will also depend on the magnitude of the genetic correlation between the challenge testing of small fish in freshwater and resistance to furunculosis outbreaks in the sea. Gjøen (personal communication) estimated a genetic correlation between the outcome of challenge tests and furunculosis outbreaks in the field of 0.90. This promising figure suggests that challenge testing of small fish may provide good records of resistance to furunculosis during the growout period.

With the current knowledge about the genetic correlations between immunological parameters plasma proteins and diseases (Table 5), it is at present not recommended to use indirect selection to improve disease resistance. However, the methodology of estimating the correlated traits may be improved and new marker traits with higher genetic correlation may be found. Combining informations from several genetic markers may also improve the correlation to disease resistance. Such improvements may increase the efficacy of indirect selection to a level where the advantage of individual selection in addition to family selection should be explored.

As records become available, breeding values for all traits that are included in the breeding goal may be calculated for each family. Broodstock will then be selected from the highest ranking families.

Response to selection

As reported earlier some selection experiments for improvement of survival has shown response. It could therefore be of interest on a theoretical basis to estimate expected response to selection. As an example selection to increase resistance to furunculose has been chosen because good estimates of phenotypic and genetic parameters is available. Given that a breeding station is available, the breeding goal is to select for increased growth rate and improved resistance to furunculosis and that family selection should be used. It is further anticipated that 150 full-sib families can be reared and that it is possible to challenge the families for resistance to furunculosis each year by using 50 fish per family; mortality 0.67 %; broodstock is selected from the best 15 families and a heritability of $h^2 = 0.33$ is used. According to Falconer (1961) the expected response to direct selection for resistance to furunculosis will be:

$$R = i \times \sigma_p \times h^2_p \times (1+(n-1)r/(\sqrt{n}(1+(n-1)t))$$

Where i = selection intensity (when a low percentage of animals are selected as parents the value of i is high, while i is low when a high percentage of animals are used as parents), at 10 % selection $i = 1.75$; σ_p = individual phenotypic standard deviation, $\sigma_p = \sqrt{p(p-1)}$ where p is the incidence of mortality/survival; h^2_p is heritability of the trait on individual basis; $(1+(n-1)r/(\sqrt{n}(1+(n-1)t))$ is a factor transforming σ_p and h^2 to family basis; r is the additive genetic relationship between family members (for full-sibs $r = 0.50$); t is phenotypic correlation between family members, ($t = 0.50 \times h^2 + c^2$, where c^2 is an environmental component common to all family members, $c^2=0$ in this example); n is number of individuals per family. One generation of selection will then give the following response:

$$R = 1.75 \times 0.47 \times 0.33 \times (1+(50-1)0.50)/(\sqrt{50}(1+(50-1)0.165)) = 0.34.$$

This estimate means that in one generation of selection, it should be possible to improve resistance to furunculosis such that the average mortality (p) could be reduced by 34 % which is remarkably high. In addition to the direct effect of selection on resistance to furunculosis, a correlated response will be obtained when selection for high growth rate is practised simultaneously.

BREEDING PLAN TO IMPROVE DISEASE RESISTANCE

Based on the present knowledge and previous discussions sentral prinsiples in a breeding plan is outlined below. It is difficult to make it general particularly since genetic parameters are only known for a few species.

Selection should be applied because:

* Several investigations have demonstrated considerable additive genetic variance in disease resistance.
* Non-additive genetic variance normally seems to be small for disease resistance.
* Challenge tests by exposing different parallells of the sib groups to one pathogen at a time have shown higher genetic variance than has the recording of survivors in field tests.

Family selection should be used because:

* Survival is an all or none trait.
* Survivors from challenge tests can not be used as broodstock in a breeding program since there is a risk that these fish may be carriers of the pathogen.

Selection of diseases to which resistance should be improved:

* One should select the diseases causing the highest economic losses. However, the costs of challenge tests must be taken into consideration, together with the magnitude of genetic variation and genetic correlation to other diseases. As an example the National Norwegian Breeding Program for Atlantic salmon includes challenge tests for furunculosis and ISA-virus.

Production of families:

* Since there may be some non-additive genetic variance in disease resistance the mating system should be hierchical in order to produce both full- and half-sib families. A large number of families must be produced to enable a strong selection.
* Facilities where families can be reared from fertilization till tagging must be available, and environmental conditions must be standardised to avoid as much as possible non-genetic differences among families.
* The fish should be tagged as early as possible.

Testing for disease resistance:

* A standardised method of challenge should be worked out for each pathogen and for each specie.
* A sample of 20 or more tagged fingerlings from each family should be challenged with each selected bacterium, virus or parasite. The sampled testfish from all families should be put together in one tank or unit. The pathogen should preferably be introduced to the testfish by means of cohabitations (Infected fish are put into the tank) rather than by injecting the pathogen into each fish. In the case of parasites they may be introduced into the tank by cohabitants or added directly to the water.
* Dead fish should be identified by its number and date of death.
* Samples of dead fish should be diagnosed to make sure that death is caused by the pathogen used.
* The facilities at the test station should allow sterilization of effluents from the challenge tests to avoid infection of the surrounding environment.

Selection of broodstock:

* A breeding value for each full-sib family should be estimated based on its survival after challenge, and the survival of the half-sib group to which it belongs.

* Selection of broodstock should be made among fish from the 10-20 families with the highest breeding value for disease resistance. Selection intensity for disease resistance will depend on how many other traits are included in the breeding goal.

If non-additive genetic variance is large for disease resistance, crossing two or more strains/lines to produce hybred progenies for farming may be advantageous. It is recommended that each of the strains/lines which is used in the crossbreeding program should be improved by selecting for disease resistance as described above.

DISCUSSION

In most cases, all animals in a population are susceptible to the initial infection but differ in their ability to limit the infection or destroy the pathogen. The best single criterion for determining the level of resistance seems to be survival rate.

The general outcome of studies in livestock, laboratory animals and fish, indicates that immune responsiveness and disease resistance are quantitative traits regulated by the effects of several genes that are influenced again by a variety of environmental factors. Selection of lines with high or low responsiveness, such as the Biozzi mouse lines (Biozzi et al., 1979), without prior selection for MHC (major histocompatibility complex) alleles, has pointed out that the MHC is only one set of genes among many (Skamene and Pietrangeli, 1991). Breeding for resistance to specific diseases seems to be associated with breeding for specific immune responsiveness and likely involves selection for certain MHC haplotypes (Outteridge, 1993). It should also be mentioned that there are several factors not belonging to the immune system that are involved in resistance to disease examples being enzymic inhibitors etc. (Kushner,1982).

Compared with livestock, fish has several advantages when it comes to improvement of disease resistance:

* The fecundity is very high in most species which make it possible to take samples from each full-sib family and test them for susceptibility to a serie of pathogens
* The economic value of young animals is low.
* Good testing procedures applying challenge tests have been developed for several diseases, parasites, species and more testing methods can easily be extended.
* It seems justifiable to challenge small fish because there are indications that there is a relatively high genetic correlation between test results obtained with small fish and resistance against the disease at a later stage.

Good genetic informations about disease resistance is available only for a few fish species, diseases and parasites. There seems, however, to be a considerable additive genetic variance for bacterial diseases (furunculosis, BKD, vibriosis and cold water vibriosis) which are the best documented, but also for virus, fungi and parasites. More information is needed particularly on the magnitude of non-additive genetic variance. Because of the limited information on non-additive genetic variance, it is not possible to recommend crossbreeding as breeding method to improve disease resistance in fish.

Since additive genetic variance is well documented for several diseases and since survival is an all or none character, it is recommended to use family selection to improve disease resistance in farmed fish.

With the present testing methods for marker traits direct selection for disease resistance is recommended. There seems to be little to gain by including correlated traits like immunological and physiological markers because of low genetic correlations with disease. The simultaneous selection for disease resistance and growth rate is likely to yield a correlated response in disease resistance.

In discussing the possibility of improving disease resistance in fish, Maclean and Penman (1990) and Jiang (1993) agree that the greatest, single contribution that gene manipulation will make to future aquaculture will be in terms of an increase of disease resistance of aquatic organisms. Furthermore

these authors state that disease resistance is, in many cases, known to depend on the possession of specific genes and yet none of these genes has been identified. Thus, the isolation of genes and producing transgenic fish which are resistant to diseases, is an intriguing possibility for future work.

The present knowledge of genetic parameters for diseas resistance is limited to a few species (Atlantic salmon, rainbow trout, tilapia, common carp, channel catfish). It is therefore urgent that research is carried out to fill this gap. To day breeding program to improve disease resistance is very rare. Taking into account the good possibiliy to improve disease resistance through selection, start of efficient breeding programs should be given high priority.

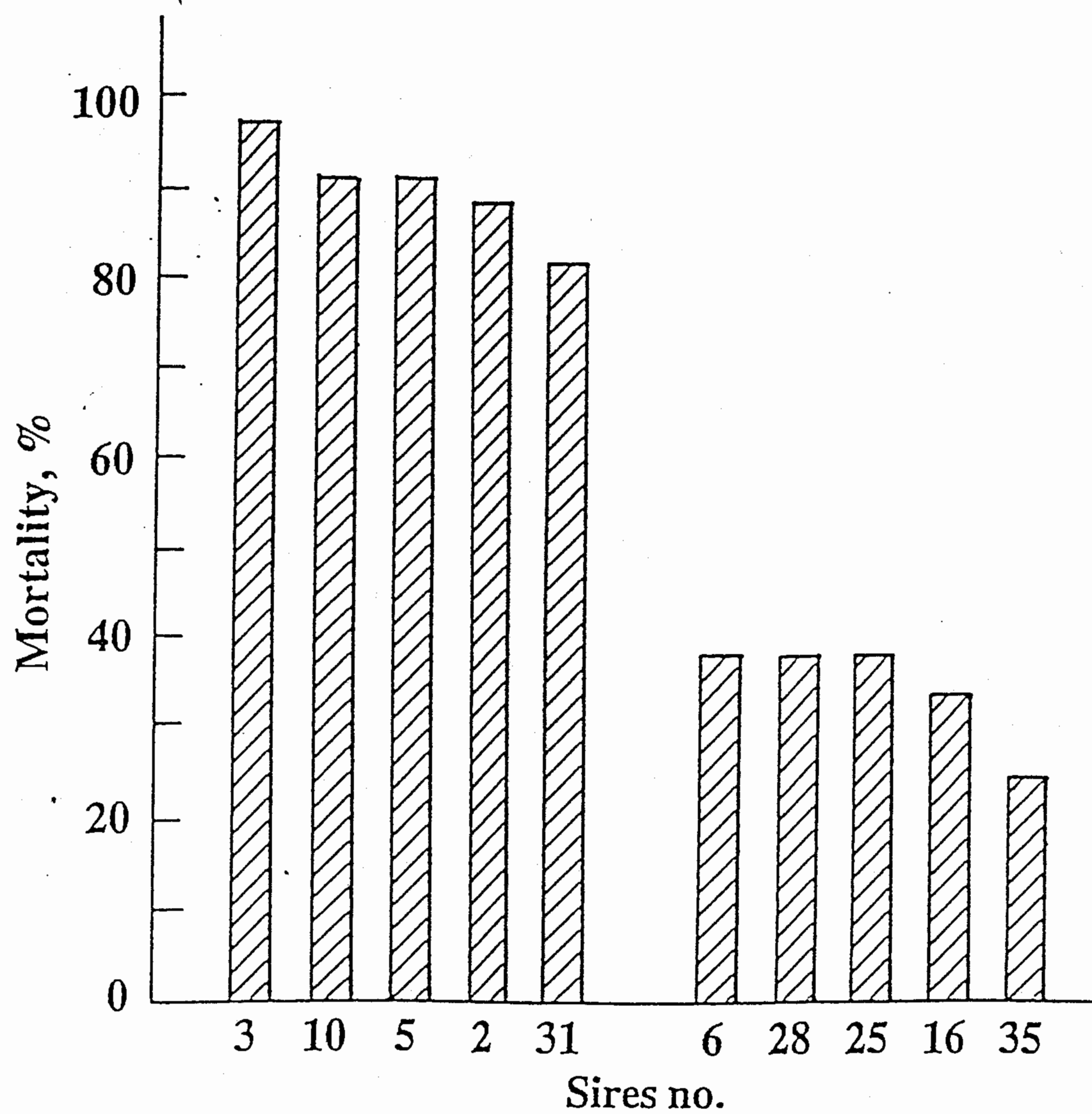

Fig. 1 Mortality of the five halfsib groups with the highest and the five with the lowest mortality (Gjedrem et al., 1991).

	Tropical fish					Subtropical fish						Cold water fish			
Diseases	Chinese carp	Common carp	Indian carp.	Tilapia	Milk fish	Grouper	Yellow tail	Eel	Sea bass	Sea bream	Catfish	Atlantic salmon	Pacific salmon	Rainbow trout	Vaccines
Bacteria:															
Aeromonas hydrophila	x	x	x	x	x			x	x		x				x
Aeromonas salmonicida												x		x	x
Pseudomonas sp.	x		x	x	x			x		x				x	
Streptoccoccus sp.				x			x	x	x	x		x	x	x	
Yersinia ruckeri												x	x	x	x
Edwardsiella tarda				x			x	x		x	x				
Myxobacteria	x	x	x	x					x		x	x	x	x	
Renibacterium salmoninarum (BKD)												x	x	x	
Pasturella sp.							x			x					
Vibrio sp.					x	x	x	x	x	x		x	x	x	x
Virus:															
IPN												x	x	x	
IHN												x	x	x	
Special virus	xx[1]										xx[2]			x[3]	
Fungi:															
Saprolgnia	x	x	x	x								x	x	x	
Parasites:															
Protozoa	x	x	x	x	x	x			x	x			x	x	
Monogena	x	x	x	x		x		x	xx	x		x			
Digena	x	x		x	x				x	x		x	x	x	
Nematoda		x		x					x	x					
Crustacea	x	x	x	x	x				x			x	x	x	

1. Haemorrhagic disease in grass carp.
2. CCVD, Channel catfish virus disease.
3. VHS, rainbow trout.

Table 2. Heritabilities for disease resistance in fish

Disease	Heritability	Species[2)]	Number of Sires	Dams	Author
Survival (unspecified)	0,08±0,02	A.s.	178	1124	Rye et.al. (1990)
	0,06±0,03	Rb.	186	770	"
"	0,04±0,01	A.s.	120	361	Kanis et.al. (1976)
"	0,14±0,03	Rb.	47	143	"
"	0,02±0,01	St.	38	115	"
"	-0,02±0,04	Bt.	32	32	Robison & Luempert (1984)
Vibriosis	0,12±0,05	A.s	42	140	Gjedrem & Aulstad (1974)
Coldwater vibriosis	0,13±0,08	A.s	32	81	Gjedrem & Gjøen (1995)
Furunculosis	0,48±0,17	A.s.	25	50	Gjedrem et.al. (1991)
	0,16±0,12	A.s.	32	81	Gjedrem & Gjøen (1994)
"	0,32±0,06	A.s.	-	42	Bailey (1986)
"	0,38[1)]	A.s.	93	171	Refstie et.al. (1993)
"	0,00±0,12	Coho	14	28	Beacham & Evelyn (1992c)
"	0.07±0,05	Chum	15	30	"
"	0,14±0,11	Chinook	15	30	"
"	0.18[1)]	Chinook	10	20	" (1992a)
BKD	0,23±0.10	A.s.	32	81	Gjedrem & Gjøen (1995)
Fungi	o.34±0.14	Ac	36	32	Nilsson (1992)

1) Average of several estimates

2) A.s. = Atlantic salmon; St. = Sea trout
Rb = Rainbow trout Bt. = Brook trout
Ac= Arctic char

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ABLE 3 **Interspecific variation of resistance of the principal salmonid species to the main viral diseases following natural challenge (waterborne virus). (after Chevassus and Dorson (1990)).**

Species	Birnavirus	Rhabdovirus			
	IPN	IHN	VHS		
			Serotype 1	Serotype 2	Serotype 3
Salmo gairdneri	S(1)	S(6)	S(9)	S(9)	S(9)
S.trutta	S(2)	?	R(10)	S(9)	S(9)
S.salar	R(2,3)	S(7)	R(11)	?	R(11)
Salvelinus fontinalis	S(1)	?	R(2)	?	R(2)
S.alpinus	S(2)	?	R(2)	?	R(2)
S.namaychus	R(2,4)	?	S(2)	?	S(2)
Oncorhynchus kisutch	R(1)	R(8)	R(10)	?	?
O.tshawytscha	R(1)	S(6)	R(12)	?	?
O. nerka	S(5)	S(6)	?	?	?
O. rhodurus	S(5)	S(6)	?	?	?

(1) Dorson, 1983 (review paper).
(2) M. Dorson, unpublished results 1986. *S.trutta* appears less susceptible to IPN compared to *S. gairdneri*. *S. namaycush* appears less susceptible to VHS compared to *S. gairdneri* and almost completely resistant to IPN.
(3) One clinical case reported, all experimental trials gave negative results (Munro et al., 1983).
(4) Silim et al., 1982.
(5) Sano, 1973.
(6) Pilcher and Fryer, 1980 (review paper).
(7) Mulcahy and Wood, 1986.
(8) Adaption of IHN to coho salmon is in progress (Hedrick et al., 1987).
(9) De Kinkelin, 1983, (review paper).
(10) De Kinkelin et al., 1974.
11) De Kinkelin and Castric, 1982. S. salar is susceptible only to injection with the virus.
(12). Ord, 1975.

S= Susceptilbe, following field or laboratory challenge, to at least one train of the virus.
R=Resistance established (no disease in infected areas nor experimental transmission).
?=No clue available concerning susceptibilities or resistance.
The different serotypes have been mentioned only when interesting differences are known.
IPN=Infectious pancreatic necrosis.
IHN=Infectious haematopoietic necrosis.
VHS=Viral haemorrhagic septicaemia.

Table 4. **Heritabilities for immune parameters in fish**

Traits	Heritability	Species[2)]	Number of Sires	Number of Dams	Author
Serum Lysozyme Activity	0,16±0,10	A.s.	31	77	Røed et.al. (1994)
	0,06[1)]	A.s.	12	34	Røed et.al. (1993)a
	0,27±0,12	Rb	30	80	Røed et.al. (1993)b
Serum Haemolytic-activity	0,15[1)]	A.s.	12	34	Røed et.al. (1993)a
	0.32±0,13	A.s.	20	57	Røed et.al. (1992)
	0.68[1)]	Rb	9	20	Røed et.al. (1990)
IgM	0.00	A.s.	31	77	Lund et.al. (1994)
Antibody-titre	0.18±0,14	A.s.	31	77	Lund et.al. (1994)
	0.16[1)]	A.s.	12	34	Strømsheim et.al. (1993)
Plasma α_2-antiplasmin activity	0.19±0.04	A.s.	20	30	Salte et.al. (1993)

[1)] Average of several estimates
[2)] A.s. = Atlantic salmon;
Rb = Rainbow trout

Table 5. **Correlation between family survival after furunculosis challenge test and average immunological and plasma protein parameters.**

Trait	Correlation with furunculosis survival	Author
Serum lycozyme activity	-0,25	Lund et.al. (1994)
	0,00[1)]	Fjalestad et.al. (1994)
Serum haemolytic-activity	0,00	Lund et.al. (1994)
	0,13[1)]	Fjalestad et.al. (1994)
IgM	0,00	Lund et.al. (1994)
"	-0,35	Fjalestad et.al. (1994)
Antibody-titre	-0,02	Lund et.al. (1994)
	0,34[1)]	Fjalestad et.al. (1994)
Plasma α_2- antiplasmin activity	0,37	Salte et.al. (1993)

[1)] Average of several estimates

Table 6. **Correlations between survival in response to different diseases using family averages after challenge test.**

Trait	Furunculosis	BKD	Coldwater vibriosis	Vibriosis
BKD	0,81[1]			
Coldwater-	0,35[1]			
vibriosis	0,43[2]	0,29[1]		
Vibriosis	0,35[2]		0,82[2]	
ILA	-0,24[2]		-0,40[2]	-0,20[2]

[1] Gjedrem and Gjøen (1995)
[2] Refstie et.al. (1993)

Table 7. **Genetic correlation between growth rate and different measurements of survival**

	Genetic correlation	Spesies	Number Sire	Dam	Author
Overall survival, fingerlings	0,30	B.t.	32	32	Robison & Luempert (1984)
Overall survival, juvenile	-0,87	B.t.	32	32	
Coldwater vibiosis, adult	0,18	A.s.	53	329	Standal & Gjerde (1987)
Overall survival, fingerlings	0,37	A.s.	187	1404	Rye et.al. (1990)
Overall survival, fingerlings	0,23	Rb.	213	1062	"
Furunculose, fingerlings, exp. challenge	0,30	A.s.	25	50	Gjedrem et.al. (1991)
Overall survival, fingerlings	0,31	A.s.	100	298	Jonasson (1993)
Fungal infection	0.50	A.c.	36	32	Nilsson (1992)

A.s. = Atlantic salmon
Rb = Rainbow trout
B.t. = Brook trout
A.c. = Arctic char

REFERENCE

ADB/NACA. 1991. Fish health management in Asia-Pacific. Report on a regional study and workshop on fish disease and fish health management. ADB Agriculture Department Report Series No. 1, Network of Aquaculture Centres in Asia-Pacific. Bangkok, thailand. 627pp.

Amend, D.F. and J. R. Nelson. 1977. Variation in the susceptibility of sockeye salmon Oncorhynchus nerka to infectious haemopoetic necrosis virus. J. Fish Biol. 11: 567-573.

Ayles, G.B. 1974. Relative importance of additive genetic and maternal sources of variation in early survival of young splake hybrids. J. Fish. Res. Board Can. 31: 1499-1502.

Ayles, G.B. and R.F. Baker. 1983. Genetic differences in growth and survival between strains and hybrids of rainbow trout (Salmo gardneri) stocked in aquaculture lakes in the Canadian prairies. Aquaculture 33: 269-280.

Bailey, J.K.1986. Differential survival among full sib Atlantic salmon (Salmo salar) families exposed to furunculosis. Salmon Genetic Research Program, Report Series, Technical Report No. 58, 6 pp.

Bakke, T. A. 1991. A review of the inter- and intraspecific variability in salmonid hosts to laboratory infections with Gyrodactylus salaris Malmberg. Aquaculture 98: 303-310.

Bakke, T. A., P.A. Jansen and L.P. Hansen. 1990. Differences in the host resistance of Atlantic salmon Salmo salar L. stocks to the monogenean Gyrodactylus salaris Malmberg, 1957. J. Fish Biol. 37: 577-587.

Bakke, T. A., L.P. Hansen and R. Nordmo. 1992. The susceptibility of a Swedish Baltic Salmon stock (Salmo salar) to Norwegian Gyrodactylus salaris Malmberg (Monogenea). In: Proceedings of the European Federation of Parasitologists VI European Multicolloquium of Parasitology, Hague, The Netherlands. Netherlands Society for Parasitologists.

Bakke, T. A. and K. MacKenzie. 1993. Comparative susceptibility of Native Scottish and Norwegian stocks of Atlantic salmon, Salmo salar L. to Gyrodactylus salaris Malmberg: Laborative experiments. Fish. Research 17: 69-85.

Beacham, T.D. and Evelyn, T.P.T.1992a. Population and genetic variation in resistance of chinook salmon to vibriosis, furunculosis, and bacterial kidney disease. Journal of Aquatic Animal Health 4, 153-167.

Beacham, T.D. and T.P.T. Evelyn. 1992b. Genetic variation in disease resistance and growth of chinook, coho, and chum salmon with respect to vibriosis, furunculosis, and bacterial kidney disease. Trans. Amer. Fish. Soc. 121: 456-485.

Beacham, T.D. and T.P.T. Evelyn. 1992c. Genetic variation in disease resistance and growth of chinook, coho, and chum salmon with respect to vibriosis, furunculosis, and bacterial kidney disease. Trans. Amer. Fish. Soc. 121: 456-485.

Biozzi, G., D. Mouton, O.A. Sant'Anna, H.C. Passos, M. Gennari, M.H. Reis, V.C.A. Ferreira, A.M. Heumann, Y. Bouthillier, O.M. Ibanez, C. Stiffel and M. Siqueira. 1979. Genetics of immunoresponsiveness to natural antigens in the mouse. Curr. Top. Microbiol. Immunol. 85: 31-98.

Bondad-Renataso, M. G. and J.R. Arthur. 1990. The parasite of Nile tilapia (Oreochromis niloticus, L.) in the Philippines, including an analysis of changes in the parasite fauna of cultured tilapia from fry to marketable size. The Second Asian Fisheries

Forum. Proceedings of the Second Asian Fisheries Forum, Tokio, Japan, 17-22 April 1989. pp 729-734.

Buchanan, D. V., J.E. Sanders, J.L. Zinn and J.L. Fryer. 1983. Relative susceptibility of four strains of summer steelhead to infection by Ceratomyxa shasta. Trans. Amer. Fish. Soc. 112: 541-543.

Chevassus, B. and M. Dorson. 1990. Genetics of resistance to disease in fishes. Aquaculture 85: 83-107.

Ching, H. L. and L. Parker. 1989. Experimental exposure of trout and salmon from 12 British Columbia stocks to the Myxozoan Parasite Ceratomyxa shasta. J. Aquatic Animal Health 1: 205-208.

Cipriano, R. C. 1983. Resistance of salmonids to Areomonas salmonicida: Relation between agglutinins and neutralizing activities. Trans. Amer. Fish. Soc. 112: 95-99.

Cipriano, R.O. and C.W.III. Heartwell. 1986. Susceptibility of salmonids to furunculosis: differences between serum and mucus responses against Aeromonas salmonicida. Trans. Amer. Fish. Soc. 115: 83-88.

Clayton, G. M. and D.J. Price. 1987. Standardization of infection and response to white spot, Ichthyophthirius multifiliis, in fish. J. Fish Biol. 31: 241-242.

De Kinkelin, P. 1983. Viral haemorrhagic septicemia. Antigens of fish pathogens. Collection Foundation Marcel Merieux, Lyon, pp. 51-62.

De Kinkelin, P. and J. Castric. 1982. An experimental study of the susceptibility of Atlantic salmon fry, Salmo salar, to viral haemorrhagic septicaemia. J. Fish Diseases 5: 57-65.

De Kinkelin, P., M. Le Berre and A. Meurillon. 1974. Septicemie hemorrragique virale: demonstration de l'etat refractaire du saumon coho (Oncorhynchus kisutch) et de la truite Fario (Salmo trutta). Bull. Fr. Piscic. 253: 166-176.

Dorson, M. 1983. Infectious pancreatic necrosis of salmonids: overview of current problems. In: D. Anderson, M. Dorson and P. Dubourget (Editors), Antigens of fish pathogens. Collection Foundation Marcel Merieux, Lyon, pp. 7-31.

Ehlinger, N.F. 1977. Selective breeding of trout for resistance to furunculosis. New York Fish and Game Journal 24: 25-36.

Embody, G.C. and C.D. Hayford. 1925. The advantage of rearing brook trout fingerlings from selected breeders. Trans. Amer. Fish. Soc. 55: 135-138.

Falconer, D.S. 1961. An introduction to quantitative genetics. Longman Group Limited, New York, NY, pp 1-340.

FAO. 1995. Review of the state of world fisheries resouces: Aquaculture. FAO Fisheries Circular No. 886.127 pp.

Ferguson, H. W. and D.A. Rice. 1980. Post-spawning mortalities in brown trout Salmo trutta L. J. Fish Disease 3: 153-160.

Fevolden, S.E., T. Refstie and K.H. Røed. 1991. Selection for high and low stress response in Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss). Aquaculture 95: 53-65.

Fevolden, S.E., T. Refstie and K.H. Røed. 1992. Disease resistance in rainbow trout (Oncorhynchus mykiss) selected for stress response. Aquaculture 104: 19-29.

Fevolden, S.E., R. Nordmo, T. Refstie and K.H. Røed. 1993. Disease resistance in Atlantic salmon (Salmo salar) selected for high or low responses to stress. Aquaculture 109: 215-224.

Fijan, N.F. 1972. Infectious dropsy of carp-A disease complex. Symposium of the Zoological Society of London, 30: 39-52.

Fjalestad, K.T., T. Gjedrem and B. Gjerde. 1993. Genetic improvement of disease resistance in fish: an overview. Aquaculture 111: 65-74.

Fjalestad, K.T., T. Gjedrem, T. Lund, K.H. Røed and T. Refstie. 1994. Immune parameters

as indirect selection criteria against furunculosis. In manuscript.

Gjedrem, T. (1967). Selection indexes compared with single trait selection. I. The efficiency of including correlated traits. Acta Agric. Scand. 17: 263-268.

Gjedrem, T. 1983. Genetic variation on quantitative traits and selective breeding in fish and shellfish. Aquaculture 33: 51-72.

Gjedrem, T. 1985. Improvement of productivity through breeding schemes. GeoJournal 10(3): 233-241.

Gjedrem, T. (1992). Breeding plan for rainbow trout. Aquaculture 100: 73-83.

Gjedrem, T. and D. Aulstad. 1974. Differences in resistance to vibrio disease of salmon parr. Aquaculture 3: 51-59.

Gjedrem, T. and H.M. Gjøen. 1995. Genetic variation in susceptibility of Atlantic salmon, Salmo salar L., to furunculosis, BKD and cold water vibriosis. Aquaculture Research 26: 129-134.

Gjedrem, T., R. Salte and H.M. Gjøen. 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. Aquaculture 97: 1-6.

Gjerde, B. 1986. Growth and reproduction in fish and shellfish. Aquaculture 57:37-55.

Gjerde, B. and T. Refstie. 1984. Complete diallel cross between five strains of Atlantic salmon. Livestock Prod. Sci. 11: 207-226.

Hedrick, R. P., S.E. Lapatra, J.L. Fryer, T. McDowell and W.H. Wingfield. 1987. Susceptibility of coho (Oncorhynchus kisutch) and chinook (Oncorhynchus tshawytscha) salmon hybrids to experimental infections with infectious hematopoietic necrosis virus (IHNV). Bull. Eur. Assoc. Fish Pathol. 7(4): 97.

Hemmingsen, A: R., R.A. Holt and R.D. Ewing. 1986. Susceptibility of progeny from crosses among three stocks of coho salmon to infection by Ceratomyxa shasta. Trans. Amer. Fish. Soc. 115: 492-495.

Hines, R.S., G.W. Wohlfarth, R. Moav and G. Hulata. (1974). Genetic susceptibility to two diseases among strains of the common carp. Aquaculture 3: 187-197.

Houghton, G., G.F. Wiergertjes, A. Groenveld and W.B. VanMuiswinkel. 1991. Differences in resistance of carp, Cyprinus carpio L., to atypical Aeromonas salmonicida. J. Fish Diseases 14: 333-341.

Ibarra, A: M., R.P. Hedrick and G.A.E. Gall. 1992. Inheritance of susceptibility to Ceratomyxa shasta (Myxozoa) in rainbow trout and the effect of length of exposure on the liability to develop ceratomyxosis. Aquaculture 104: 217-229.

Ibarra, A. M., R.P. Hedrick and G.A.E. Gall. 1994. Genetic analysis of rainbow trout susceptibility to the myxosporean, Ceratomyxa shasta. Aquaculture 120: 239-262.

Ilyassov, Y.I. 1987. Genetic principles of fish selection for disease resistance. Proceeding of World Symposium on Selection, Hybridization, and genetic engineering in aquaculture, Bordeaux 27-30 May, 1986, Berlin. Vol. I, 455-469.

Jiang, Y. 1993. Transgenic fish - gene transfer to increase disease and cold resistance. Aquaculture 111: 31-40.

Jonasson, J. 1993. Selection experiments in salmon ranching. I. Genetic and environmental sources of variation in survival and growth in freshwater. Aquaculture 109, 225-236.

Kanis, E., T. Refstie and T. Gjedrem. (1976). A genetic analysis of egg, alevin and fry mortality in salmon (Salmo salar), sea trout (Salmo trutta) and rainbow trout (Salmo gairdneri). Aquaculture 8, 259-268.

Kinghorn, B.P. 1983. A review of quantitative genetics in fish breeding. Aquaculture 31: 283- 304.

Kirpichnikov, V.S., K.A. Factorvich, Y.I. Ilyassov and L.A. Shart. (1976). Selection of common carp (Cyprinus carpio) for resistance to dropsy. FAO Technical Conference on Aquaculture, Kyoto, Japan 26 May-2 June. 628-632

Kirpichnikov, V. S., Y.I. Ilyassov, L.A. Shart, A.A. Vikhman, M.V. Ganchenko, L.A. Ostashevsky, V.M. Simonov, G.F. Tikhonov and V.V. Tjurin. 1993. Selection of krasnodar common carp (Cyprinus carpio L.) for resistance to dropsy: principal results and prospects. Aquaculture 111: 7-20.

Klupp, R. 1979. Genetic variance for growth in rainbow trout (Salmo gairdneri). Aquaculture 18: 123-134.

Kushner, I. 1982. The phenomenon of the acute phase response. Ann. N.Y. Acad. Sci. 389:39-.

Lewis, R.C. 1944. Selective breeding of rainbow trout at Hot Creek Hatchery. California Fish and Game 30, 95-97.

Lightner, D., R. Redman, L. Mohney, G. Dickenson and K. Fitzsimmons. 1988. Major diseases encountered in controlled environment culture of tilapias in fresh- and brackishwater over a three-year period in Arizona. In The Second International Symposium on tilapia in Aquaculture, Bangkok, Thailand, 16-20 March 1987. pp 111-116.

Llewellyn, L. C. 1980. A bacterium with similarities to the redmouth bacterium and Serratia liquefaciens (Grimmes and Hennerty) causing mortalities in the hatchery reared salmon in Australia. J. Fish Disease 3: 29-39.

Lund, T., T. Gjedrem, H.B. Bentsen, D.M. Eide, H.J.S. Larsen and K.H. Røed. 1995. Genetic variation in immune parameters and associations to survival in Atlantic salmon. J. Fish Biol. 46: 748-758.

Maclean, L. and D. Penman. 1990. The application of gene manipulation to aquaculture. Aquaculture 84: 1-20.

McIntyre, J. D. and D.F. Amend. 1978. Heritability of tolerance for infectious hematopoietic necrosis in sockeye salmon (Oncorhynchus nerka). Trans. Amer. Fish. Soc. 1o7: 305-308.

Moav, R. and G.W. Wohlfarth. 1974. Magnification through competition of genetic differences in yield capacity in carp. Heredity 33: 181-202.

Moav, R. and G.W. Wohlfarth. 1976. Two way selection for growth rate in the common carp (Cyprinus carpio). Genetics 82: 83-101.

Mulcahy, D. and J. Wood. 1986. A natural epizootic of infectious haematopoietic necrosis in imported Atlantic salmon, Salmo salar, reared in the enzootic regions. J. Fish diseases 9: 173-175.

Munro, A. I. S., D.A. Smail, I.F. Waldell and K. Elson. 1983. Studies of IPN virus in farmed Atlantic salmon in Scotland. Proc. 4th Coprag Meeting on Fish and Shellfish Diseases. Oct. 1981, Cadiz, Acuigrup, Madrid, pp. 33-42.

Natividad, J.M., M.G. Bondad-Reantaso, and J.R. Arthur. 1986. Parasites of Nile tilapia (Oreochromis niloticus) in the Philippines. Proceedings of the First Asian Fisheries Forum, Manila, Philippines, 26-31 May 1986.pp 255-259.

Nilsson, J. 1992. Genetic variation in resistance of Arctic char to fungal infection. J. Aquatic Anim. Health 4: 126-128.

Novotny, A. 1975. Net-pen culture of pacific salmon in marine waters. Marine Fish. Review, 37 (1): 36-47.

Okamoto, N., T. Tayama, M. Kawanobe, N. Fujiki, Y. Yasuda and T. Sano. 1993. Resistance of a rainbow trout strain to infectious pancreatic necrosis. Aquaculture 117: 71-76.

Olivier, G., G.W. Friars and J. Bailey. 1988. Genetic basis for resistance to furunculosis in Atlantic salmon (Salmo salar). Bull. Aqua. Assoc. Can. Bull. Assoc. Aquacole Can. no. 88-2, pp.88.

Ord, W. 1975. Resistance of chinook salmon (Oncorhynchus tshawytscha) fingerlings experimental infected with viral hemorrhagic virus. Bull. Fr. Piscic. 257: 149-152.

Outteridge, P. M. 1993. High and low responsiveness to vaccines in farm animals. Immunol. Cell Biol. 71: 355-366.

Pilcher, R. S. and J.L. Fryer. 1980. The viral diseases of fish: a review through 1978. Part II. Disease in which a viral etiology is suspected but unproven. Part I. Disease of proven viral etiology. Reprinted from CRC Crit. Rev: Microbiol. 7(4):287-364; 3(1): 1-25. ORES U-R 80-019.

Pillay, T.V.R. 1990. Aquaculture, principles and practices. Fishing News Books, 576 pp.

Plumb, J. A., O.I. Green, R.O. Smitherman and G.B. Pardue. 1975. Channel catfish virus experiments with different strains of channel catfish. Trans. Amer. Fish. Soc. 104: 140-143.

Plumb, J. A. and D,J. Sanchez. 1983. Susceptibility of five species of fish to Edwardsiella ictaluri. J. Fish diseases 6: 261-266.

Pojoga; J. 1972. Race metis et hybrides chez la carpe. Bull. Fr. Piscic. 244: 134-142.

Poppe, T., A.F. Frøslie, N. Koppang and T. Håstein. 1985. Muskeldegenerasjon med eksudativ hemorrhagisk diatese hos oppdrettslaks-»Hitrasjuke». Norsk Veterinærtidsskrift 97(3): 159-165.

Price, D. J. 1985. Genetics of susceptibility and resistance to disease in fish. J. Fish Biol. 26: 509-519.

Refstie, T. 1982. Preliminary results: differences between rainbow trout families in resistance against vibriosis and stress. In: W. B. Van Muiswinkel and E. L. Cooper (Editors), Conf. on Immunology and Immunization of Fish. 22 June 1981, Wageningen, The Netherlands. Dev. Comp. Immunol. Suppl. 2: 205-209.

Refstie, T. (1990). Application of breeding schemes. Aquaculture 85: 163-169.

Refstie,T., B. Gjerde and T. Gjedrem. 1993. Selection for improved disease resistance in farmed salmon. (Seleksjon for bedre motstandsevne mot sjukdom hos oppdrettslaks). Project report: MB-913 40026. AKVAFORSK, Norway. (In Norwegian).

Roberts, R.J. and C. Sommerville. 1982. Diseases of tilapias.p. 247-263. In R.S.V. Pullin and R.H. Lowe-McConnell (eds) The biology and culture of tilapias: ICLARM Conference Proceedings 7. International Center for Living Aquatic Resources Management, Manila, Philippines.

Robison, O.W. and L.G. Luempert 1984. Genetic variation in weight and survival of brook trout (Salvelinius fontinalis). Aquaculture 38: 155-170.

Rye, M., K.M. Lillevik and B. Gjerde. 1990. Survival in early life of Atlantic salmon and rainbow trout: estimates of heritabilities and genetic correlations. Aquaculture 89: 209-216.

Røed, K.H., E. Brun, H.J.S. Larsen and T. Refstie. 1990. The genetic influence on serum haemolytic activity in rainbow trout. Aquaculture 85: 109-117.

Røed, K.H., K.T. Fjalestad, H.J.S. Larsen and L. Midthjell. 1992. Genetic variation in haemolytic activity in Atlantic salmon (Salmo salar L.) J. Fish Biol. 40: 739-750.

Røed, K.H., K.T. Fjalestad and A. Strømsheim. 1993a. Genetic variation in lysozyme activity and spontaneous haemolytic activity in Atlantic salmon (Salmo salar). Aquaculture 114, 19-31.

Røed, K.H., H.J.S. Larsen, R.D. Linder and T. Refstie. 1993b. Genetic variation in lysozyme activity in rainbow trout (Oncorhynchus mykiss). Aquaculture 109, 37-244.

Salte, R., H.M. Gjøen, K. Norberg, and T. Gjedrem. 1993. Plasma protein levels as potential marker traits for resistance to furunculosis. J. Fish Diseases 16, 561-568.

Sanders, J.E., J.L. Fryer and R.W. Gold. 1970. Occurrence of the myxosporidian parasite Ceratomyxa shasta in salmonid fish from Columbia river basin and Oregon coastal streams. In a Symposium on diseases of fishes and shellfishes, ed. S.F. Snieszko, pp. 133-141, Special publication no. 5. Washington, D.C.: Amer. Fish. Soc.

Sano, T. 1973. Studies on viral diseases of Japanese fishes. IV. Infectious pancreatic necrosis of rainbow trout: susceptibility of fresh water salmonids of genus Oncorhynchus. Bull. Jpn. Soc. Sci. Fish. 39 (2): 117.

Schaperclaus, W. 1961. Lehrbuch der Tierchwirtschaft. Berlin, P. Parey, pp 1-582.

Schaperclaus, W. 1962. Traite de Pisciculture en Etang. Vigot Freres, Paris, pp.208-227.

Silim, A., M.A.S.Y. Elazhary, and A. Lagace. 1982. Susceptibility of trouts of different species and origins to various isolates of infectious pancreatic necrosis virus. Can. J. Fish. Aquat. Sci. 39: 1580-1584.

Singhal, R.N., S. Jeet and R.W. Davies. 1987. Experimental transmission of Saprolegnia and Achlya to fish. Aquaculture 64: 1-7.

Skamene, S. and C.E. Pietrangeli. 1991. Genetics of the immune response to infectious pathogens. Curr. Opin. Immunol. 3: 511-517.

Smoker, W.W. 1986. Variability of embryo development rate, fry growth, and disease susceptibility in hatchery stock of chum salmon. Aquaculture 57: 219-226.

Snieszko, S. F., C. N. Dunbar and G.L. Bullock. 1959. Resistance to ulcer disease and furunculosis in eastern brook trout, Salvelinus fontinalis. Prog. Fish Cult. 21: 111-116.

Sovenyi, J.F., M. Bercsenyi and J. Bakos. 1988. Comparative examination of susceptibility genotypes of carp (Cyprinus carpio L.) to infection with Aeromonas salmonicida. Aquaculture 70, 301-308.

Standal, M. and B. Gjerde. 1987. Genetic variation in survival of Atlantic salmon during the sea-rearing period. Aquaculture 66, 197-207.

Strømsheim, A., D.M. Eide, K.T. Fjalestad, H.J.S. Larsen and K.H. Røed. 1993. Genetic variation in the immune response against Aeromonas salmonicida A-layer in Atlantic salmon (Salmon salar). Dr.s thesis. Norwegian College of Veterinary Medicine, Oslo, Norway,

Supriyadi, H. 1986. The susceptibility of various fish species to infection by the bacterium Aeromonas hydrophia. Proceedings from The First Asian Fisheries Forum , Manila, Philippines, 26-31 May, p 241-242.

Tave,D., J.E. Bartels and R. O. Smitherman. 1983. Saddleback: a dominant, lethal gene in rotherodon aureus (Steindachner) (=Tilapia aurea). J. Fish Diseases 6: 59-73.

Winter, G. W., C. B. Schreck and J.D. McIntyre. 1980. Resistance of different stocks and transferrin genotypes of coho salmon, Oncorhynchus kisutch, and steelhead trout, salmo gairdneri, to bacterial kidney disease and vibriosis. Fish. Bull. 77: 795-902.

Thune, R.L., T.E. Graham, L.M. Riddle and R.L. Amborski. 1982. Effects of Aeromonas hydrophila extracellular products and endotoxins. Trans. Amer. Fish. Soc. 111: 749-754.

Yamamoto, S., I. Sanjyo, R. Sato, M. Kohara and H. Thara. 1991. Estimation of the heritability for resistance to infectious hematopoietic necrosis in rainbow trout. Nippon Suisan Gakkaishi Bull. Jap. Soc. Sci. Fish. 57: 1519-1522.

Zinn, J.L., K.H. Johnson, J.E. Sanders and J.L. Fryer. 1977. Susceptibility of salmonid species and hatchery strains of chinook salmon (Oncorhynchus tshawytscha) to infections by Ceratomyxa. J. Fish. Resh. Board Can. 34: 933-936.

Wolf, L. E. 1954. Development of disese resistant strains of fish. Trans. Amer. Fish. Soc. 83: 342-249.

Woo, P. T. K. 1992. Immunological responses of fish to parasitic organisms. Annual Review of Fish Diseases, pp.339-366.

Woo,P. T. K. and M. Shariff. 1990. Lernea cyprinacea L. (Copepoda: Caligidea) in Helostoma temmincki Cuvier and Valenciennes: the dynamics of resistance in recovered and native fish. J. Fish Diseases 13: 485-493.

Attachment 12.

Outline of guiding principles for conducting the "Research and development of salt tolerant Nile tilapia or for any aquaculture development in brackish/marine waters in general"

Guiding Principles for Conducting the Research and Development of Salt Tolerant Nile Tilapia or for any Aquaculture Development in Brackish/Marine waters in General

Development. Before any aquaculture development is undertaken in brackish/marine waters, the goals and market need to be established. The benefits could be more than the economic return from the project, food, employment and relieve fishing pressure.

Coastal lagoons, estuaries, inland saline lakes and regions with saline soil represent resources that are often poorly utilized and to which aquaculture could safely be applied.

The species that will be selected for culture in brackish/marine waters should be based on a number of criteria which include: (i) availability, (ii) ease of cultured/domestication, (iii) market demand; and (iv) potential risk to environment and the native species.

While Nile tilapia, for a variety of reasons, will be the species of choice for base material on which to found a selective breeding programme for salinity tolerance, it is unlikely to be the most appropriate species in all such situations.

The use of exotic species and GMO, IMO, etc. should be evaluated against the use of local species. International codes of practice and guidelines should be applied to the use of exotic species and GMOs.

Several unknown exist concerning the impact and benefits of development of a new strain for aquaculture (genetic, ecological, biological), therefore a precautionary approach should be adopted and the benefits should be great enough to risk failure.

Technology can and should be applied to increase aquaculture production. The choices range from acclimation to saline waters to growout only, to selection in both farms and nature, to hybridization, to chromosome manipulation, to gene transfer. Choice will be determined by state of knowledge, ease, consumer attitudes, and risk to environment.

Development plans for aquaculture in brackish/saline areas will be influenced by background of the proponents. Therefore, it is necessary to have proposal consistent with an overall integrated area management plan.

Proposal or development plan should consult the aquaculture industry for similar on-going activities and for its view of the plan.

The genetic architecture of salinity tolerance is poorly understood and should be investigated. Nile tilapia and related species would be good candidate species for this exercise.

Conservation. The valuable aquatic resources should be protected and conserved.

The levels of acceptable impacts on environments and aquatic biodiversity should be defined and agreed.

It is assumed that cultured species will eventually escape into nature. Hence, development plan should strive to minimize risk to the environment and biodiversity. Some option includes production of sterile animals (or others not capable of reproducing in saline waters), location of hatchery in freshwater area and have only grow-out in saline area and utilization of local species rather than exotic.

Utilization of brackish/saline water may involve several of the suggested solutions and there will be different solutions for different areas.

Attachment 13.

A compilation of Abstracts from Master of Science Theses.

ABSTRACTS OF COMPLETED MSc THESIS USING GIFT MATERIAL

AFAN, LILIBETH B.	Fingerling Production of Genetically Improved Farmed Tilapia (GIFT) Nile tilapia *(Oreochromis niloticus L.)* Broodstock as Influenced by the Frequency of Zeolite Application
AGUSTIN, LIZA Q.	Assessment of the Growth Rate and Nutritional Status of Six Strains of Oreochromis niloticus reared under different Agroclimatic Conditions[2, 3]
BAAY, MIGUEL O.	Growth and Yield of Genetically Improved Farmed Tilapia (GIFT) Nile tilapia *(Oreochromis niloticus L.)* at Varying Fertilization Trials[1]
DE VERA, MARIETTA P.	Effects of culture environments, sex and initial size on relative growth and survival of Nile tilapia (Oreochromis niloticus)[2, 3]
DIONISIO, EDNA E.	Progeny Sex Ratio in a Complete Diallel Cross with Eight Diverse strains of Nile tilapia (*Oreochromis niloticus L.)*[3]
FERNANDEZ, JAIME S.	Response of Genetically Improved Farmed Tilapia (GIFT) Nile tilapia *(Oreochromis niloticus L.)* to Varying Dietary Protein and Gross Energy Levels[1]
FRANCISCO, MELBA E.	Growth Performance and Survival of Genetically Improved Farmed Tilapias (GIFT) Nile tilapia *(Oreochromis niloticus L.)* Raised in Cages at Various Stocking Densities[1]
GARCIA, LILIAN C.	Growth and survival of Genetically Improved Farmed Tilapia (GIFT): Nile tilapia *(Oreochromis niloticus L.)* at Three Stocking Densities[1]
LONGALONG, FELICISIMA M.	Response to Selection for Low and High Occurrence of Sexual Maturation at a Fixed Age in Nile tilapia *(Oreochromis niloticus L.)*[2, 3]
MENDOZA, ALMA M.	Growth and Survival of Genetically Improved Farmed Tilapia: Nile tilapia *(Oreochromis niloticus L.)* In Cages fed with Practical Diets
PAPA, DELFIN C.	Caudal fin bar as indicator of growth performance in seven strains of Nile tilapia (Oreochromis niloticus *L.)*[3]

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1 thesis financial grants provided by Fishery Sector Program (FSP) of the Philippine Department of Agriculture
2 thesis studies given outstanding marks by the university
3 thesis studies which are complementary to GIFT Project objective

ABSTRACT

AFAN, LILIBETH BALAJADIA, Institute of Graduate Studies, Central Luzon State University, Philippines, April 1996. **FINGERLING PRODUCTION OF GENETICALLY IMPROVED FARMED TILAPIA (*Oreochromis niloticus L.)* BROODSTOCK AS INFLUENCED BY THE FREQUENCY OF ZEOLITE APPLICATION.**

Adviser: Prof. Danilo C. Monje

Breeding of Genetically Improved Farmed Tilapia was conducted in 12 earthen ponds for a period of 60 days. Four treatments with three replicates were randomly assigned to 12 breeding ponds. The treatments used were: Treatment I - control, no zeolite application; Treatment II, weekly application; Treatment III, biweekly application and treatment IV, monthly application.

Results show that zeolite application had no significant effect (P.0.05) on fingerling production of *O. niloticus* (GIFT) broodstock across all treatments after 60 days breeding period. Treatment I gave a mean production of 119, 640, Treatment II, 61, 772; Treatment III, 69, 653 and Treatment IV 93, 925. However, fingerling production of the four treatment did not differ significantly.

The water quality parameters measured/monitored were all at the ideal range for tilapia culture. The mean pH reading 3 hours before and 3 hours after zeolite application were 7.66 and 8.17 respectively which was still within the range of 6.5 to 9 as the desirable range for pond fish culture (Boyd, 1990 and Cagauan, 1992). However, the increase of 0.51 on the pH reading 3 hours before and after application maybe attributed to the reaction of zeolite application, (Arctus Enterprises Inc., 1996). Cacations were released in exchange for NH_3 that were absorbed by the zeolite, thus increasing the pH. The mean NH_3 reading hours before and after application of zeolite were 0.39 and 0.24 mg/li. Respectively which was still low to create ammonia toxicity and still on the safe level for tilapia culture. However, it still shows the absorbing capabilities of zeolite to ammonia, because it lowered the ammonia level in all the treatments wherein zeolite was applied.

The cost and return analysis shows that Treatment I gave the highest net return which only suggest that no application of zeolite on fingerling production of tilapia is economical.

The study therefore concludes that zeolite as a water quality enhancer/pollutant absorber does not significantly (P>0.05) affect the increase of fingerling production of *O. niloticus* (GIFT) broodstock. This may suggest that the management practices used in this study as regards water quality management water at the desired level for breeding.

ABSTRACT

AGUSTIN, LIZA Q., Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, Philippines, October 1991, **ASSESMENT OF THE GROWTH RATE AND NUTRITIONAL STATUS OF SIX STRAINS OF *OREOCHROMIS NILOTICUS* REARED UNDER DIFFERENT AGROCLIMATIC CONDITIONS.**

Advisers: Dr. Gloria N. Pocsodio
Dr. Julie Macaranas

Four African strains (Egypt, Ghana, Kenya and Sénégal) and two Philippine strains (Singapore and Taiwan) of Nile tilapia *Oreochromis niloticus* were assessed for their growth rate and nutritional status under different agroclimatic conditions. This was done using primarily muscle **RNA:DNA** ratios correlated with other growth and nutritional parameters such as specific growth rate, protein content and condition factor (K).

Individually tagged fish of the same were reared in three test environments namely: Slow Growth (SG) Environment - Bay, Laguna, Fast Growth (FG) Environment - Muñoz, Nueva Ecija and a stressful Low Temperature (LT) Environment - La Trinidad, Benguet representing different levels of fertilizers input and ambient temperature conditions. A controlled laboratory feeding experiment provided the standards for comparisons. Control fish fed *ad libitum* had a mean **RNA:DNA** ratio of 4.37 while control fish totally starved had a mean **RNA:DNA** ratio of 3.29.

As observed, fish reared in a good agroclimatic condition had high growth rates and were in good nutritional status while fish reared in poor environment exhibited very low growth rates poor nutritional status. Results in FG confirmed that fish were in good nutritional status, relatively well-fed and rapidly growing with an **RNA:DNA** ratio of 3.45 and a specific growth rate of 2.14. In SG. The fish were slightly in starving condition with an **RNA:DNA** ratio of 3.18 and a specific growth rate of 1.06. Fish reared in poor environment like LT had very low **RNA:DNA** ratio of 1.50 and low specific growth rate of -0.01 indicating very poor nutritional status with severe starvation.

The use of muscle **RNA:DNA** ratios provided accurate indices of growth rate and nutritional status in *O. niloticus.* Significant correlations were observed between specific growth rate and **RNA:DNA** ratio and protein content of fish. The condition factor was sensitive to factors like maturation of fish, feeding activity, etc. and was not a good index of the growth performance and nutritional status of tilapia.

Significant differences in growth performance were observed in the six strains of tilapia. Kenya was observed to be the fastest growing strain while Singapore strain the slowest in both in the control feeding experiment and when reared in test environments.

The test of the strains were intermediate. There were no significant strain differences in their **RNA:DNA** ratios and protein content.

GROWTH AND YIELD OF GENETICALLY IMPROVED FARMED TILAPIA *(Oreochromis niloticus)* AT VARYING FERTILIZATION TRIALS

MIGUEL O. BAAY

ABSTRACT

Growth and production of Genetically Improved Farmed Tilapia *(O. niloticus)* were evaluated under four (4) different fertilization inputs for 120 days in earthen ponds.

Twelve (12) earthen ponds measuring 500m^2 each were stocked with Genetically Improved Farmed Tilapia (GIFT) fingerlings with initial weight ranging from 1.46 to 3.92g at a density of 2 fish per m^2.

The study showed a harvest mean weight of 28.66 in Treatment IV as the lowest with 95.54g in Treatment I as the highest in four months. The highest mean total yield of 1.951.60 kg/ha/crop was obtained from Treatment I (inorganic fertilizers) followed by Treatment II (chicken manure) with 1, 606.40 kg/ha/crop, Treatment III (sugarcane bagasse alone) with 1, 492.0 kg and 706.60 kg/ha/crop, respectively. Statistical analysis showed significant differences in mean weight among treatments.

Among the water quality parameters, DOPM (r= -0.79), SDVAM (r= -0.69), SDVPM (r= -0.71), pHAM (r= 0.74) and pHPM (r= 0.81) gross photosynthesis (r= 0.98), net photosynthesis (r= 0.97) and respiration (r= 0.89) were found to have the greatest influence in fish yield.

Economic analysis showed that Treatment I gave the highest net return of P 97, 139/ha/yr, followed by Treatments II (P 66, 046) and III (P 42, 825) per ha/yr, while Treatment IV gave a return of (P 2, 890.00).

However, if sugarcane bagasse were given free and located near the project site, Treatment III would have obtained a net return of P 66, 644/ha/yr while Treatment IV, had P 29, 050/ha/yr.

ABSTRACT

DE VERA, MARIETTA, PALADA, Institute of Graduate Studies, Central Luzon State University, Philippines, October 1992. EFFECTS OF CULTURED ENVIRONMENTS, SEX AND INITIAL SIZE ON RELATIVE GROWTH AND SURVIVAL OF DIFFERENT STRAINS OF NILE TILAPIA *(Oreochromis niloticus L.).*

Advisers: Prof. Remedios Bolivar
Dr. Ambekar E. Eknath

The premise of this thesis was to understand individual growth characteristics of different strains of Nile tilapia *(Oreochromis niloticus L.)* under actual production conditions. Under this broad topic of determining the mechanisms of growth variation, the focus was on studying the effects of initial size and age on subsequent growth performance, the size-, sex- and strain-specific growth characteristics and the general implications of relative growth performance of individuals for genetic selection programs.

A total of 7, 652 individually tagged fingerlings (initial body weight, 1.6 to 14.8 g; and age 98 to 121 days) of seven strains of *Oreochromis niloticus:* Egypt, Ghana, Senegal, Israel, Singapore, Taiwan and Thailand were reared communally in 19 different culture environments. The number of replications per environments was variable. Stocking density ranged from 0.5 to 50 fish/m^2. Sampling was done every three weeks during the 90-day production cycle. Growth of strains, sexes, size groups and mortality across and with each environments were analyzed following generalized linear models (GLM). Sex ratios were analyzed using chi-square test. Repeatability of individual growth by strain and sex within each environments was determined by serial correlation of individual growth performance.

The a priori expectation of the present study that relatively smaller individuals at stocking may be predominantly females was not fully confirmed. The observed trend in sex ratio seemed to indicate relatively higher proportion of females in the smaller six group (SG1) and higher proportion of males in the largest size group (SG4). Overall, however the sex ratios among the different size groups were not significantly different from 1:1.

Mortality among the different size groups were not significantly different indicating that mortality was independent of relative size at stocking. Furthermore, there were no differences in the mortality among size groups within strains and within environments. Mortality was also not sex-specific reflected by the 1:1 sex ratios across all environments. The most important factor influencing mortality was the type of culture environment which ranged from "stressful" low temperature pond systems to relatively "normal" tilapia farming environments. Mortality was relatively low (< 20%) in cage environments; high in integrated rice-fish environment (about 50%) and intermediate (about 30-50%) in pond systems (except the low temperature pond). Although there were no significant differences in mortality among strains, Israel strain seemed to suffer

greater mortality in most environments; survival of Ghana strains were relatively higher in ponds and cages; and survival of Senegal strain was highest in rice-fish environment.

Divergence of growth performance between males and females was strain and environment-specific. Divergence occurred earlier in "normal" environments than in "stressful" environments. Within strains, divergence accurred earlier in slow growing strains, Ghana and later in fastest growing strain, Egypt.

Results from this study indicates that relative growth performance of individuals at the start of the production cycle was poor predictor of later growth performance. In general repeatability of growth performance was high from day 42 post-stocking (age 150 days) onwards when the divergence of growth between sexes was firmly established. The mean body weight at which the relative growth performance becomes "repeatable" was sex and environment-specific. The growth performance of *Oreochromis niloticus* was unaffected by initial size differences.

ABSTRACT

DIONISIO, EDNA ELIGADO, Institute of Graduate Studies, Central Luzon State University, Philippines, October, 1995, **PROGENY SEX RATIO IN A COMPLETE DIALLELE CROSS WITH EIGHT DIVERSE STRAINS OF NILE TILAPIA *(Oreochromis niloticus L.)***

Adviser: Prof. Antonio V. Circa
Co-Adviser: Dr. Ambekar E. Eknath

The objective of this study was to determine the proportion of males in the progeny from a complete diallel cross with eight strains of Nile tilapia. The eight strains include four new strains recently imported to the Philippines from Egypt, Ghana, Kenya and Sénégal and four Asian farmed strains popularly known in the Philippines as 'Israel', 'Singapore', 'Taiwan' and 'Thailand'. This study was conducted in two phases: a) Phase I involved a complete diallel cross of eight strains and b) Phase II involved repeat breeding of the strains combinations that generated a relatively high proportion of males during Phase I.

During Phase I, five batches of fry were produced from 6 to 12 matings (1 male:2females) per strain combination. Fry collected during short episodes of spawning was referred to as a batch. Batches of progenies from each cross were reared separately in the hapas until the fish reached a mean body weight of 3 to 5 grams when they were tagged. A total of 20, 928 individually tagged fingerlings was reared in different test environments for 90 days. The proportion of males across batches and 64 strain combinations was determined at harvest.

The association between proportion of males and survival at early stages (before tagging) and at the harvest was tested by Pearson correlation analysis. The proportion of males was independent of survival ($p < 0.05$). The effects of batch, the use of parent strains either as sires (SST) or dams (DST) and their interactions (SST*DST) on the variation in proportion of males were determined by General Linear Models (GLM) procedure. Only SST and DST significantly affected the proportion of males ($p < 0.05$). The overall mean proportion of males across all effect was 57.4 percent, indicating a slightly skewed sex ratio in favour of males across all experimental groups (n=183). Significantly higher proportion of males was observed when Thailand strain was used as sires ($p < 0.05$). Sénégal strain, when used as dams produced significantly lower proportion of males as compared to all other strains ($p < 0.05$).

Across all 64 strain combinations, the highest proportion of males was produced by the Asian crossbreds (PP) and the lowest by the African crossbreds (AA). Among Asian crossbreds, significantly higher proportion of males (62 to 76%) was observed when Thailand and Taiwan strains were used as sires ($p < 0.05$). The proportion of males in the reciprocal crosses of African sires x Asian dams (AP) and Asian sires x African dams (PA) did not differ significantly from each other ($p < 0.05$). The proportion

of males in the eight purebreds and the 12 African crossbreds did not deviate significantly from the expected 50 percent of males ($p < 0.05$).

During Phase II, a total of 19 strains combinations that had produced significantly high proportion of males during phase I was put for repeat breeding. Only 10 crosses yield progeny during this phase. Overall, the proportion of males observed during Phase II and Phase I was consistent indicating that high proportion of males in the main experiments (Phase I) may be repeated. This suggests that sex ratio is a repeatable trait.

RESPONSE OF GENETICALLY IMPROVED FARMED TILAPIA *(O. niloticus)* TO VARYING DIETARY PROTEIN AND GROSS ENERGY LEVELS

JAIME S. FERNANDEZ

ABSTRACT

A 90-day feeding experiment was conducted in twenty seven (27) 30x75x30 cm aquaria to determine the effects of varying levels of dietary protein and energy on the growth of GIFT fish. Nine diets were prepared each containing varying levels of protein gross energy . Levels of protein evaluated were 25, 30 and 35%, while the gross energy levels tested were 4000, 4200 and 4400 kcal/kg.

Results revealed better growth of GIFT fish at higher levels of protein (30-35%). Growth of GIFT fish, on the other hand did not differ at varying levels of gross energy. Results also revealed that survival rate and feed conversion of GIFT fish were not significantly affected by the varying levels of protein and gross energy.

Results suggest that protein requirement of GIFT fish (between 30-35%) is similar to the protein requirement of strains of *O. niloticus*. Its gross energy requirement, however, is lower (4000-4400 kcal/kg).

ABSTRACT

FRANCISCO, MELBA ESPERO, Institute of Graduate Studies, Central Luzon State University, Nueva Ecija, Philippines, April 1996. **GROWTH PERFORMANCE AND SURVIVAL OF GENETICALLY IMPROVED FARMED TILAPIA : Nile tilapia (*Oreochromis niloticus L.)* RAISED IN CAGES AT VARIOUS STOCKING DENSITIES.**

Adviser: Prof. Rodora M. Bartolome

This study was conducted to evaluate the growth performance and survival of Genetically Improved Farmed Tilapia (GIFT strain) in cages at various stocking densities. Growth, yield, survival and food conversion ratio of GIFT strain were compared to commercial strain at 10, 20, 30 and 40 fish/m^3 stocking densities.

The experimental fish were fed daily with commercial diet containing 30% crude protein at the rate of 5% of the fish biomass for the first and second months and at 3% during the last half of the culture until harvest. Daily ration was divided into two equal portions and given to the fish in the morning and in the afternoon.

Results of the study showed that GIFT fish at stocking density of 10 fish/m^3 gave the highest gain in weight of 60.45g with a corresponding daily gain in weight of 0.50g. The lowest gain in weight was obtained in commercial strain at stocking density of 40fish/m^3 (38.65g) with a corresponding daily gain in weight of 0.32g.

Analysis of variance in mean gain in weight revealed significant differences among the four stocking densities and between the two strains at 5% levels of significance. However, interaction effect was not significant.

Results on survival did not differ significantly between the main factors - strain and stocking density as well as the interaction effect.

Computed FCR of the GIFT strain was generally higher than the computed FCR of the commercial strain; however, differences were not significant.

Analysis of variance on FCR showed that FCR of stocked at lower stocking density was significantly higher than the FCR of fish stocked at higher stocking density at 5% level of significance.

Total production of the GIFT was significantly higher than that of commercial at various stocking densities evaluated.

Water quality parameters monitored in the experimental site throughout the culture period were within the desirable range for fish culture.

Simple cost and return analysis showed that the highest cost of production was incurred in GIFT strain at stocking density of 40 fish/m^3 of P 239.55 and the lowest was in commercial strain at stocking density of 10 fish/m^3 of P 89.55.

GIFT strain stocked at 20 fish/m^3 gave the highest net earning of P 54.20, followed by the same strain stocked at 10 fish /m^3 which had a net farming earning of P 40.55 while commercial strain stocked at 10 fish /m^3 had a net farming earning of P 89.55.

GIFT strain stocked at 40 fish /m^3 yielded the highest sale of P 196.00 while commercial strain stocked at 10 fish /m^3 yielded the lowest sale of P 87.00.

GIFT strain stocked at 20 fish /m^3 gave the highest net farming earning at P 54.20, followed by the same strain stocked in 10 fish /m^3 with a net farming earning of P 40.55. The commercial strain stocked at 10 fish /m^3 had a net farming earning of P 16.55.

ABSTRACT

GARCIA, LILIAN CRUZ, Institute of Graduate Studies, Central Luzon State University, May 1996, **GROWTH AND SURVIVAL OF GENETICALLY IMPROVED FARMED TILAPIA: NILE TILAPIA *(Oreochromis niloticus L.)* AT THREE STOCKING DENSITIES.**

Adviser: Prof. Eduardo A. Lopez

The study was conducted to test the response on growth, survival and size variability of Genetically Improved Farmed Tilapia (GIFT) at various levels of stocking density laid in a 2x3 factorial experiment. Eighteen (18) six m^3 fixed cages installed in a 1, 000 m^3 pond were used. The study had six treatment combinations and with three replicates each. Three stocking densities were tested namely: 20 pcs/m^3, 30 pcs/m^3 and 40 pcs/m^3 . Genetically Improved Farmed Tilapia of Nile tilapia *(Oreochromis niloticus L.)* served as the test species while Thailand strain (termed as THAI) of the same species served as the control. Treatment combinations were as follows: T_I - GIFT at 20 pcs/m^3 (GIFT20); T_{II} - GIFT at 30 pcs/m^3 (GIFT30); T_{III} - GIFT at 40 pcs/m^3 (GIFT40); T_{IV} - Thailand at 20 pcs/m^3 (THAI20); T_V - Thailand at 30 pcs/m^3 (THAI30); and T_{VI} - Thailand at 40 pcs/m^3 (THAI40).

For growth in final weight, GIFT is significantly better than Thailand strain ($P < 0.05$.) with corresponding values of 70.52g and 56.76g, respectively. All levels of stocking density namely: 20 pcs/m3, 30 pcs/m3, and 40 pcs/m3 are significantly different (72.66 g, 64.26 g and 54.31 g, respectively). It was observed that individual gain in weight decreases as density increases. Strain x density interaction shows no statistical difference. Mean final standard length, mean net production and Food Conversion Ratio (FCR) shows significant superiority of GIFT than Thailand strain. All density levels shows significant difference on mean final standard length and mean net production but non with FCR and in strain and density interaction.

Percentage survival shows GIFT is significantly a better survivor than THAI ($P < 0.05$) with corresponding values of 94.31% and 83.77%. No statistical difference was observed among densities and between strain x density interaction.

A general lower percentage of fish reached 100g and above (preferred marketable size) even so, GIFT was higher than THAI (11.80% and 4.91%, respectively). Among densities, 20 pcs/m3 had highest percentage of 15.28%, followed by 30 pcs/m^3 (7.1%) and 40 pcs/m^3 (2.16%) in reaching marketable size.

Economic analysis showed very low profitability cage culturing both strains in an unfertilized ponds, however, GIFT at 40 pcs/m^3 had highest profitability index yet closer with GIFT at 20 pcs/m^3 and 30 pcs/m^3.

Within the confines of this study temperature and dissolved oxygen in the early morning were negatively correlated with fish body weight, total production and average production per cage. Total ammonia-nitrogen showed otherwise. Result is not conclusive and need to be verified.

ABSTRACT

LONGALONG, FELICISIMA MANALASTAS, Institute of Graduate Studies, Central Luzon State University, Philippines, April 1996, **RESPONSE TO SELECTION FOR LOW AND HIGH OCCURRENCE OF SEXUAL MATURATION AT A FIXED AGE IN NILE TILAPIA *(Oreochromis niloticus L.)***

Adviser: Dr. Tereso A. Abella
Co-Adviser: Dr. Ambekar E. Eknath

The primary objective of this study was to determine the response to selection for frequency or early maturing females (FMF) at a given age by comparing the FMF in the progeny of two separate lines - the high line and the low line - screened previously for this trait. The parental high and low lines were drawn from on-going selection program for improving the growth performance. The high line composed of families which on an average had a high FMF (> 75%), while the low line represented parental families with allow FMF (< 20%).

Twenty five pairs of breeders from each line were mated (1 male:1 female) in 1 m3 fine mesh hapas. The swim-up fry were collected, counted, bulk weighed and reared in hapas until they reached the size of about 3 to 5 g when they could be tagged. A total of 3, 179 individually tagged fingerlings from 25 full-sibs families (9 families from low line and 16 from high line) was communally stocked in three 600m2 ponds. Draining of ponds and sampling of all individuals after the first appearance of fry were done as follows: in Pond 1 after 4 weeks, in Pond 2 after 3 weeks. The maturity condition of females was determined by examining their genital papilla. The other traits recorded at this intermediate sampling were age, length, weight, survival and sex of the fish. All surviving fish were reared further for about 60 days before final harvest. Significant age differences between the two lines were observed due to asynchronous spawning. The two lines were compared for their various performance traits (FMF, growth, survival and proportion of females) after correction for ages differences and the pond effects using a general linear model procedure.

The highest FMF was observed in the high line in pond 1 (71%) and the lowest in low line in pond 2 (16%). The difference in FMF between the two lines across all ponds represents the response to bi-directional selection for FMF. It was 21.4%. The correlated response in body weight was highly significant. At intermediate sampling, the body weights of males in the high and low lines were 158.2 and 145.2 g, respectively. Similarly, for females the body weights were 126.2 g and 113.5 g. This suggests that selection for low FMF will result in low mean body weights. It is therefore important to include the two traits (FMF and growth) simultaneously in an applied breeding program. There were no correlated responses in survival and proportion of females.

ABSTRACT

MENDOZA, ALMA MACALINCAG, Institute of Graduate Studies, Central Luzon State University, Philippines, October 1996, **GROWTH AND SURVIVAL OF GENETICALLY IMPROVED FARMED TILAPIA: NILE TILAPIA *(Oreochromis niloticus L.)* IN CAGES FED WITH PRACTICAL DIETS**

Adviser: Prof. Rodora M. Bartolome

A 120 day feeding experiment was conducted to determine the growth performance, percentage survival, food conversion ratio (FCR), and cost and return analysis of GIFT strain *O. niloticus* fed with four formulated and commercial feeds in cages installed in pond. Each 2m x 2m x 1m B-net cage was stocked with 120 GIFT fingerlings weighing 8.95g to 10.13g.

Four isonitrogenous diets containing 30% protein were formulated using locally available feed ingredients such as fish meal, meat and bone meal, soybean meal, copra meal and rice bran. Commercial feeds with crude protein of 30% was used as control. The different treatments were: Treatment I, commercial feeds; Treatment II, 5% fish meal, 28% soybean meal, 9% meat & bone meal, 27% copra meal, 31% rice bran; Treatment III, 30% fish meal, 25% copra meal, 45% rice bran; Treatment IV, 45% meat & bone meal, 30% copra meal, 25% rice bran; Treatment V, 46% soybean meal, 27% copra meal, 27% rice bran. Test diets were fed to the fish for 120 days culture period.

Treatment III, 30% fish meal, 25% copra meal, 45% rice bran; Treatment IV, 45% meat & bone meal, 30% copra meal, 25% rice bran; Treatment V, 46% soybean meal, 27% copra meal, 27% rice bran. Test diets were fed to the fish for 120 days culture period.

Results show that weight gain, specific growth rate, and total biomass of fish were significantly affected (P <0.01) by the protein source of feeds. Likewise, feeds intake (FCR) of GIFT fingerlings was also affected (P >0.01) by the feed composition and protein source of feeds. Survival in all the test diets did not differ significantly.

Water quality parameters such as dissolved oxygen, temperature, and pH monitored during the feeding experiment were within the desirable range of optimum growth of tilapia. However, the Secchi disc visibility of the water was far from the recommended range for fish culture.

The cost and return analysis showed that fish ration containing 45% meat & bone meal, 30% copra meal and 25% rice bran (T IV) obtained shorter payback period and high return of investments (ROI) compared to other treatments.

These findings imply that practical diets made from locally available feed ingredients such as fish meal, copra meal, soybean meal, meat and bone meal and rice

bran are cheaper than commercial feeds. Utilizing practical diets, result to a reduction in feeds and farm production costs and would ultimately increase the profits of fishfarmers.

ABSTRACT

PAPA, DELFIN CARRASCO, Institute of Graduate Studies, Central Luzon State University, Philippines, March 1990, **CAUDAL FIN BAR AS INDICATOR OF GROWTH PERFORMANCE IN SEVEN STRAINS OF NILE TILAPIA *(Oreochromis niloticus* L.)**

Advisers: Prof. Renato D. Recometa
Dr. Ambekar E. Eknath

Caudal fin bar as a meristic character in *Oreochromis niloticus (L.)* was investigated to determine whether it could serve as indicator of growth performance. Seven strains, namely: Egypt, Ghana, Israel, Sénégal, Singapore, Taiwan and Thailand were used as experimental fish. Individuals having 3 and 4 number of caudal fin bars, initially, were represented equally from each strain. All individuals were tagged and communally in net cages installed in a fertilized pond at BFAR/ICLARM - Genetic Improvement of Farmed Tilapia (GIFT) project's site at CLSU campus. The experiment was replicated twice. Feeding was employed twice daily at 10% of total body weight using tilapia experimental diet of 36% crude protein. Data on body weight (g), standard body length (cm), number of caudal fin bar, sex and survival were collected from all fish samples at bimonthly intervals for 88 days growing period.

Analysis of variance on General Linear Models (GLM) procedure under SAS Program was used to assess the data on growth and number of caudal fin bar. Phenotypic correlations were computed using Linear Correlation procedure to establish relationships among selected variables. Sex ratio differences was tested using Chi-square analysis.

From the results, significant test of differences indicated better growth performance in female strains of Egypt, Israel, Sénégal, Singapore and Thailand with higher initial number of caudal fin bars. Egypt with highest growth in body weight was not significantly different ($P < 0.05$) with Thailand, Taiwan, Israel and Singapore. Irrespective of initial number of caudal fin bars, male strains had higher growth performance. This fact has been established in tilapia species. Egypt, consistently, had the highest growth, while Ghana and the lowest.

All male strain had more number of caudal fin bars, however, its variations from initial caudal fin bars was not significantly different. Irrespective of initial number of caudal fin bar's variations and sexes, Thailand had the highest more number of bars, but not significantly different from Egypt, Israel and Taiwan.

Phenotypic correlations among body weight, standard length, number of caudal fin bar with time showed high significant degree of associations.

Highest survival was observed in Ghana strain, while Egypt, Sénégal and Taiwan with the lowest survival among other strains. Higher proportion of female individuals was evident in all strains with less initial number of caudal fin bar.

Implications of these results for broodstock selection and management are discussed.

Attachment 14.

Breeding Plans for Nile tilapia, Mrigal and Silver barb.

BREEDING PLAN FOR NILE TILAPIA *(OREOCHROMIS NILOTICUS)* IN VIETNAM: COMBINED MULTI-TRAIT SELECTION

Report No. 1

Hans Bernhard Bentsen
Trygve Gjedrem
Tran Mai Thien
Nguyen Cong Dan

INTERNATIONAL NETWORK ON GENETICS
IN AQUACULTURE
July 1996

FOREWORD

The International Network on Genetics in Aquaculture (INGA) was established in 1993 and is being coordinated by the International Center for Living Aquatic Resources Management (ICLARM), with the objective to contribute through collaborative research, to the domestication and sustainable performance of tropical finfish species farmed in developing countries and to strengthen national capabilities for genetic enhancement of farmed fish through exchange of germplasm, methodologies and through training and interactive forums.

Studies undertaken in recent years for improving breeds of salmon in Norway and tilapia in Philippines by ICLARM and collaborating Philippine and Norwegian institutions, have led to increased awareness among researchers the need for undertaking programs for improvement of breeds of species that are of aquaculture importance in their countries. INGA has been assisting the member countries in developing national breeding programs. This report on Breeding Plan for Nile Tilapia *(Oreochromis niloticus)* in Vietnam: Combined Multi-Trait Selection is an outcome of such an effort and has been prepared by Drs. Hans Bernhard Bentsen and Trygve Gjedrem of the Institute of Aquaculture Research (AKVAFORSK), Norway and Dr. Tran Mai Thien and Mr. Nguyen Cong Dan of Research Institute for Aquaculture No.1 of Vietnam. Dr. A.E. Eknath of ICLARM has assisted INGA and the authors in planning and development of this breeding program, which is greatfully acknowledged.

We hope that this document will be useful to other researchers and planners in developing breeding programs in their countries.

DR. M.V. GUPTA
INGA Research Coordinator

BREEDING PLAN FOR NILE TILAPIA IN VIETNAM: COMBINED MULTITRAIT SELECTION

PREPARED FOR
THE INTERNATIONAL NETWORK OF GENETICS IN AQUACULTURE (INGA)

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AKVAFORSK Report No. 20/96

INTRODUCTION

Total freshwater aquaculture production in Vietnam in 1995 was about 300 000 tons and total brackish water aquaculture production was about 115 000 tons. Total tilapia production was about 15 .000 tons, mainly produced in fresh water. Until now, most of the production has been based on Mozambique tilapia, that was introduced in Vietnam in 1951 and after the introduction of Nile tilapia from Taiwan in 1973, also on hybrids between Mozambique and Nile tilapia. Because of the small size of the fish at sexual maturation, when the fish has to be harvested, the consumers' demand for tilapia has been limited. Recent experiences with culture of Nile tilapia after the reintroduction in 1994 of the GIFT, Thai and Egypt strains have been encouraging.

Fingerlings have been stocked in mangrove brackish water ponds in the rainy season (salinity may be up to 15-20 ppm). Size at stocking has been about 10 grams and the stocking density about 1 fish per square meter. After a culture period of 5 months using supplementary feeding with rice bran, the fish has reached a size of 250-300 grams. The quality of brackish water farmed tilapia is appreciated by the consumers. The demand for Nile tilapia fingerlings is rapidly increasing, in particular in the brackish water areas. A total area of 340 000 ha is considered suitable for brackish water aquaculture.

Rice-fish culture with tilapia is also practised. A total area of 580 000 ha of paddy fields are suited for this kind of culture, but only a small fraction is presently used for this purpose.

In the cities, in particular in the Thanh Tri district, Hanoi, tilapia has also been stocked in the sewage areas with good results. The fish will feed on the organic waste in the sewage areas and make the water cleaner. It will grow to a size of 200-250 grams in 4 months. Studies have shown no harmful residuals in the fish meat, and the fish is used for human consumption.

In North Vietnam, the culture period for tilapia is limited by the temperature. Fry production may star in April/May, and the grow-out season will last until December. In South Vietnam, tilapia may be cultured throughout the year.

BASE POPULATION

Through ICLARM and INGA, RIA 1 has established contacts with the GIFT project in the Philippines. A request for a transfer of a complete tagged family material of the latest generation from the GIFT project to RIA 1 should be forwarded as soon as possible. As soon as possible after arrival

at RIA 1, at least 2 males and 2 females from each full sib family should be electronically tagged (e. g. with PIT-tags). Efforts should be made to transfer the material before the end of 1996 or in early 1997. This will then form the base population for a breeding program for Nile tilapia in Vietnam. The Nile tilapia stocks presently available at RIA 1 may be considered as complementary broodstock and may be tested as breeders in the program if desired. The number of non-GIFT broodstock tested in the breeding program in each generation should be limited.

BREEDING GOAL

At present, the breeding goal of the GIFT program is to increase growth rate and reduce the frequency of early maturing females. This goal should be maintained in the Vietnamese breeding program. In addition, the great potential for brackish water culture of Nile tilapia calls for breeding for improved growth performance at high salinity levels. The survival at high salinity levels should also be recorded. In North Vietnam, the growth performance during the cold months (January, February and March), is greatly reduced. In addition, occasional periods of temperatures below 11 °C have caused extensive mortalities in shallow ponds. Increased cold tolerance should therefore be included in the breeding goal, in particular the ability to survive short periods (1-2 weeks) of low temperature. During the initial generations, all families should be tested in both fresh and brackish water in both Northern and Southern Vietnam, as well as in a cold water challenge test. The importance of genotype by environment interactions (the correlation of the performance of the families under different environmental conditions) may then be evaluated. If substantial genotype by environment interaction is found between important target environments (negative correlations or correlations close to zero), it should be considered to split the breeding program in two or more programs with different breeding goals. This may be separate breeding programs for Northern and Southern Vietnam or for freshwater and brackish water culture.

SELECTION METHOD

The breeding method should be a combination of individual and family selection. This will require tagging of all test fish. Family selection is required for improvement of frequency of early maturing females, for parallel testing in brackish water ponds and for challenge testing at low temperatures. The breeding values of the broodstock will be computed based on the growth performance of the individual and it's full sibs and half sibs, and the frequency of early maturing females, the survival rate in a low temperature challenge test and the growth performance and survival in a brackish water

field test of the full and half sibs of the breeding candidates. The relative weighting of the different traits and sources of information will be determined by the economic importance of the traits for the fish farmers.

START OF BREEDING PROGRAM

The breeding program should be started by random mating of breeders transferred from the GIFT project, avoiding mating of full or half sibs. All full sib families should be represented among the breeders. The production of the progeny generation of full sib families should follow the procedure described below.

PRODUCTION OF FAMILIES

The production of families will follow the design developed in the GIFT project (Figure 1). A training program for staff members from RIA1 and RIA 2 should be requested from the GIFT staff. Before mating, the breeders will be conditioned. One male will then be stocked with 2 females in a 1x1x1 m breeding hapa. A total number of 100 breeding hapas will be installed in a pond. All hapas will be inspected once every week for swim-up fry. Swim-up fry will be collected separately from each hapa and transferred at a standardised stocking density to 1x1x1 m rearing hapas, one hapa for each full sib group. The date of collection of swim up fry should be recorded. The spent females will be removed from the breeding hapas. Totally, this should result in some 150-200 full sib groups. After 3-4 weeks in the rearing hapas, the fry will be transferred at a reduced stocking density to B-net hapas for further rearing until an average body weight of 5 grams. The fingerlings will then be individually tagged, following the method developed by the GIFT project. A total of 130 fingerlings will be tagged per full sib family, amounting to 20-25 000 tagged fingerlings per generation. Of these, 50 fingerlings per full sib family will be communally stocked in a pond at RIA 1, 40 fingerlings per full sib family will be sent to the Research Institute for Aquaculture No. 2 in Ho Chi Minh City (RIA 2) for testing in fresh water (20) and brackish water (20). Furthermore, 20 fingerlings per family should be sent to the Cua Lo station of RIA 1 for brackish water testing and 20 fingerlings per full sib group will be kept for low temperature challenge testing at RIA 1 (Fig 2).

Low temperature challenge test: Experiments will have to be carried out at RIA 1 to establish a suitable method for low temperature challenge testing. Preferably, a single communal test should be carried out with 20 tagged fish from each test family at a small size. Alternatively, if larger fish has to be used in the test, the test fish may be split in e.g. two groups. All full sib families should then be equally represented in each group. The number of fish from each full sib family should be recorded at the start of the challenge test. The suitable size of the test fish and the rate of the decrease of the temperature in the water will have to be determined experimentally. Water may be cooled by ice or by an electrical cooling system. At about 50 percent mortality, the family identity of all dead fish should be recorded. The surviving fish may then be kept at a low temperature (temperature to be determined) to record the survival time of each individual.

Frequency of early maturing females: Fifty tagged fingerlings from all families will be communally stocked in one large pond at RIA 1. The pond should be continuously inspected for occurrence of swim-up fry. The pond should be drained about three weeks after the first occurrence of swim-up fry, and all females should be scored and recorded as sexually mature or non-mature according to the method developed in the GIFT project. All fish should then be restocked in a neighbouring pond.

Brackish water field tests, north and south: In both field test stations (RIA 1 and RIA 2), 20 tagged fingerlings from each family should be communally stocked in one brackish water pond. Because of possible problems with entangling of tags, ponds without mangrove vegetation may have to be used. Body weight and survival of all test fish should be recorded in the first week of November, and the records should be forwarded to RIA 1 immediately. The test fish may then be slaughtered or restocked for further grow-out. At RIA 2, the test fish should be kept for later use as broodstok for mass production of progeny that may be disseminated as broodstock to collaborating hatchery operators (see the chapter about dissemination below).

Fresh water field test, south: At RIA 2, 20 tagged fingerlings from each family should be communally stocked in a fresh water pond. Body weight of all test fish should be recorded in the first week of November, and the records should be forwarded to RIA 1 immediately. The test fish should be kept for later use as broodstock for mass production of progeny that may be disseminated as broodstock (see the chapter about dissemination below). If the genotype by environment interaction (see above) between fresh water pond culture in the north and the south is found to be insignificant during the initial generations, the focus of the fresh water test at RIA 2 may be shifted to other freshwater target farm environments of importance (cage culture, low pH).

Fresh water test of the breeding nucleus: In early December, the body weight of all fish in the pond at RIA 1 should be recorded and pre-selection may be carried out (see below).

SELECTION OF BROODSTOCK

In late November, full sib and half sib family records of low temperature tolerance, frequency of early maturing females, body weight in brackish water at RIA 1 and RIA 2 and in fresh water at RIA 2 will be available. The families may then be preliminary ranked according to their combined breeding values (full sib family selection index developed by AKVAFORSK). At final recording of body weights in the pond at RIA 1, a pre-selection may then be performed based on the breeding values of the full sib families. All fish from the 50 top ranked full sib families should be pre-selected and restocked, each sex separately, until final breeding values have been computed. The best of the remaining breeders should be kept for mass production of broodstock for hatchery operators.

Final selection of broodstock to produce the next generation in the breeding program will be based on a selection index developed and initially computed at AKVAFORSK, including the following traits (Figures 2 and 3):

Body weight of the individual and its full and half sibs recorded at RIA 1
Low temperature tolerance of full and half sibs in challenge test at RIA 1
Frequency of early maturing females in the full and half sib families at RIA 1
Body weight and survival of full and half sibs in brackish water test Cua Lo Station of RIA 1
Body weight of full an half sibs in freshwater pond at RIA 2
Body weight and survival of full and half sibs in brackish water test at RIA 2

The non-selected breeders should be kept for mass production of broodstock for hatchery operators.

The routines for production of the next generation of 150-200 full sib families will then be repeated as described earlier (Figure 1).

DISSEMINATION OF IMPROVED SEED TO THE FISH FARMERS

Because of the relatively low fecundity of the tilapia, dissemination of improved seed will have to be based on distribution of improved broodstock to hatchery operators (Figure 3). After the production of the full sib families for the breeding program have been completed, the selected parents should be used for mass production of broodstock for hatchery operators. The progeny of the selected parents will be top genetic quality broodstock, followed by the progeny of the discarded breeders during the final selection (see above) and the discarded breeders during the pre-selection (see above). RIA 2 will serve as the center for dissemination in South Vietnam. This may be done by keeping the test fish until the full sib family breeding values have been computed (see above). Fish from the best 50 % of the full sib families may then be stocked in a breeding pond for mass production of fingerlings that may be disseminated as broodstock to collaborating hatchery operators.

CONTROL TO ESTIMATE GENETIC GAIN

The PIT-tagged fish transferred from the GIFT project may be kept alive at RIA 1 for several years. This will secure the availability of the material for other INGA members in the future. In addition, these breeders may be used for repeated matings after 2-4 generations of selection in the Vietnamese breeding program. The genetic gain in the breeding program may then be estimated by fin clipping of e.g. 20 progeny from each of 50 pairs of random breeders from the originally transferred material with e.g. 20 fin clipped progeny from each of 50 random pairs from the latest reproduction of families in the breeding program. The production of these groups may be carried out after the completion of the production of the families in the breeding program. After fin clipping, the two groups may be communally stocked in one fresh water test pond at RIA 1 and, if feasible, in one brackish water test pond e.g. at RIA 2 for grow out. The frequency of early maturing females in the two groups may be determined in the pond at RIA 1 as described earlier. If desired, the number in each of the test groups may be increased to carry out a communal low temperature challenge test. The difference between the two test groups in the various traits recorded will represent an estimate of response to selection.

After some years, the original GIFT breeders will become too old to be successfully reproduced. Before that, it must be decided if a new generation of progeny of randomly mated original GIFT breeders (using all available breeders) should be produced to serve as a continued source of genetic control. Other methods of control of genetic gain should then be considered as well.

1 2 100

BREEDING HAPAS ♂ ♀♀ ♂ ♀♀ .... ♂ ♀♀

REARING HAPAS (FULL SIB GROUP NO)

1 2 3 4 200

B-NET HAPAS

TAGGING OF 130 FINGERLINGS PER FULL SIB FAMILY

FIGURE 1. MATING AND REARING DESIGN

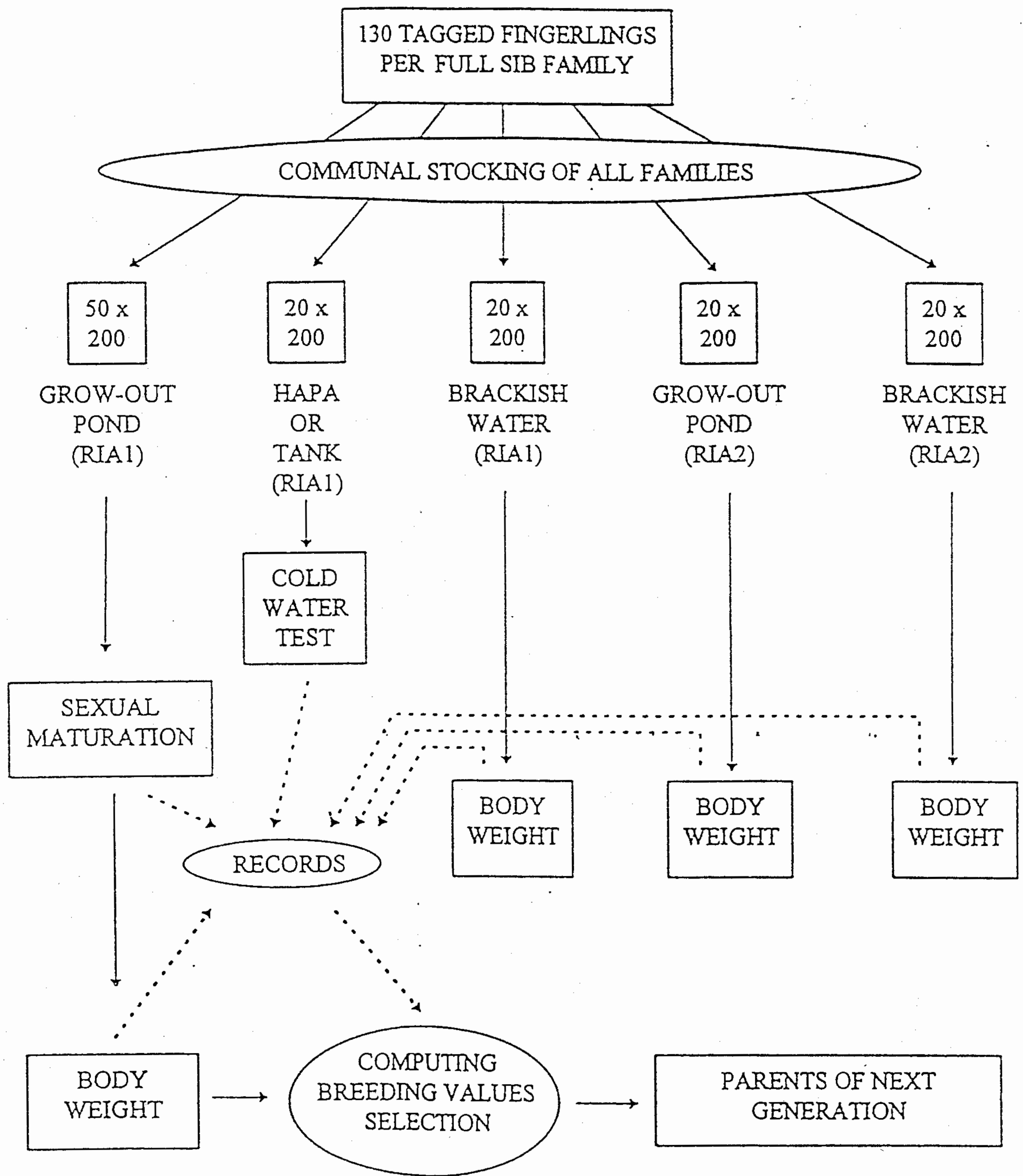

FIGURE 2. TESTING, RECORDING AND COMPUTING OF BREEDING VALUES

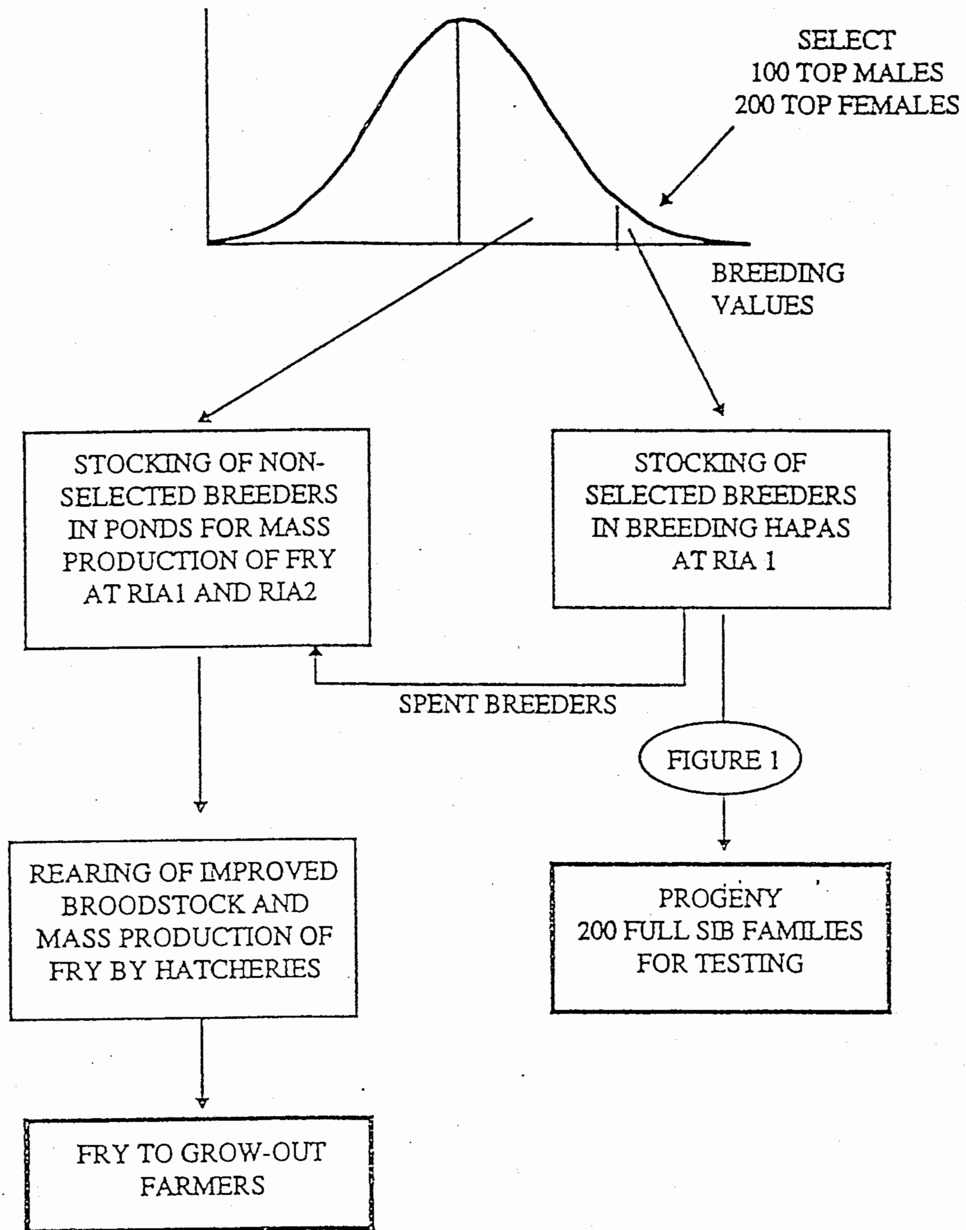

FIGURE 3. SELECTION OF BREEDERS AND DISSEMINATION OF GENETICALLY IMPROVED TILAPIA TO FARMERS

BREEDING PLAN FOR MRIGAL (*CIRRHINUS MRIGALA*) IN VIETNAM: INDIVIDUAL (MASS) SELECTION TO IMPROVE GROWTH RATE

Report No. 2

Hans Bernhard Bentsen
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INTERNATIONAL NETWORK ON GENETICS IN AQUACULTURE
July 1996

FOREWORD

The International Network on Genetics in Aquaculture (INGA) was established in 1993 and is being coordinated by the International Center for Living Aquatic Resources Management (ICLARM), with the objective to contribute through collaborative research, to the domestication and sustainable performance of tropical finfish species farmed in developing countries and to strengthen national capabilities for genetic enhancement of farmed fish through exchange of germplasm, methodologies and through training and interactive forums.

Studies undertaken in recent years for improving breeds of salmon in Norway and tilapia in Philippines by ICLARM and collaborating Philippine and Norwegian institutions, have led to increased awareness among researchers the need for undertaking programs for improvement of breeds of species that are of aquaculture importance in their countries. INGA has been assisting the member countries in developing national breeding programs. This report on **Breeding Plan for Mrigal *(Cirrhinus mrigala)* in Vietnam: Individual (Mass) Selection to Improve Growth Rate** is an outcome of such an effort and has been prepared by Drs. Hans Bernhard Bentsen and Trygve Gjedrem of the Institute of Aquaculture Research (AKVAFORSK), Norway and Dr. Tran Mai Thien and Mr. Nguyen Cong Dan of Research Institute for Aquaculture No.1 of Vietnam. Dr. A.E. Eknath of ICLARM has assisted INGA and the authors in planning and development of this breeding program, which is greatfully acknowledged.

We hope that this document will be useful to other researchers and planners in developing breeding programs in their countries.

DR. M.V. GUPTA
INGA Research Coordinator

BREEDING PLAN FOR MRIGAL CARP IN VIETNAM: INDIVIDUAL (MASS) SELECTION TO IMPROVE GROWTH RATE

PREPARED FOR
THE INTERNATIONAL NETWORK OF GENETICS IN AQUACULTURE (INGA)

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AKVAFORSK Report No. 21/96

Total freshwater aquaculture production in Vietnam in 1995 was about 300 000 tons and from brackish water aquaculture about 115 000 tons. Of the freshwater aquaculture, about 200 000 tons were produced in North Vietnam. Mrigal production is mainly located in North Vietnam. Total production of mrigal was about 45 000 tons, mainly from pond culture in the lowland region. However, mrigal is also a suitable species in the midland and highland area, even in the cold season. The species is a mid prized product (11 000 Dong per kg, as compared to 14 000-16 000 Dong for tilapia and 15 000-20 000 Dong for common carp). Production of mrigal is mainly for local consumption. All carp hatcheries will produce mrigal seed. The broodstock in the hatcheries in North Vietnam all originates from RIA1.

In North Vietnam, spawning season is in April/May until late August. Sexual maturity occurs at 2 years of age. Broodstock will normally be used for 3 breeding seasons, and breeding may be repeated three times in one season. The third breeding in the season is considered to give poorer quality seed than the first and second breeding. Because young and small breeders normally mature and spawn earlier in the season, and early produced fry are better paid, seed producing farmers tend to prefer to use this early maturing and small sized broodstock as much as possible. This may have caused a selection for small size and early maturation.

Spawning is induced by injecting the females and males with pituitary gland extract or manufactured hormones (Ovaprim from Canada or LRH-A from China). Two injections are administered to the females at an interval of 4-6 hours. About 75 percent of the females will spawn after injection. Males are injected once at the same time as the second injection of the females with a dose of 1/5-1/6 of the dose given to the females. All males will normally respond to the injection. The females and males are then stocked together in a spawning tank at a ratio of 1:1 according to body weight. Natural spawning occurs after 4-6 hours depending on the water temperature. Fertilised eggs are collected and stored for hatching in incubation tanks with high water flow, and will hatch after 12-15 hours depending on the water temperature (31-27 °C). The hatchlings are kept in the circular incubation tanks for 3-4 days until the yolk sac has been absorbed. At the end of this period, the larvae is start-fed with powder from boiled chicken egg yolk. At a total age of 5-6 days after fertilisation, the larvae is transferred to nursery ponds at a density of 150-200 per square meter, where they are fed for one week with a mixture of cooked rice bran, rice powder, soya been powder, and sometimes with fish meal mixed with water. The mixture is sprayed on the water surface. The same mixture without cooking are then fed for another 3 weeks until they reach fry stage (2-3 cm). The fry may be sold or transferred to rearing ponds at a density of 15-20 per square meter until fingerling size (6-8 cm) at an age of 8 weeks

or large fingerlings (10-12 cm) at an age of 12 weeks, still with feeding and additional natural food by fertilising the pond. Fingerlings are then stocked in grow-out ponds at a density of 1.5-2 fish per square meter in mono culture. Harvest may start in January at an age of 8-9 months and a size of 300-500 grams.

BASE POPULATION

About 1 000 fingerlings of mrigal carp were imported in 1984 from the Thangon State Fish Farm in Laos to Thu Duc Fish Farm of the Research Institute for Aquaculture No. 2, Ho Chi Minh City with the support of the Interim Mekong Committee. After quarantine and acclimatisation, the fish was transferred to the Cai Be Fish Farm of RIA 2 in Tien Giang Province. Another import of 10 pairs of breeders were imported from the same source in 1986. Mrigal is not considered an important species in South Vietnam. In June 1986, 6 males and 6 females of the first import were transported from RIA 2 to RIA 1 at a size of 700-1000 grams, where they were spawned two weeks after transportation. Since then, about 4 to 5 generations have been produced at RIA 1. Broodstock has been randomly chosen. Observations indicate that since the introduction, growth rate has declined year by year, and the fish has become mature at lower age and lower body weight. Deformities has been observed in about 5-7 percent, but has not increased.

Before the start of a breeding program, the base population should be upgraded to broaden the genetic base and eliminate inbreeding. The possibility of acquiring wild mrigal directly from India should be investigated. RIA 1 has institutional contacts with Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, and Central Institute of Fisheries Education (CIFE), Bombay. If possible, a request for direct transfer of fry from three different river systems should be forwarded. A number of 2 000 fry per river strain will be sufficient. The imported strains should be grown separately at RIA 1 until maturation.

BREEDING GOAL AND SELECTION METHOD

The two most important features of an aquaculture stock of mrigal carp are a high growth rate and a high proportion of females. Males grow to 60-70 percent of the female body weight. An additive genetic improvement program will have to focus on growth rate in both sexes. Sex ratio will have to be manipulated by other techniques. The additive genetic variation of growth rate in mrigal has not

been studied. If the variation is similar to that of rohu and common carp, growth rate of mrigal may easily be improved by individual (mass) selection.

The breeding program should then be designed to avoid loss of genetic variation and avoid rapid accumulation of inbreeding. This may be done by securing a large effective population size. In the proposed breeding plan, a large effective population size is achieved by using a large number of breeders in each generation (2x75=150 pairs), and a restricted number of progeny per pair (on average 35-40 surviving progeny per pair at the end of the grow-out test). Furthermore, the variation between pairs in the number of progeny is kept as low as possible by separate incubation of the progeny groups through the early life phase, when the mortality is high. A fixed number of progeny per pair may then be counted and communally stocked for nursing, rearing and grow-out testing. The design is expected to result in a rate of inbreeding of less than 1% per generation.

Furthermore, maintaining two separate batches of breeding candidates originating from different sets of parents throughout every generation, as proposed in the present breeding plan, will permit a mating design that will exclude mating between sibs. This will prevent that mating between breeders from the breeding program could occasionally result in high inbreeding coefficients in some groups of progeny.

START OF THE PROGRAM

The relative performance of the RIA 1 population and the introduced strains in Vietnamese fish farms will not be known at the start of the program. However, in some strain comparison tests with other tropical species, little evidence has been found of strong genotype by environment interaction. Rather than spending a lot of time and efforts (e.g. on developing tagging or branding methods) to carry out a strain comparison test, it is recommended to start forming a synthetic (mixed) base population for selection from the early beginning of the program.

If the introduced material arrives at RIA 1 before the end of 1996, mating may start in April/May 1998. All matings should then be strain crosses, using all possible combinations in a 4 by 4 complete crossing design without pure-breds (Table 1). Single pairs will be mated by stripping of eggs and milt and artificial fertilisation in trays or by natural mating in hapas.

For each of the 12 reciprocal crosses, a first round of 6-7 pairs will be mated separately, producing about 75 full sib families. This means that at least 25 females and 20 males from each strain should be injected. Each male should be mated to one female only, and each female to one male only. Matings will take place within 2 days. About 1 000 fertilised eggs from each pair should be transferred to a separate incubation jar (Figure 1). The fertilised eggs will be hatched and the larvae will be kept in the jars until absorption of the yolk sac. After the absorption of the yolk sac, 100 larvae from each incubation jar should be communally stocked in a nursery pond. This will be the first batch of progeny. The procedure will then be repeated by mating another set of 75 pairs the following week. A second nursery pond will be used for the progeny from this set of breeding pairs. This will be the second batch of progeny. The two batches should be kept separately until the fish are sexually mature (Figure 1).

At stocking in nursery ponds, each batch will consist of 75 full sib families of 100 larvae, making up a total of 7500 larvae. At a stocking density of 100 larvae per square meter, this will require two nursery ponds (or hapas) of 75 square meters each. Assuming a larvae survival of 60 percent until transfer to rearing ponds, a total of 4500 fry will have to be stocked in each of the two rearing ponds. At a stocking density of 10 fry per square meter, this will require two rearing ponds of 450 square meters each. Assuming a survival rate of 80 percent until stocking in grow-out ponds (i.e. a total number of 3600 fingerlings in each of the two grow-out ponds) and a stocking density of 2.0-2.5 fingerlings per square meter, two grow-out ponds of 1300-1400 square meters each will be required for production of broodstock for selection.

SELECTION OF BROODSTOCK

In commercial mrigal farming, production target is to harvest the fish in January, at an age of 8 months or a size of 300-500 grams. Consequently, the body weight of all fish in the two grow-out ponds should ideally be recorded some time in January. However, since it is difficult to determine the sex of the fish at this age, and since sexual dimorphism in body size has already started to occur, strong selection at this stage may result in a low number of males among the selected broodstock. Assuming a survival rate of 80 percent from stocking of fingerlings in the grow-out ponds until 8 months of age, each of the two grow-out ponds will contain about 3300 fish. By applying a pre-selection of 50 percent (discarding the 50 percent of the fish with the lowest body weight), the stocking density in the grow-out ponds will be reduced to about 1 per square meter. At the same time as the pre-selection, 40 fish with an average body weight (average of all fish before pre-selection) should be fin-clipped and restocked with the pre-selected broodstock in each pond (Figure 2).

Final selection should then be carried out in March/April at 22 months of age. Body weight and sex should be recorded for all fish, and the 80 largest males and the 110 largest females from each of the two grow-out ponds should be stocked separately by pond and sex in hapas (4 hapas) until mating. All the selected fish should be sexually mature and ready to spawn. The final selection intensity is then expected to be about 6-8 % (Figure 2). With an assumed heritability for body weight of 0.3 and a coefficient of variation of 30 percent, as shown in many other fish species, the expected mean genetic gain in the progeny should then amount to about 16-18 % compared to the mean of the parent generation (16-18 % genetic gain per generation).

At the same time as the final selection, all fin-clipped males from the first pond (batch 1) that are sexually mature and ready to spawn should be kept and stocked together with the selected males from the same pond until mating. All fin-clipped females from the other pond (batch 2) that are sexually mature and ready to spawn should be kept and stocked together with the selected females from this pond until mating. These breeders will be used to produce a control group (see below).

PRODUCTION OF THE NEXT GENERATION

The production of the next generation should be carried out by single pair mating of 75 selected females from the first grow-out pond with 75 selected males from the second grow-out pond to produce a new batch 1 (Figure 3). This means that 110 females and 80 males should be injected to induce spawning. Again, each full sib group will be hatched in a separate incubation jar and 1000 larvae will be kept in the jar until the yolk sac is absorbed. From each jar, 100 larvae will be counted and pooled together in one nursery pond. In the following week, the routine will be repeated, this time with 75 selected males from the first grow-out pond and 75 selected females from the second grow-out pond, to produce a new batch 2 (Figure 3). The two batches should again be kept separate all the way until sexual maturity, selection and mating (Figure 1).

DISSEMINATION OF IMPROVED SEED TO THE FISH FARMERS

The surplus eggs from the all together 150 selected females may be incubated in ordinary, large scale circular tank incubators for hatching. The larvae may then be sold to fish farmers through collaborating hatcheries. Assuming that a total of 150 females with an average body weight of 1.2 kg will spawn, that 250 000 eggs will be spawned per kg body weight per spawning, that each female

will spawn two times during the spawning season and that the survival from fertilisation to post larvae will be 60 percent, this should amount to 54 mill post larvae available for the industry. This will all be progeny of the best selected breeders, and may be sold directly to grow-out farmers (Figure 3). The best non-selected broodstock at the final selection may be sold to collaborating hatchery operators. To avoid inbreeding in the hatcheries, males from one pond should be supplied together with females from the other pond to each individual hatchery operator.

CONTROL TO ESTIMATE REALISED GENETIC GAIN

Establishing a procedure for control of genetic gain is not required to obtain response to selection in a breeding program. However, a lot of assumptions have been made about unknown parameters in the present program. Including a routine for genetic control will make it possible to check if these assumptions are valid, or if the program needs adjustments for other reasons.

The progeny of the average breeders (see above) may serve as a control to estimate genetic gain from each generation of selection. At the same time as the new batch 1 is produced (see above), about 15 pairs of fin-clipped breeders (males from pond 1 and females from pond 2) should be mated, and 1 000 fertilised eggs from each pair should be hatched in separate jars. After absorption of the yolk sac, 40 larvae from each jar containing progeny of the first batch of selected breeders and 200 larvae from each jar containing progeny of a pair of average breeders should be counted from the jars as shown in Figure 4. The 3 000 progeny of the selected breeders should then be pooled and randomly divided in 3 equally sized groups of 1 000 larvae for stocking in 3 nursery hapas (replicates) as shown in Figure 4. The same procedure should be repeated with the progeny of the average breeders. The 6 nursery hapas should be placed together in the same pond and given the same treatment.

At fry size, about 330 fry from each nursery hapa should be transferred to separate rearing hapas and reared until fingerling size. The fry should be reared in the hapas until they have grown to a size when they may be fin clipped or branded to separate the two progeny groups from each other (e. g. by clipping the pectorial fin on one side in the progeny of selected breeders and the other side in progeny of average breeders). About 100 fin clipped fingerlings from each hapa (i. e. 300 progeny of selected breeders and 300 progeny of average breeders) should then be communally stocked in a grow-out pond until market size. The fish should be stocked at a low density and under proper feeding and management conditions to ensure good growth performance. At 8 months of age, the comparison test may be terminated by harvesting all fish and recording of individual body weights and fin clip marking to compare the two groups (Figure 4). This should provide an estimate of the realised response to selection in this generation. The procedure may be repeated in each generation.

TABLE 1. MATING DESIGN FOR PRODUCTION OF THE BASE POPULATION.

Females from location No.	Males from location No.			
	1	2	3	4
1	-	X	X	X
2	X	-	X	X
3	X	X	-	X
4	X	X	X	-

X: Mating of 6-7 pairs for each reciprocal cross

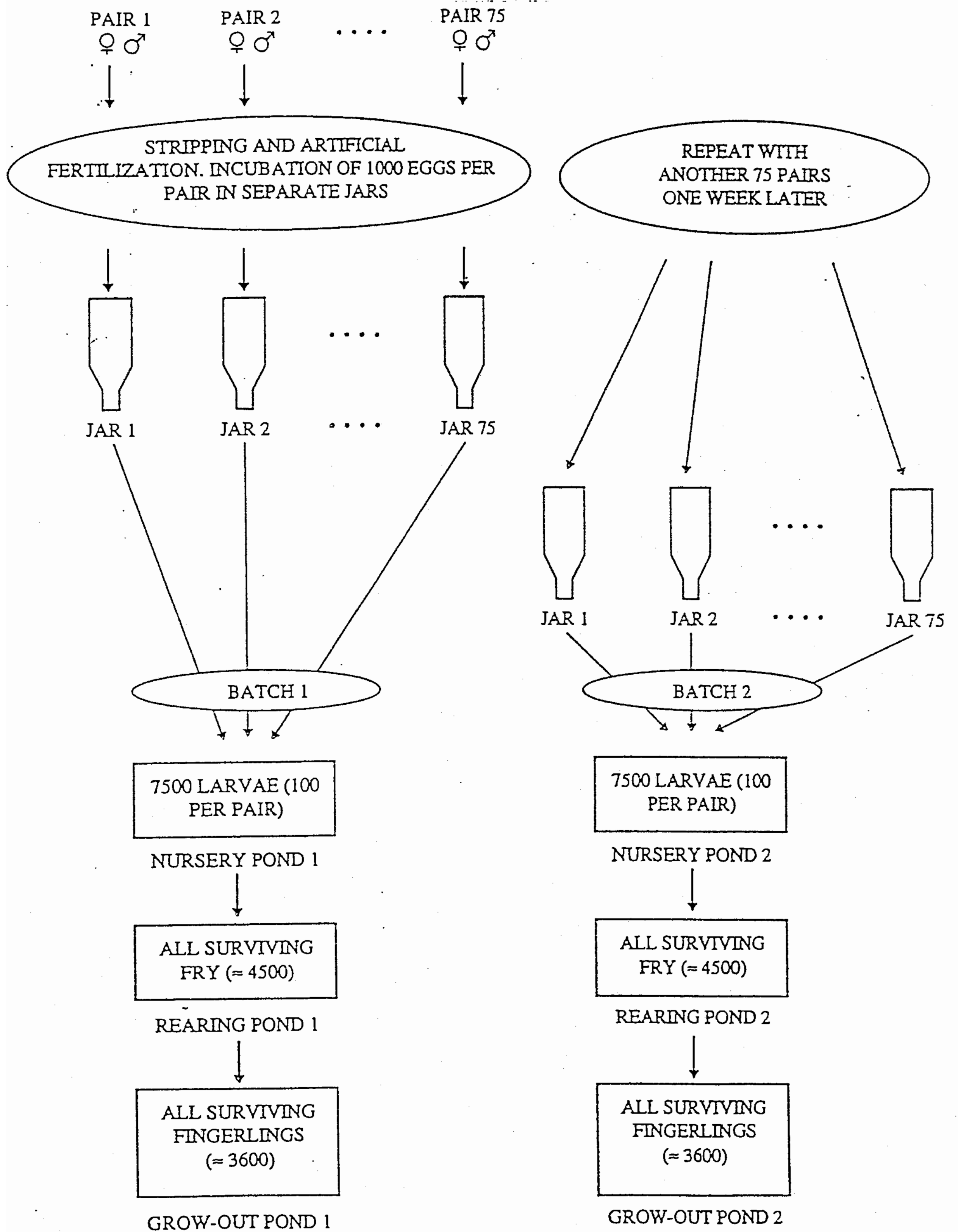

FIGURE 1. MATING AND REPRODUCTION OF THE BROODSTOCK AND REARING AND TESTING OF THE PROGENY

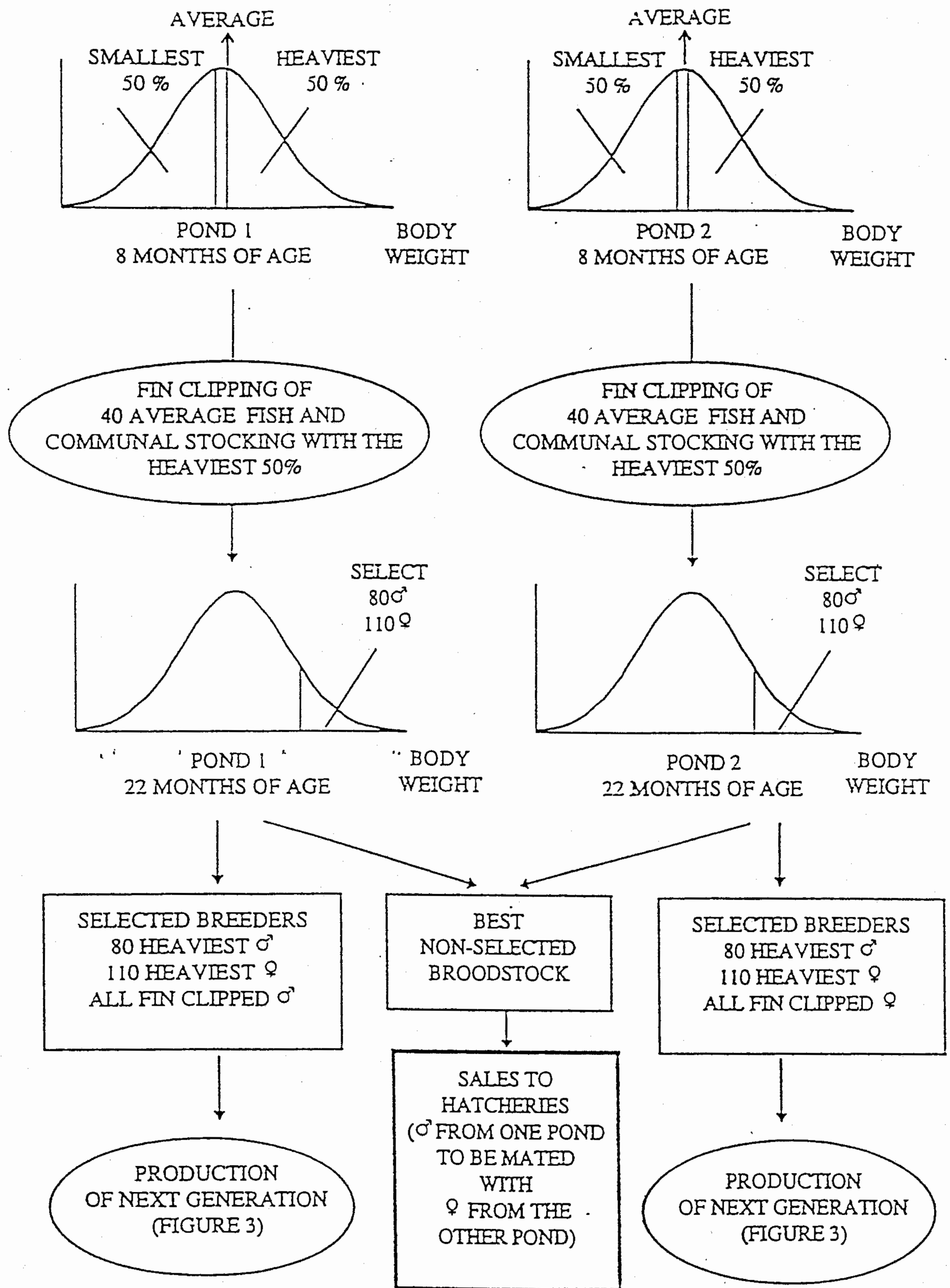

FIGURE 2. SELECTION OF BROODSTOCK FOR PRODUCTION OF THE NEXT GENERATION

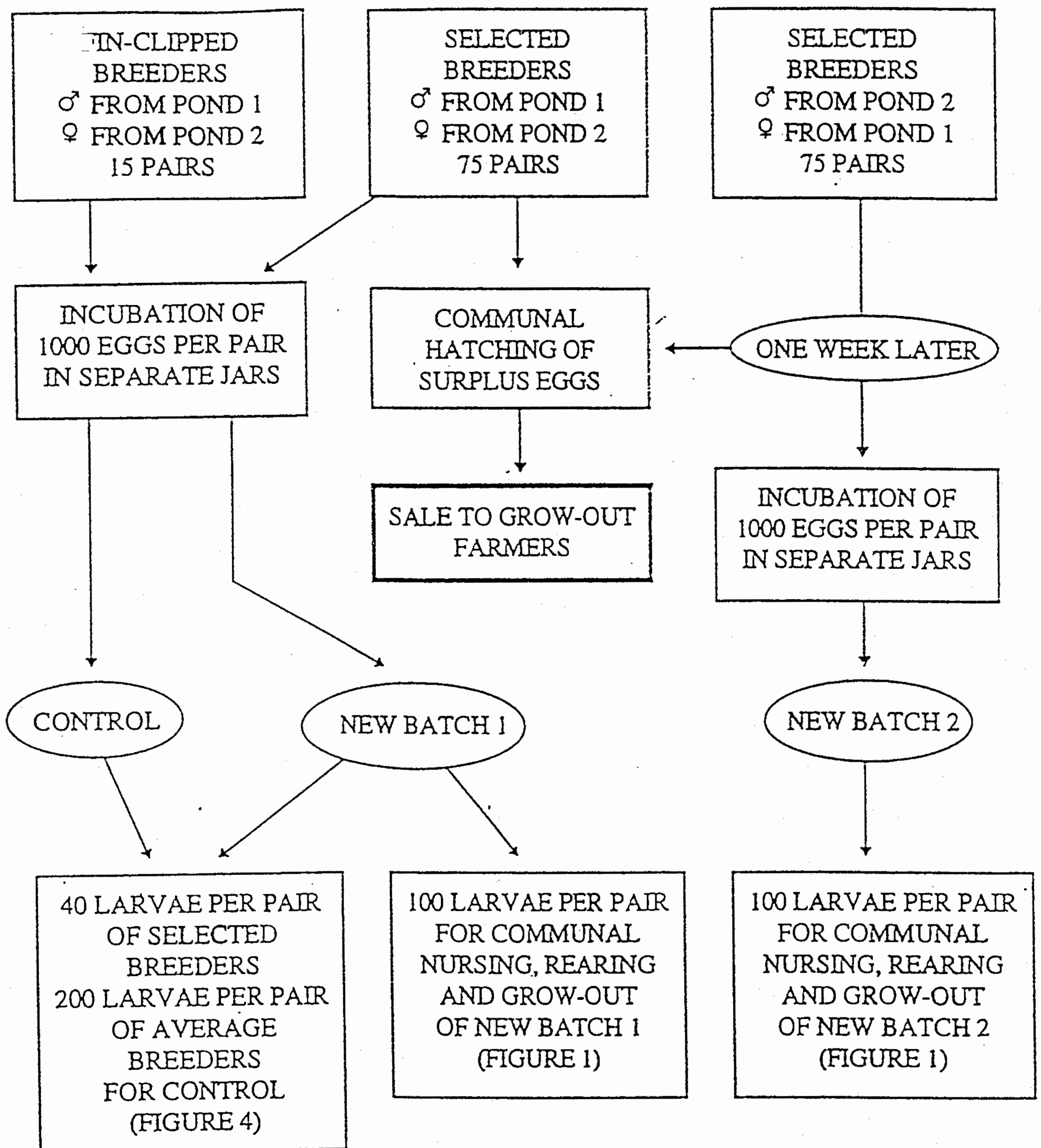

FIGURE 3. PRODUCTION OF THE NEXT GENERATION

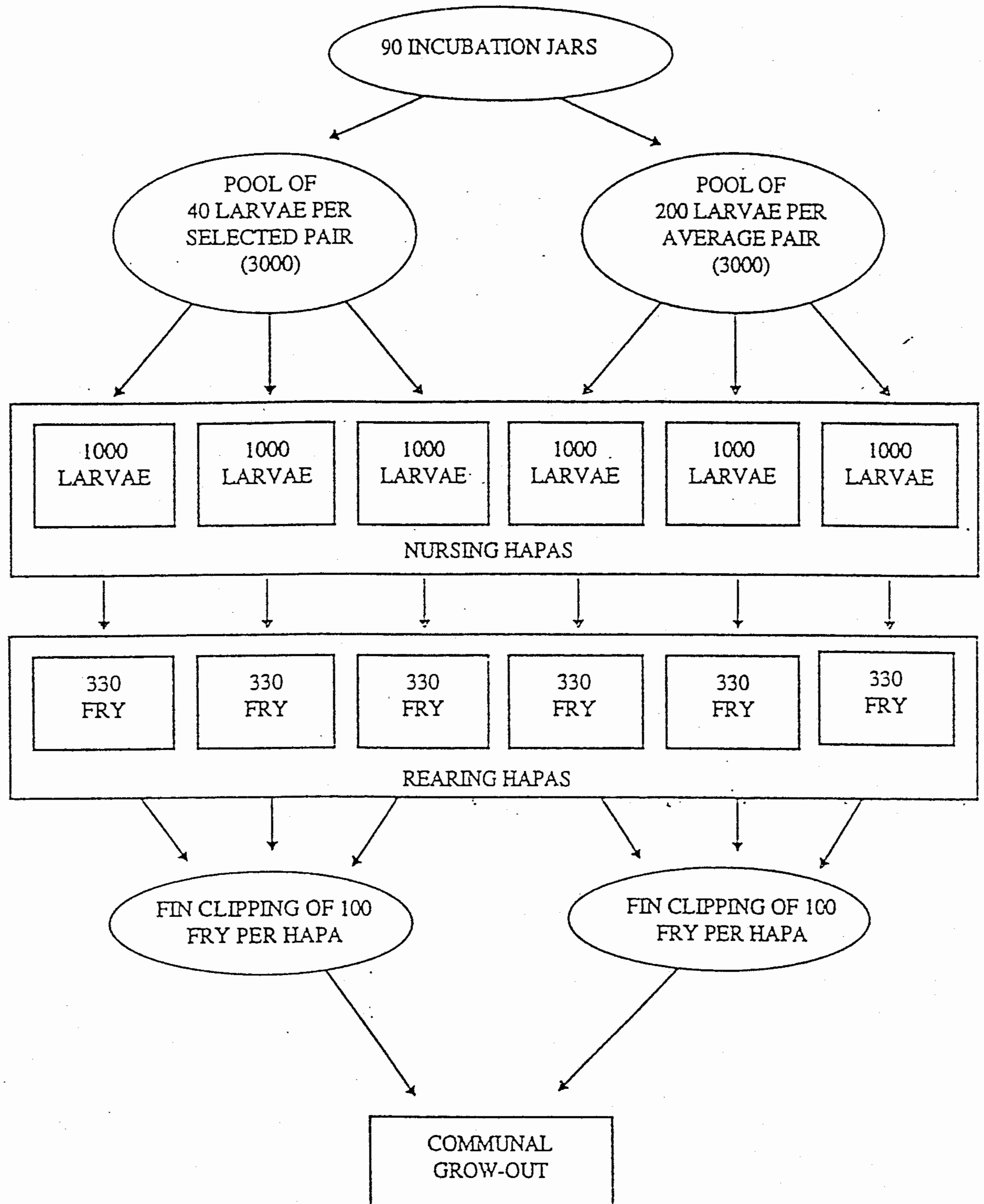

FIGURE 4. CONTROL COMPARISON OF PROGENY OF SELECTED BREEDERS WITH PROGENY OF AVERAGE BREEDERS

BREEDING PLAN FOR SILVER BARB (*PUNTIUS GONIONOTUS*) IN VIETNAM: INDIVIDUAL (MASS) SELECTION TO IMPROVE GROWTH RATE

Report No. 3

Hans Bernhard Bentsen
Trygve Gjedrem
Nguyen Van Hao

ERNATIONAL NETWORK ON GENETICS
IN AQUACULTURE
July 1996

FOREWORD

The International Network on Genetics in Aquaculture (INGA) was established in 1993 and is being coordinated by the International Center for Living Aquatic Resources Management (ICLARM), with the objective to contribute through collaborative research, to the domestication and sustainable performance of tropical finfish species farmed in developing countries and to strengthen national capabilities for genetic enhancement of farmed fish through exchange of germplasm, methodologies and through training and interactive forums.

Studies undertaken in recent years for improving breeds of salmon in Norway and tilapia in Philippines by ICLARM and collaborating Philippine and Norwegian institutions, have led to increased awareness among researchers the need for undertaking programs for improvement of breeds of species that are of aquaculture importance in their countries. INGA has been assisting the member countries in developing national breeding programs. This report on **Breeding Plan for Silver Barb *(Puntius gonionotus)* in Vietnam: Individual (Mass) Selection to Improve Growth Rate** is an outcome of such an effort and has been prepared by Drs. Hans Bernhard Bentsen and Trygve Gjedrem of the Institute of Aquaculture Research (AKVAFORSK), Norway and Mr. Nguyen Van Hao of Research Institute for Aquaculture No.2 of Vietnam. Dr. A.E. Eknath of ICLARM has assisted INGA and the authors in planning and development of this breeding program, which is greatfully acknowledged.

We hope that this document will be useful to other researchers and planners in developing breeding programs in their countries.

DR. M.V. GUPTA
INGA Research Coordinator

BREEDING PLAN FOR SILVER BARB IN VIETNAM: INDIVIDUAL (MASS) SELECTION TO IMPROVE GROWTH RATE

PREPARED FOR
THE INTERNATIONAL NETWORK OF GENETICS IN AQUACULTURE (INGA)

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AKVAFORSK Report No. 22/96

INTRODUCTION

Silver barb (*Puntius gonionotus*) is widely distributed in Southeast Asia. In addition to Vietnam, the species is found in Thailand, Cambodia, Lao, Malaysia, Indonesia, Bangladesh etc. In Vietnam, the species is abundantly represented in the Mekong Delta and has a wide distribution in Southern Vietnam (Dong Nai, Tien Giang, Vinh Long, Can Tho, Soc Trang, An Giang etc.)

Silver barb is one of the 40 economically important species in the Mekong Delta. The species has several characteristics that makes it suitable for culture. It grows well on low protein diets, whether feeding on certain aquatic plants or given supplementary feeding. If properly acclimatised, it can tolerate a wide range of environmental conditions: Water temperatures from 15 to 41.5 °C (suitable temperature from 25 to 30 °C), salinity levels from 0 to 7 ppm, pH levels from 5.5 to 9 (suitable pH from 7 to 8), and dissolved oxygen levels down to 0.656 mg/l (suitable oxygen levels above 2 mg/l). The species also seems to be resistant against diseases and tolerates handling and captivity. In pond culture, silver barb grows well during the first year and may reach an individual body weight of 150-200 grams in 12 months. In paddy fields with high water level during the rainy season, the body weight may reach 500 grams after 6-8 months. Some experiments suggest that the growth rate is lower during the second year.

In the Mekong Delta, the spawning season of silver barb is from March until September. Sexual maturity occurs at about 12 months of age in both males and females. Broodstock will normally be used for 2 to 3 breeding seasons, and spawning may be repeated 4 to 5 times in one season. The spawning interval may vary from 30 to 45 days.

Spawning is induced by environmental manipulation (high speed of running water) or by injecting both sexes with pituitary gland extract or manufactured hormones (HCG, LR-A from China). Females are injected twice and males are injected once at the same time as the second injection of the females. Males and females are then stocked together in a Chinese design spawning tank at a ratio of 1:1. Natural spawning occurs after 6-8 hours depending on the water temperature. All males and about 75-80 percent of the females will normally spawn after injection. The females will produce about 200 000 to 300 000 eggs per kg of body weight. The eggs will hatch after 17-18 hours at a water temperature of 27-29 °C. The hatchlings will be kept in the circular incubation tank for 3-4 days until the yolk sac has been absorbed.

Further rearing and grow-out will follow the techniques usually applied to farmed freshwater fish in Southern Vietnam.

Silver barb is a popular species among fish farmers in the Mekong Delta. The species is widely appreciated by the consumers because of it's long history from inland fisheries. In recent time, silver barb has become the main species in rice-fish culture in the Mekong Delta because of the favourable effects on the total income of the farmers. The impacts of a genetically improved farm stock of silver barb may consequently be substantial. The first priority breeding goal should be increased growth rate. In the long term, other traits like disease resistance, meat quality etc. should also be considered.

BASE POPULATION

At present, silver barb originating from various locations in the Mekong Delta is reproduced in captivity for aquaculture purposes. Broodstock will be collected from two such populations at Cai Be fish farm (Tien Giang province) and Can Tho fish farm (Can Tho province). However, to secure the genetic variability of the base population, and to eliminate possible inbreeding, wild silver barb broodstock will also be collected from 3 different locations in the Mekong River: Hau River in the Chau Doc district, Tien River in the Cao Lanh district, and plain of reed in the Hong Ngu district. Broodstock will also be collected from the Tri An reservoir in the Dong Nai River. The collected broodstock will be kept at the Cai Be fish farm of RIA 2 for mating. All together 100 males and 100 females from the 6 different locations (16-17 males and 16-17 females from each location) will be cross mated as shown in Table 1 to form a heterogeneous, outbred base population for the breeding program.

BREEDING GOAL AND SELECTION METHOD

The most important breeding goal for an aquaculture stock of silver barb is to improve the growth rate. Since spawning of the breeders in the breeding nucleus may be synchronised (induced spawning), all the test fish in the following generation will be of the same age. Growth rate may then be recorded for each individual test fish as body weight on a fixed recording day. No tagging will be necessary to correct for age differences. The additive genetic variation of growth rate in silver barb has not been studied. If the variation is similar to that of several other fish species, growth rate may easily be improved by individual (mass) selection, i.e. without tagging. The breeding program should then be designed to avoid loss of genetic variation and to avoid rapid accumulation of inbreeding.

This may be done by securing a large effective population size. In the proposed breeding plan, a large effective population size is achieved by using a large number of breeders in each generation (100 pairs), and a restricted number of progeny per pair (on average 20-30 surviving progeny per pair at the end of the grow-out test). Furthermore, the variation between pairs in the number of progeny is kept as low as possible by separate nursing and rearing of the progeny groups through the early life phases, when the mortality is high. A fixed number of progeny per pair may then be counted and communally stocked for grow-out testing. The design is expected to result in a rate of inbreeding of less than 1% per generation.

Future broadening of the breeding goal to include less heritable traits or traits that may not be recorded in the breeding candidates will require development of tagging or branding techniques for pedigree recording. This may be done as a continuation of the proposed breeding program, since loss of genetic variation and accumulation of inbreeding will be kept under control.

START OF THE PROGRAM

The relative performance of the 6 collected strains in various farm environments will not be known at the start of the program. However, several strain comparison tests with other fish species have given little evidence of strong genotype by environment interactions. Rather than spending a lot of time and efforts (e.g. on developing tagging or branding methods) to carry out a strain comparison test in a range of farm environments, it is recommended to start forming a synthetic (mixed) base population for selection from the early beginning of the program.

If the collected strains are available at RIA 2 (Cai Be fish farm) before the end of 1996, mating may start in April/May 1997. All matings should be strain crosses, using all possible strain combinations in a 6 by 6 complete crossing design without pure-breds (Table 1). About 100 single pairs will be mated by stripping of eggs and milt and artificial fertilisation in trays. About 1 000 fertilised eggs from each pair will be transferred to separate incubation jars, where they will be hatched and kept until absorption of the yolk sac (Figure 1).

For each of the 30 reciprocal crosses, 3-4 pairs will be mated separately, producing about 100 full sib families (Table 1). This means that at least 23 females and 17 males from each location must be injected to induce spawning. Each male should be mated to one female only, and each female to one male only. All matings will take place within one day. The hatchlings will be kept in the jars until the yolk sac is absorbed. The number of larvae per sib group may then be standardised.

Even if the number of larvae from each sib group is standardised after yolk sac absorption, the high mortality of larvae from yolk sac absorption until fry size (about 60 % in an ordinary nursery pond or hapa) may cause the number of fry per sib group to be quite variable at the end of the nursing period, and some sib groups may be entirely lost. Consequently, methods of separate nursing of larvae from each incubation jar until fry size should be investigated. A possible method may be to transfer the larvae from each jar to a separate, small nursery hapa (Figure 1). All 100 nursery hapas should then be installed in the same pond. The number of larvae to be transferred from each jar to the separate nursery hapas will depend on the mortality in the hapas until fry size. If the mortality is similar to that under communal nursing, 150 larvae should be counted from each jar and stocked in each nursery hapa. At fry size, 50 fry should then be counted from each hapa and communally stocked in a rearing pond. At fingerling size, all surviving fingerlings should be communally stocked in a grow-out pond until sexual maturation at 11-12 months of age (Figure 1). If nursing of the sib groups in separate hapas is found to be impossible, 125 larvae may be counted from each of the 100 jars (from each sib group), and communally stocked in a nursery pond or hapa until the fry may be transferred to a communal rearing pond and later on to a grow-out pond (Figure 1). At a stocking density of 100 larvae per square meter, this will require a nursery pond (or hapa) of 125 square meters.

The number of fry to be communally stocked for rearing in a rearing pond will be about 5 000, both if the sib groups are nursed separately (50 fry from each of 100 nursery hapas) or communally (125 larvae from each of 100 jars communally stocked in one nursery pond, 60 % mortality before transfer to the rearing pond). At a stocking density of 10 fry per square meter, this will require a rearing pond of 500 square meters. Assuming a survival rate of 60 % until stocking of the fingerlings in a grow-out pond, the total number of fingerlings will be about 3 000. At a stocking density of 2 fingerlings per square meter, one grow-out pond of 1 500 square meters will be required for testing and production of broodstock for selection. It is essential to secure the best possible environment (density, feeding, water quality etc.) during grow-out, to make sure that the test fish will express their growth potential and reach sexual maturity at about 11 months of age.

SELECTION OF BROODSTOCK

Assuming a survival rate of 80 % in the grow-out pond from stocking of the fingerlings until the fish has reached an age of 11-12 months, 2 400 breeders will be available for selection (no pre-selection). Body weight, sex, and sexual maturity should then be recorded for all fish. Among the sexually mature breeders that are ready to spawn, the 110 largest males and the 130 largest females should be

selected and injected to induce spawning (Figure 2). This will result in an expected selection intensity of about 8-10 %. With an assumed heriabilty for body weight of about 0.3 and an assumed coefficient of variation of 30 percent, as shown in many fish species, the expected genetic gain in the progeny should amount to about 15-17 % compared to the mean of the parent generation (15-17 % genetic gain per generation). Preferably, selection and mating should be carried out at 11-12 months of age to maintain a generation interval of about one year. This will make it possible to carry out the mating of the selected breeders at about the same time during the spawning season every year. If the generation interval has to be extended to more than a year, mating will have to take place later in the spawning season for every generation that passes, and eventually it will have to be postponed with 6-7 months until the next spawning season occurs.

At the same time as the largest breeders are selected, 20 sexually mature males with average male body weight and 25 sexually mature females with average female body weight should be selected and injected to induce spawning. These breeders will be used to produce a control group.

PRODUCTION OF THE NEXT GENERATION

The production of the next generation should be carried out by single pair mating of 100 of the largest selected males and 100 of the largest selected females and 15 average males with 15 average females (Figure 2). Again, separate incubation and hatching of 1 000 eggs counted from each pair into incubation jars and nursing of 150 larvae counted from each incubation jar into separate nursery hapas should be carried out following the design in Figure 1. At fry size, 50 progeny from each of the 100 selected pairs should be communally stocked in a rearing pond until fingerling size and then transferred to a grow-out pond for testing.

If separate nursing of the progeny from each jar until fry size is not possible, 125 larvae should be counted from each jar for each pair of selected breeders and communally stocked in one nursery pond or hapa, and all surviving fry after the nursing period should be transferred to a rearing pond and later to the grow-out pond for testing as shown in Figure 1.

For comparison with a genetic control, 30 additional larvae should be counted from each incubation jar containing progeny of selected breeders and 200 larvae should be counted from each jar containing progeny of average breeders. The further procedure for control of genetic gain is described below and in Figure 3.

The surplus eggs from the 100 selected females may be incubated in ordinary, large scale circular tank incubators for hatching. The larvae may then be sold directly from the breeding station to fish farmers (Figure 2). The selected males and females may also be used in repeated spawnings for commercial mass production of postlarvae throughout the spawning season. Assuming that a total of 100 females with an average body weight of 0.3 kg will spawn, that 200 000 eggs will be spawned per kg of body weight per spawning, that each female will spawn 3 times during the spawning season, and that the survival from fertilisation to postlarvae will be 60 %, this should result in a production of about 10 million postlarvae that will be available for the grow-out farmers. This will all be progeny of the best selected breeders. If the demand from the farmers exceeds this supply, the best non-selected broodstock may be reproduced commercially at the breeding station or sold to collaborating hatcheries for production of postlarvae (Figure 2). Progeny of the best selected breeders may also be reared and used as broodstock by collaborating hatcheries.

CONTROL TO ESTIMATE GENETIC GAIN

Establishing a procedure for control of genetic gain is not required to obtain response to selection in a breeding program. However, a lot of assumptions have been made about unknown parameters in the present program. Including a routine for genetic control will make it possible to check if these assumptions are valid, or if the program needs adjustments for other reasons.

The progeny of the average breeders (see above) may serve as a control to estimate genetic gain from each generation of selection. After the progeny of single pair matings of selected and average broodstock has been hatched in separate jars, 30 larvae from each jar containing progeny of selected breeders and 200 larvae from each jar containing progeny of a pair of average breeders should be counted from the jars as shown in Figure 3. The 3 000 progeny of the selected breeders should then be pooled and randomly divided in 3 equally sized groups of 1 000 larvae for stocking in 3 nursery hapas (replicates) as shown in Figure 3. The same procedure should be repeated with the progeny of the average breeders. The 6 nursery hapas should then be placed together in the same pond and given the same treatment .

At fry size, about 330 fry from each nursery hapa should be transferred to separate rearing hapas and reared until fingerling size. The fry should be reared in the hapas until they have grown to a size when

they may be fin clipped or branded to separate the two progeny groups from each other (e. g. by clipping the pectorial fin on one side in the progeny of selected breeders and the other side in progeny of average breeders). About 100 fin clipped fingerlings from each hapa (i. e. 300 progeny of selected breeders and 300 progeny of average breeders) should then be communally stocked in a grow-out pond until market size. The fish should be stocked at a low density and under proper feeding and management conditions to ensure good growth performance. The difference in mean body weight between the two groups will then represent the response to the previous round of selection. The procedure may be repeated in each generation.

TABLE 1. MATING DESIGN FOR PRODUCTION OF THE BASE POPULATION.

Females from location No.	Males from location No.					
	1	2	3	4	5	6
1	-	X	X	X	X	X
2	X	-	X	X	X	X
3	X	X	-	X	X	X
4	X	X	X	-	X	X
5	X	X	X	X	-	X
6	X	X	X	X	X	-

X: Mating of 3-4 pairs for each reciprocal cross

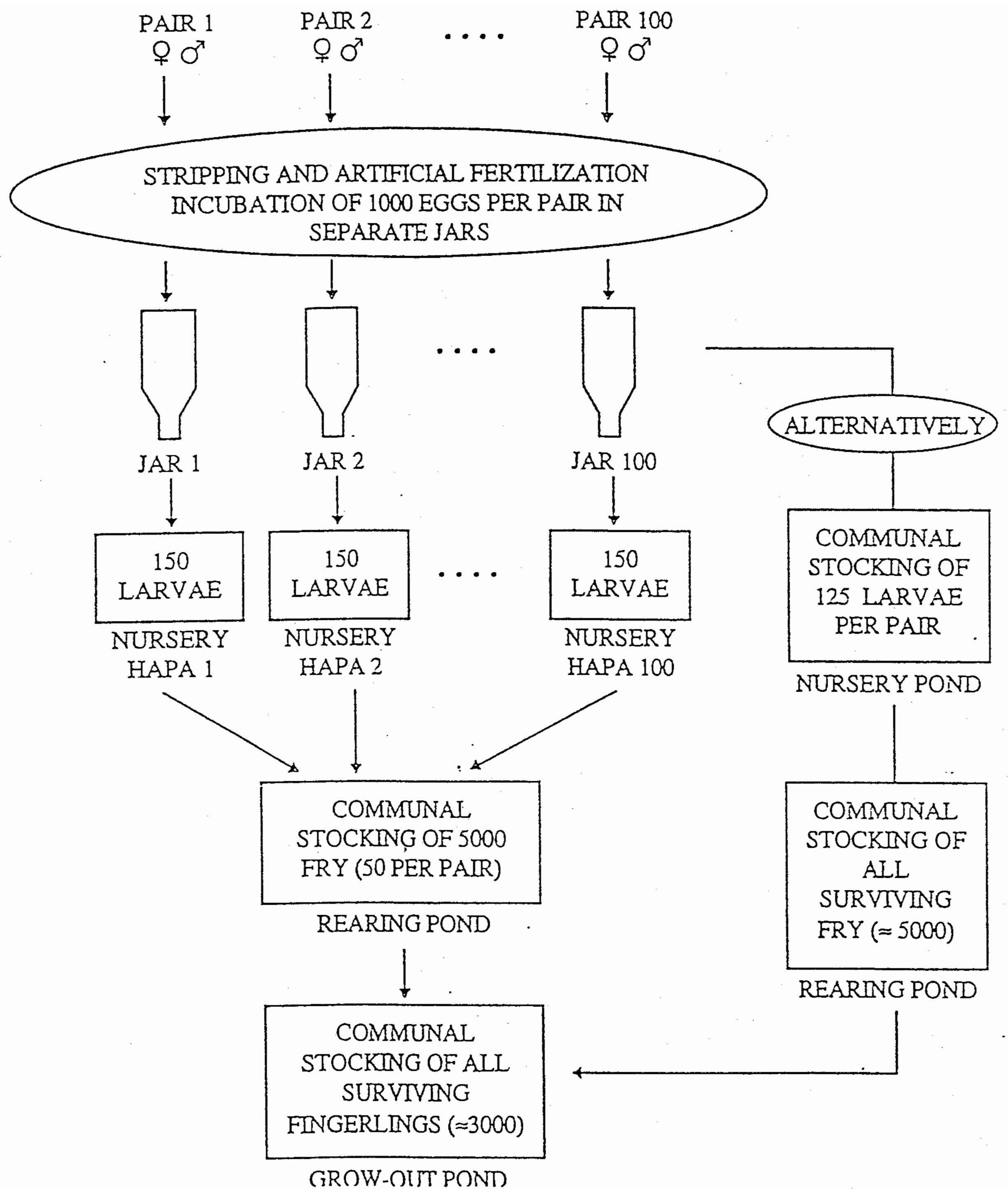


FIGURE 1. MATING AND REPRODUCTION OF THE PARENT BROODSTOCK AND REARING AND TESTING OF THE PROGENY

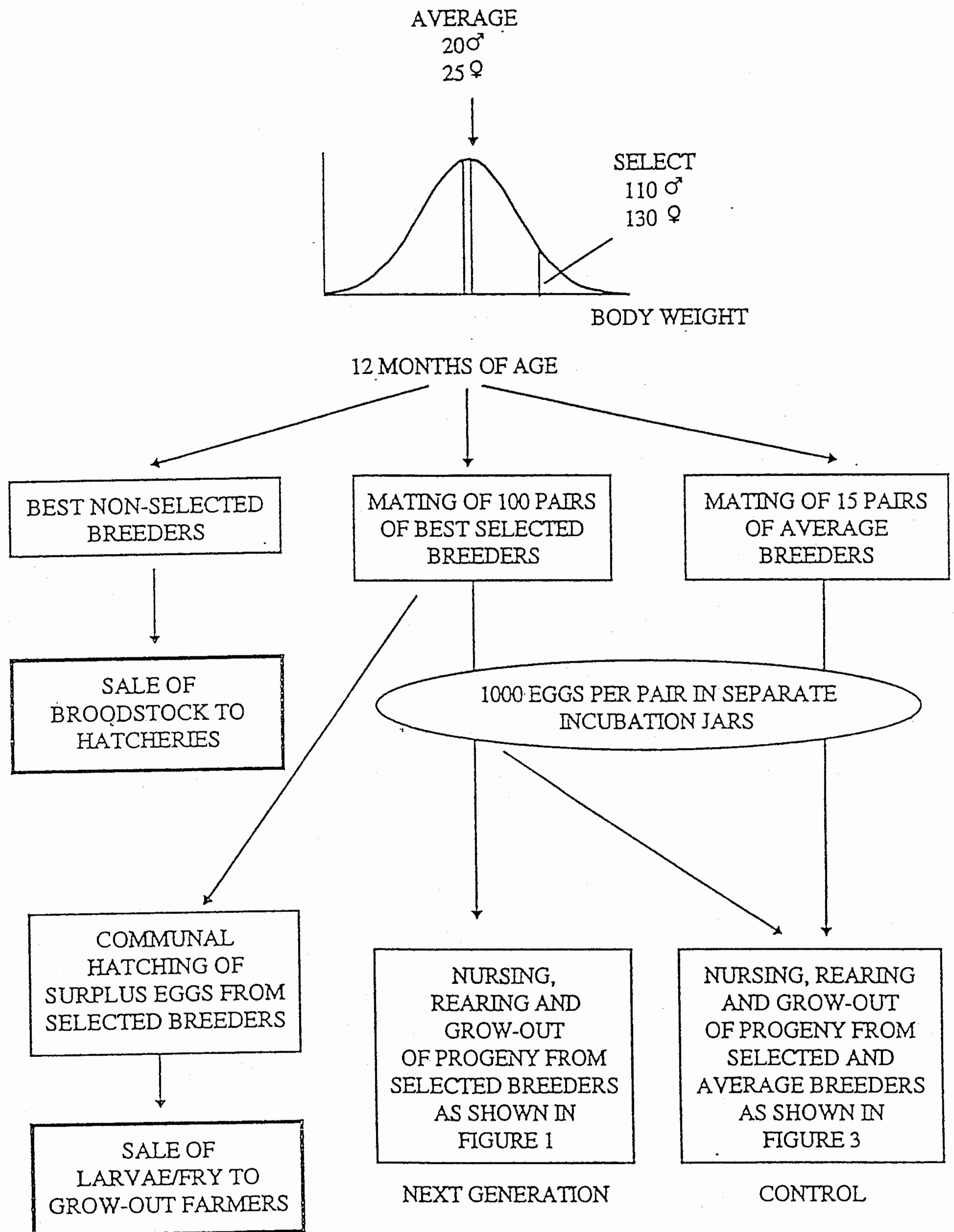


FIGURE 2. SELECTION OF BROODSTOCK FOR PRODUCTION OF THE NEXT GENERATION. DISSEMINATION OF IMPROVED BROODSTOCK TO FARMERS

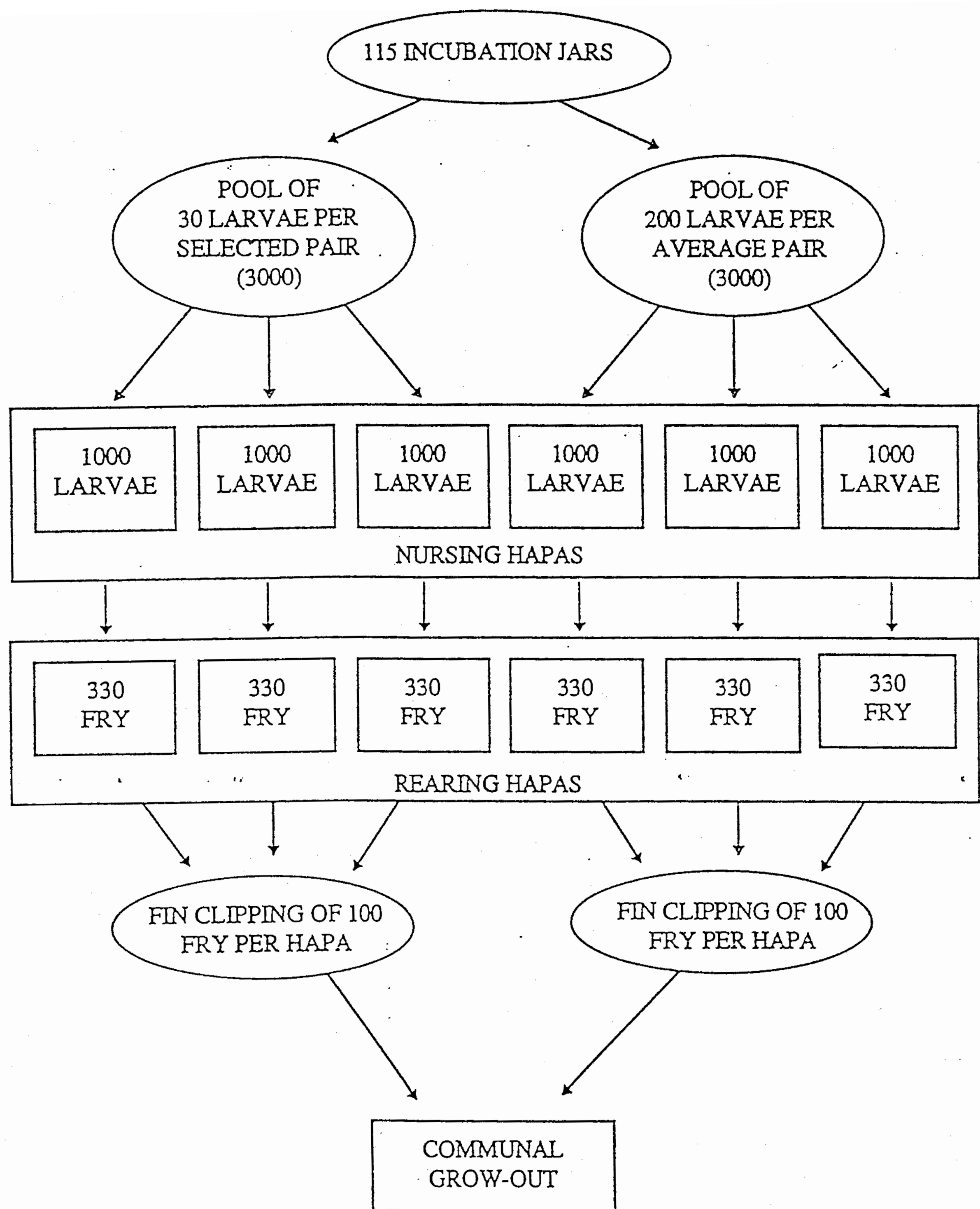


FIGURE 3. CONTROL COMPARISON OF PROGENY OF SELECTED BREEDERS WITH PROGENY OF AVERAGE BREEDERS

BREEDING PLAN FOR NILE TILAPIA *(Oreochromis niloticus)* IN INDONESIA: INDIVIDUAL SELECTION

Report No. 4

INTERNATIONAL NETWORK ON GENETICS
IN AQUACULTURE
February 1997

FOREWORD

The International Network on Genetics in Aquaculture (INGA) was established in 1993 with the objective to contribute through collaborative research, to the domestication and sustainable performance of tropical finfish species farmed in developing countries and to strengthen national capabilities for genetic enhancement through exchange of germplasm, methodologies and through training and interactive forums.

To realize this objective, the network has been assisting the member countries in developing regional research programs and plans for national breeding programs. INGA fielded a mission to Indonesia in November 1996, consisting of the INGA Research Coordinator Dr. Modadugu V Gupta and Drs. Trygve Gjedrem and Hans Magnus Gjoen of Institute of Aquaculture Research (AKVAFORSK), As, Norway, to assist national scientists in prioritizing aquaculture species for genetic improvement and develop plans for selective breeding of the prioritized species.

Tilapia (*Oreochromis niloticus* and *O. mossambicus*) contributes about 64,000 tons per year to aquaculture production (fresh and brackishwater) or about 13% of the finfish production from aquaculture. Initial trials undertaken by the Indonesian institutions indicated better performance of GIFT strain of Nile tilapia as against the local strains and hence the government is interested in developing a breeding program for the species.

This report details plans for selective breeding of GIFT strain Nile tilapia in Indonesia using individual selection and has been prepared by Drs. Trygve Gjedrem and Hans Magnus Gjoen of AKVAFORSK and Drs. Atmadja Hardjamulia, Ir. Sudarto, Ani Widiaty, Rudi Gustiano, Anang Hari Kristanto, Lies Emmawati and Wartono Hadie of the Research Institute for Freshwater Fisheries (RIFF), Sukamandi, Indonesia.

INGA acknowledges the support provided by the Indonesian Network of Fish Genetics Research and Development (INFIGRAD) to the mission and in preparing these plans,

We hope that this document will be useful to researchers in Indonesia for implementing the plans developed and informative to others involved in selective breeding in other countries.

Modadugu V Gupta
INGA Research Coordinator

BREEDING PLAN FOR NILE TILAPIA (*Oreochromis niloticus*) IN INDONESIA: INDIVIDUAL SELECTION

INTRODUCTION

Total aquaculture production in Indonesia in 1994 was 493 000 tons of finfish, 169 000 tons of crustacea, mainly shrimp and 115 000 tons of seaweed with an estimated value of 2 075 million US $. In 1994 the production of Nile tilapia was 17 600 tons and for Mozambique tilapia it was 46 800 .

The area of Fresh waters, which consist of lakes, dams, rivers, swamps and other water basins, is estimated to be 14 mill. ha. Potential areas for fish culture is estimated to 338,121 ha. with a production potential of 8-900 000 tons each year. (Source: Directorate General of Fisheries, Indonesia.)

Nile tilapia (*Oreochromis niloticus*) in Indonesia is cultured both in earthen ponds and in cages. It is assumed that production in cage culture will grow fastest in the years to come. The market for Nile tilapia is considered to be increasing. Nile tilapia from Thailand (Chitralada strain) was introduced in Indonesia in 1990, while GIFT strain was introduced from Philippines in 1994.

The strategic plan of AARD (Agency for Agricultural Research and Development) for 1995 - 2005 gives high priority to improvement of genetic potential of fish, livestock, crop and micro-organisms (AARD, 1994). In accordance with this, the Indonesian Network of Fish Genetics Research and Development (INFIGRAD) in co-operation with the International Network on Genetics in Aquaculture (INGA) has taken the initiative to establish a breeding program for Nile tilapia in Indonesia. In this program, INFIGRAD will have its role as research co-ordinator, and the members may contribute in the research and development under the responsibility of RIFF.

The effect of a breeding program depends very much on how it is organised to get the obtained improvement transferred rapidly to the farmers. AIAT (Assessment Institute for Agricultural Technology) and FADC (Freshwater Aquaculture Development Centre) are given a central role in this dissemination of the improved fish. It is important that INFIGRAD co-ordinate this process.

In this proposal, the Research Institute of Freshwater Fisheries (RIFF) serve as the breeding centre where mating, testing and selection takes place. After the first generations, as RIFF develops and improves the

breeding program, mating, testing and selection will become more and more standard routine work. This activity will also take up much of the research capacity of RIFF. At that stage, INFIGRAD may assist in reorganising the breeding program in order to reduce the routine breeding work at RIFF and in developing other breeding centres.

The *generation interval* (see Appendix), will depend on the time needed to reach sexual maturation. Today this is 9-12 months for the strains that are in common use in Indonesia, but it is not known what it will be with the GIFT-fish, which has an improved growth rate compared to the strains used today.

BREEDING GOAL

The most important economic traits for production of Nile tilapia in Indonesia are:

- Growth rate
- Delayed sexual maturation
- Disease resistance
- Dressing percentage

Growth rate should be recorded at the marketed size, about 0.3 kg, which is usually obtained at half year of age.

As discussed under the next paragraph, Selection Method, individual selection is the method of choice for Nile tilapia in Indonesia. Thus, growth rate is the only trait that can be efficiently selected for. Late sexual maturation, dressing percentage and disease resistance may, however, be included later if desired, but family tagging must then be used in the breeding program.

Preliminary results from the GIFT project in the Philippines, may indicate that delayed sexual maturation has a negative genetic correlation to growth rate. This should be carefully monitored in the present breeding program, and at a later stage it should be decided if the breeding program should be expanded to a family selection program in order to efficiently improve this trait.

Before disease resistance could be included in the breeding goal, there is need for investigations and development of methods for testing, e.g. challenge tests. Even though disease resistance is not included as specific and recorded traits, a natural individual selection will take place in the breeding population. Genetic gain in this trait may therefore still be expected in the proposed breeding plan for Nile tilapia.

Dressing percentage has, in several investigations in fish, shown to be genetically positive correlated to growth rate, and is thus expected to improve as a correlated response to selection on growth rate.

SELECTION METHOD

Results from the Genetically Improved Farmed Tilapia (GIFT) project in the Philippines have shown large genetic variation and large response to selection for growth rate. This genetic variation may, however, also be utilised easily by individual selection, i.e. without the necessity of tagging. The breeding program should then be designed to control and restrict accumulation of inbreeding and thus avoid loss of genetic variation and inbreeding depression. This is done by securing a large effective population size. In the proposed breeding plan, a large effective population size is achieved by using many breeders in each generation, and a restricted number of progeny per pair to be available for selection at the end of the grow-out test. The variation in the number of progeny between pairs is kept as low as possible by separate rearing of the family groups through the early life phases, when the mortality is high. A fixed number of progeny per pair may then be counted and communally stocked for grow-out testing. Assuming the heritability to be 0.2 for growth rate, the design of this breeding program is expected to result in a rate of inbreeding of less than 1% per generation, which will be sufficient to maintain the genetic variation and thus ensure a sustainable genetic progress.

Individual selection is chosen rather than a combined individual and family selection because of research and test capacity reasons. Furthermore, as cage culture becomes more and more important in freshwater aquaculture, early sexual maturation becomes less of a problem in this farming system.

As mentioned before, a future broadening of the breeding goal by delayed sexual maturation, improved disease resistance and increased dressing percentage, will require tagging or branding for pedigree recording and family selection. This may be done as a continuation of the proposed breeding program, since loss of genetic variation and accumulation of inbreeding will be kept under control and the breeding population may serve as a future base population for a program that employ family selection.

BASE POPULATION

Through the International Centre for Living Aquatic Resources Management (ICLARM) and INGA, RIFF has established contacts with the GIFT project in the Philippines. A request for transfer of a broad sampling of families of the latest generation from the GIFT project to RIFF (Sukamandi) has been forwarded. This should represent all the families in the GIFT project and each family should be represented with at least 5 fish (about 1000 in total). The fish may be family tagged to avoid fullsib

mating, but this is strictly not necessary. The imported GIFT fish will then form the base population for a breeding program for Nile tilapia in Indonesia.

MATING SYSTEM

Natural mating, i.e. mouth brooding, should be practised by placing the broodstock in breeding hapas (1x1x1 m^3). Before mating, the breeders will be conditioned, which means they are given better space and feed, to improve and increase rate of sexual maturation. One male will then be stocked with 2 females in each of the breeding hapas (Fig. 1). A total of 75 breeding hapas should be installed in one pond. All hapas should be inspected once every week for swim-up fry. Swim-up fry will be collected separately from each hapa and transferred at a standardised stocking density (300 fry) to 1x1x1 m rearing hapas, one hapa for each fullsib group. The date of collection of swim up fry should be recorded. The spent females shall be removed from the breeding hapas. Breeding should continue until 100 fullsib groups are produced. After 3-4 weeks in the rearing hapas, the fry of families collected in the 1st week will be transferred at a reduced number (200) to an earthen pond. The fry from families collected the 2nd an 3rd week will likewise be transferred to a 2nd and 3rd pond.

When the fish reach about 10 g (after *ca* 1.5 months), fish in each pond should be transferred to 3 cages in RIFF's floating net cage unit. The fish will be reared in the cages for 3-4 months until they reach an average size of 250-300 g which is considered to be the market size. Assuming 50 % survival from the time of transfer to the ponds to the end of the grow-out period, it is expected that on an average 100 fish from each family will be present at time of selection.

SELECTION OF BROODSTOCK

At the time of selection, there will be about 3 300 fish in each cage, averaging 250-300 g. To allow some mortality from time of selection of broodstock until mating, in total 150 males and 300 females should be selected and transferred to Sukamandi. In addition, the second heaviest fish should be selected for purpose of dissemination and should be transported to the organization that will perform the dissemination (see Fig. 2 and also next section).

Since the fish in the 3 cages on average are considered to have the same genetic value, equal number of males (50) and females (100) should be selected from each cage. Likewise, fish to be used for dissemination should also be equally selected from each of the 3 cages.

In order to find the fish with the highest body weight, it is not necessary to record body weight of all fish. A grading system could for example save one third of the heaviest fish as possible broodstock. These fish should then be weighed and the heaviest ones from each cage should be selected as described above. Mating should be performed between males from one cage and females from another cage in order to decrease the risk of inbreeding depression.

DISSEMINATION OF IMPROVED SEED TO THE FISH FARMERS

In order to maximise the benefit from the breeding program, the genetic improvement should reach the fish-farmers without delay. From RIFF there are mainly three routs for dissemination, namely trough AIAT, FADC and directly to private hatcheries. For this purpose, only improved broodstock should be used.

Because of the relatively low fecundity of Nile tilapia, dissemination of improved seed will have to be based on distribution of improved broodstock to hatchery operators (Figure 2). After production of fullsib families for the breeding population have been completed, the selected parents should be used for mass production of seed for hatchery operators. The progeny of the selected parents will, when reaching sexual maturation, be top genetic quality broodstock, followed by the progeny of the discarded breeders from the best one third of the population (see above).

It is very important that the collaborating breeding centres are informed and educated in how to use the new broodstock. The new breed should not be used as broodstock in the same manner as the old pure strains inasmuch as offspring of the selected broodstock should not be re-entered into breeding. It is also important that the hatchery managers and multipliers understand that it is of significance to use top quality seed from the breeding system.

CONTROL TO ESTIMATE GENETIC GAIN

Establishing a procedure for control of genetic gain in a breeding program is not required to obtain response to selection. Including a routine for genetic control will, however, make it possible to check if the assumptions that are made are valid, or if the program needs adjustments for other reasons.

At the same time as the largest breeders are selected, 20 sexually mature males with average male body weight and 20 sexually mature females with average female body weight should be selected (The average should be determined from a sample of at least 200 fish). These breeders will be used to produce a control group. Their progeny may be used to estimate genetic gain from each generation of

selection. After the progeny of single pair matings of selected and average broodstock has been nursed in separate hapas, 30 larvae from each hapa containing progeny of selected breeders and 150 larvae from each hapa containing progeny of a pair of average breeders should be counted from the hapas as shown in Figure 3. The 3 000 (30 x 100) progeny of the selected breeders should then be pooled and randomly divided in 3 equally sized groups of 1 000 larvae for stocking in 3 nursery hapas (replicates) as shown in Figure 3. The same procedure should be repeated with the 3000 (20 x 150) progeny of the average breeders. The 6 nursery hapas should then be placed together in the same pond and given the same treatment .

At fry size, about 330 fry from each nursery hapa should be transferred to separate rearing hapas and reared until fingerling size. The fry should be reared in the hapas until they have grown to a size when they may be fin clipped to separate the two groups from each other (e. g. by clipping the pectoral fin on one side on progeny of selected breeders and the other side on progeny of average breeders). Both groups should be fin clipped to eliminate any effect of this on growth rate. About 100 fin clipped fingerlings from each hapa (i. e. 300 randomly sampled progeny of selected breeders and 300 randomly sampled progeny of average breeders) should then be communally stocked in a grow-out pond until the market size. The fish should be stocked at a low density and under proper feeding and management conditions to ensure good growth performance. The difference in mean body weight between the two groups will then represent the response to the previous round of selection. The procedure may be repeated in each generation.

In addition to this control group, a sample of the GIFT fish in the Philippines should be brought to Indonesia for example every 5th generation. This will give interesting estimates of how individual selection is doing compared to a combined individual and family selection which is applied in the tilapia breeding program in the Philippines.

EXPECTED BENEFIT FROM A BREEDING PROGRAM

When additive genetic variation is present in a trait, there will always be response to selection if efficient selection methods are applied. In the literature there are several estimates of response to selection in growth rate in large scale breeding experiments and breeding programs. The following estimates should be mentioned (given as genetic gain in percentage per generation of selection): For coho (Pacific) salmon, 10.1; for rainbow trout, 13; for Atlantic salmon, 10.6-14.2; for channel catfish, 12-20; and for Nile tilapia, 17 %. An average figure of these estimates are 15 % genetic gain per generation for growth rate. This means that it should be possible to double the growth rate in less than 7 generations. This is a larger genetic gain than usually obtained in farm animals, and it is achieved because fish and shellfish

have larger genetic variation in growth rate and have higher fecundity; consequently, it is possible to apply a much higher selection intensity.

The benefits of genetic improvement in growth rate are reduction in both fixed costs and production costs, the latter due to lower energy requirement for maintenance for the entire life span. Often also a correlated response can be observed as an improved feed conversion rate.

In the Norwegian breeding program, which today supply genetically improved eggs of Atlantic salmon and rainbow trout to more than 70 % of the fish farming industry, has a cost/benefit ratio of 1/15. Similar estimates are also found from breeding programs in farm animals. This ratio will depend largely on the total production output that benefit from the genetic improvement. In view of the large production of Nile tilapia in Indonesia, an even better ratio may be expected for the proposed breeding program.

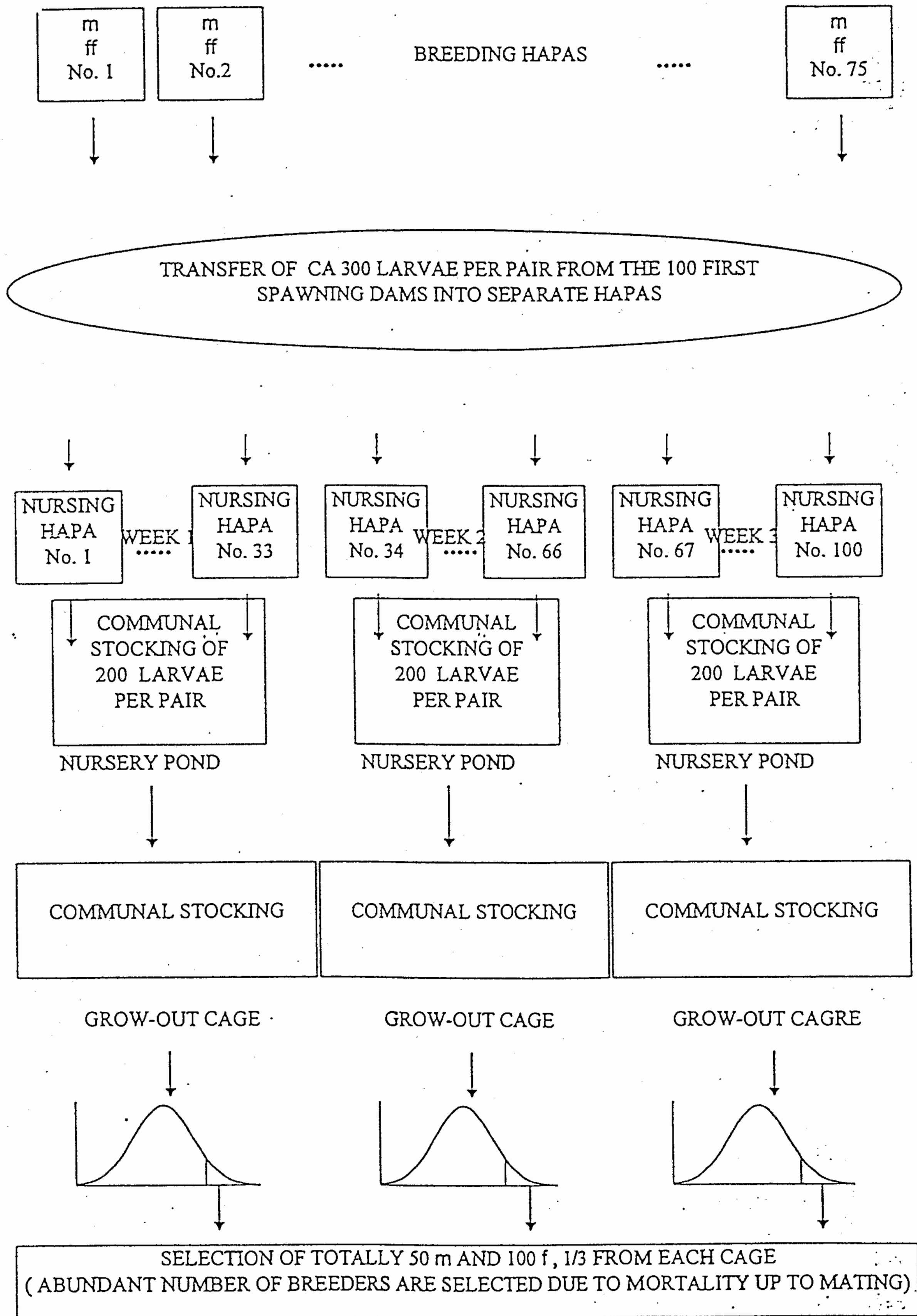


FIGURE 1. MATING OF THE PARENT BROODSTOCK AND REARING OF PROGENY UNTIL NEXT ROUND OF SELECTION

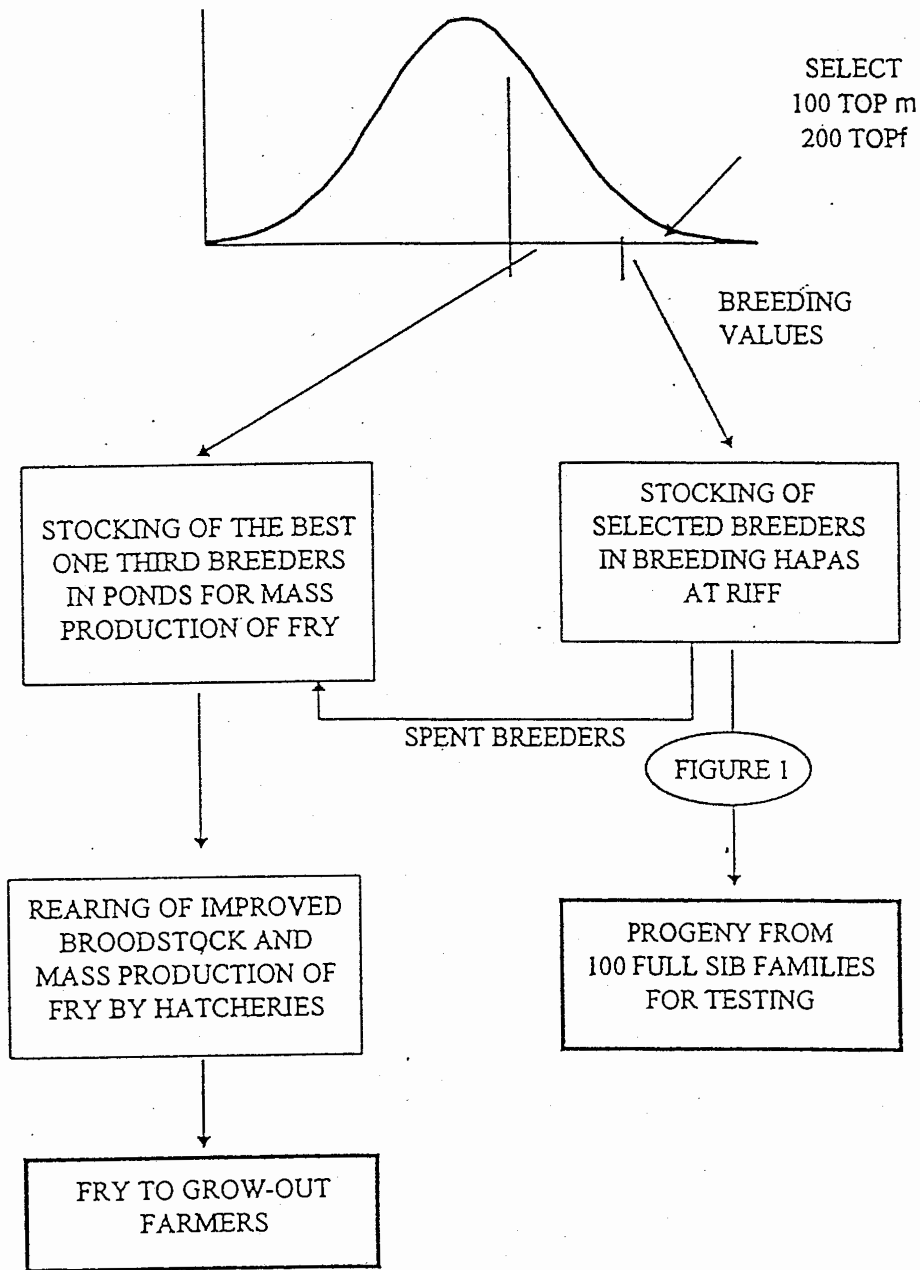


FIGURE 2. SELECTION OF BREEDERS AND DISSEMINATION OF GENETICALLY IMPROVED TILAPIA TO FARMERS

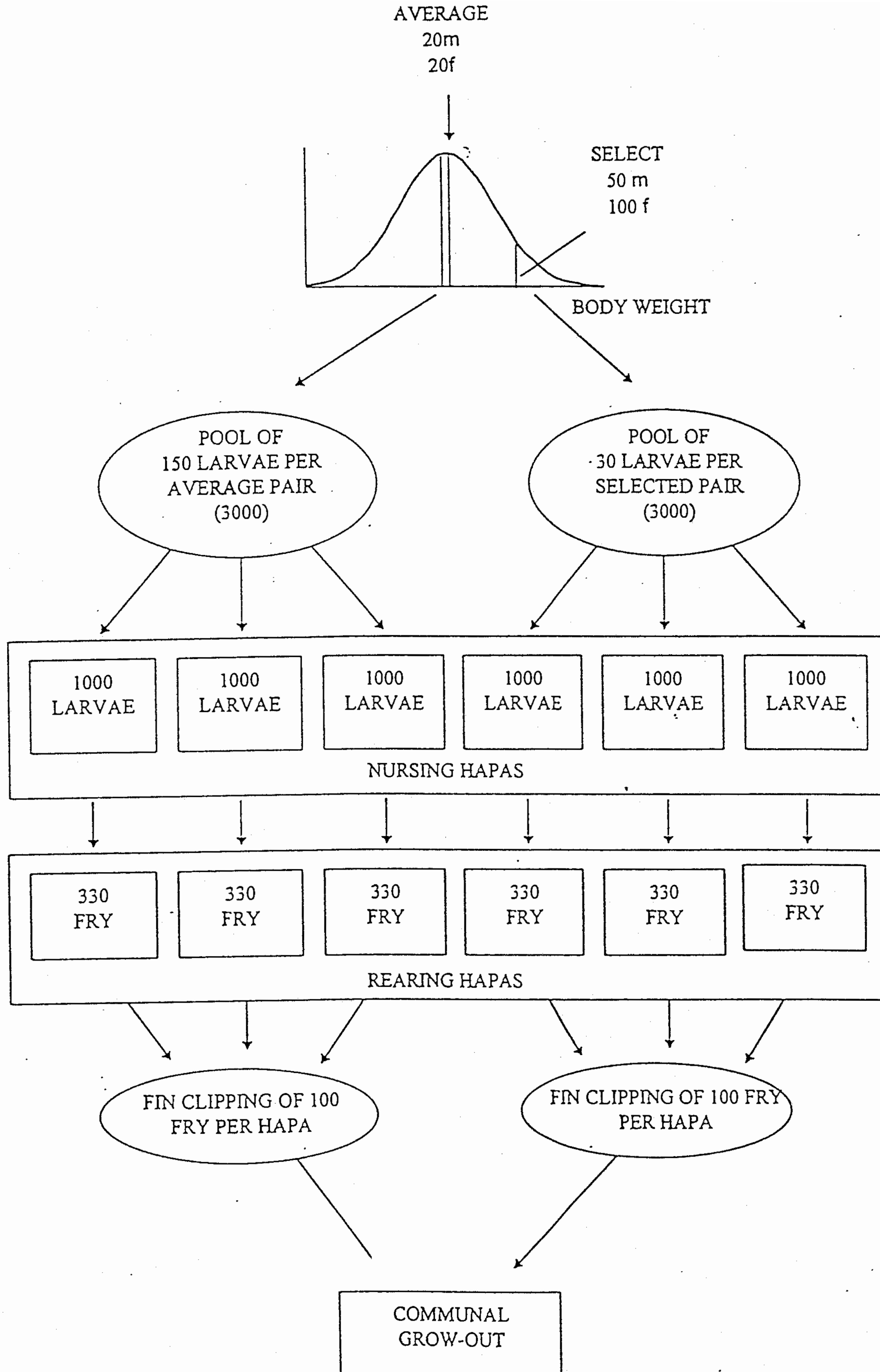


FIGURE 3. CONTROL OF GENETIC GAIN. COMPARISON OF PROGENY OF SELECTED BREEDERS WITH PROGENY OF AVERAGE BREEDERS

Appendix

Some definitions of common expressions from quantitative genetics and selective breeding plans:

Base population: The initial random-mating population that form the base for the selection experiment or selection program. It is customary to assume that the inbreeding coefficient is zero in the base population, and this is therefore the reference for estimation of inbreeding in later generations. This requirement may not always be met, but all efforts should be made to establish a base population of unrelated individuals.

Family selection: Selection based on information from fullsibs and/or halfsibs to estimate the breeding value (may also include information from other relatives). The selection is among families and not within family since no information is available to distinguish between family members (it can, however, be combined with other information in a multiple-trait index which enables us to select the best overall breeding candidate within family). The method requires that information of relatives are recorded, which means that fullsib groups must be reared separately until the fish have reached a size for which a marking system can be applied.

Fullsibs: Offspring from the same sire and dam, i.e. same pair of parents.

Dam: Female parent.

Generation interval: The average age of the parents at the birth of their selected offspring.

Halfsibs: Offspring from one sire (i.e. paternal halfsibs) or one dam (i.e. maternal halfsibs). This means that within a halfsib family some of the fish may also be fullsibs, e.g. when one sire is mated to two dams.

Heritability expresses the extent to which phenotypes, i.e. the observed value of that trait, are determined by the genes transmitted from the parents. It is given by the ratio: (additive genetic variance) / (phenotypic variance)

Hierarchical mating structure: A mating structure where for instance one male is mated with two females. Simulation studies have shown that this structure gives maximised genetic gain.

Inbreeding means the mating together of individuals that are related to each other by ancestry. This is unfavourable for two reasons: 1) it leads to inbreeding depression (low performance) in many traits, especially fitness traits (e.g. survival and fertility), and 2) it leads to decreased genetic variation.

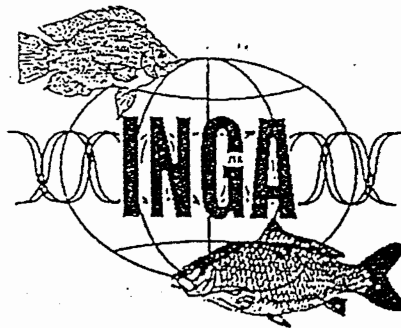
Index selection: Selection based on a combination of sources of information to estimate the breeding value, from the individual itself, and from relatives, especially fullsib or halfsib-information. The method requires that genetic relationship among the individuals must be recorded, which means that fullsib groups must be reared separately until the fish have reached a size for which a marking system can be applied.

Individual selection: Selection based on the performance of the individual itself only, to be distinguished from *Family* and *Index selection*. The method do not require a marking system, but the number of offspring from each family that are allowed to contribute their genes to the next generation must be restricted in order to control *inbreeding*.

Sire: Male parent.

BREEDING PLAN FOR COMMON CARP *(Cyprinus carpio)* IN INDONESIA: MULTIPLE-TRAIT SELECTION

Report No. 5



INTERNATIONAL NETWORK ON GENETICS IN AQUACULTURE
February 1997

FOREWORD

To improve and sustain the performance of tropical finfish species farmed in developing countries, the International Network on Genetics in Aquaculture (INGA) has been assisting the member-countries of the network (Bangladesh, Peoples Republic of China, Cote d'Ivoire, Egypt, Fiji, Ghana, India, Indonesia, Malaysia, Malawi, Philippines, Thailand and Vietnam) in developing selective breeding research and development programs.

At the request of the Government of Indonesia, INGA fielded a mission to Indonesia in November 1996, consisting of INGA Research Coordinator Dr. Modadugu V Gupta and Drs. Trygve Gjedrem and Hans Magnus Gjoen of Institute of Aquaculture Research (AKVAFORSK), As, Norway, to assist national scientists in prioritizing aquaculture species and traits for genetic improvement and develop plans for selective breeding of the prioritized species.

Common carp (*Cyprinus carpio*) is an important species for aquaculture in ponds and cages in Indonesia contributing 135,200 tons to fish production in 1994 (27.4% of total finfish production from fresh and brackishwater aquaculture). The Freshwater Fisheries Research Institute (RIFF), Sukamandi, Indonesia has identified 20 strains of the species from islands of Java and Sumatra, with different colours and body form. In view of the importance of the species in aquaculture and the liking of the Indonesian population for the species, it has been identified as a priority species for genetic improvement through selective breeding.

This report details the plan for selective breeding of common carp using multiple trait selection and has been prepared by Drs. Trygve Gjedrem and Hans Magnus Gjoen of AKVAFORSK and Drs. Atmadja Hardjamulia, Ir. Sudarto, Rudhy Gustiano, Anang Hari Kristanto and Lies Emmawati of the Research Institute for Freshwater Fisheries, Sukamandi and Maman Suparta of the Faculty of Fisheries, University of Pajajaran, Indonesia.

INGA acknowledges the support provided by the Indonesian Network of Fish Genetics Research and Development (INFIGRAD) to the mission and in preparing these plans.

We hope that this document will be useful to researchers in Indonesia for implementing the plans developed and informative to others involved in selective breeding in other countries.

Modadugu V Gupta
INGA Research Coordinator

BREEDING PLAN FOR COMMON CARP (*Cyprinus carpio*) IN INDONESIA: MULTIPLE-TRAIT SELECTION

INTRODUCTION

Total aquaculture production in Indonesia in 1994 was 493 000 tons of finfish, 169 000 tons of shellfish, mainly shrimp and 115 000 tons of seaweed with an estimated value of US$ 2 075 million.
In 1994, the production of common carp (*Cyprinus carpio*) was 135 200 tons, from ponds (52 600 tons), cages (28 600 tons) and ricefields (54 000 tons).

The potential for aquaculture in Indonesia is large. The mangrove forest area is estimated to be around 4.29 million ha. It is recommended to use only 20 % of the total mangrove area for aquaculture production (830 900 ha). To day only 37 % of this area is used for aquaculture. The most suitable species for brackishwater aquaculture are shrimp and milkfish. The area for mariculture is 81 million ha, with potential production of about 47 million tons of fish, shellfish and seaweed. The total area of freshwaters is 55 million ha. consisting of lakes, dams, rivers, swamps and other water bodies. Potential area for freshwater pond fish culture is estimated to be 338,121 ha., with a production potential of 8-900 000 tons per year (DGF, Indonesia, 1994).

Common carp is the most important freshwater fish, consisting of many strains with a variety of colours and body forms. The research Institute of Freshwater Fisheries (RIFF) collected 20 strains from the islands of Java, Sumatra and Bali. The results of the evaluation of the four strains: Rajadanu, Wildan Cianjur, Sutisna Kuningan and Cangkringan Yogyakarta are promising, while Majalaya as the existing populat cultivated strain, has wide distribution.

Common carp can spawn all the year round in Indonesia, and each broodfish can spawn naturally every three months without hormone injection. Simultaneous spawning of common carp can be induced by injecting the females and males with pituitary gland extract or manufactured hormones (Ovaprim from Canada or LRH-A from China). Two injections are administered to the females at an interval of 4-6 hours. More than 75 percent of the females spawn after injection. Males are injected once at the same time as the second injection for the females with a dose of one fifth to one sixth of the dose given to the females.

One of the main problem of common carp culture in Indonesia is early sexual maturation, particularly in males. Inbreeding might be a problem that can effect growth rate since fish farmers in general have

small number of broodstock, sometimes less than 10 fish. Fish diseases, notably *Aeromonas hydrophilla*, are a common problem, especially at fry stage.

The strategic plan of AARD (Agency for Agricultural Research and Development) for 1995 - 2005 gives high priority to improvement of genetic potential of fish, livestock, crop and micro-organisms (AARD, 1994). In accordance with this, the Indonesian Network of Fish Genetics Research and Development (INFIGRAD) in co-operation with the International Network on Genetics in Aquaculture (INGA) has taken the initiative to establish a breeding program for common carp in Indonesia. In this program, INFIGRAD will have its role as research co-ordinator, and the members may contribute in the research and development under the responsibility of RIFF, Sukamandi, which will serve as the breeding centre where mating, testing and selection takes place.

As RIFF develops and gets experience in running the breeding program, mating, testing and selection will become more and more standard routine work. This activity will also occupy much of the research capacity at RIFF. At that stage, INFIGRAD may assist in reorganising the breeding program in order to reduce the routine breeding work at RIFF and develop other breeding centres.

The effect of a breeding program depends very much on how it is organised to disseminate the obtained genetic improvement rapidly to the farmers. AIAT (Assessment Institute for Agricultural Technology) and FADC (Freshwater Aquaculture Development Centre) can play a central role in the dissemination of the improved fish. It is important that INFIGRAD co-ordinate this process.

BREEDING GOAL

The most important economic traits in common carp are:

- Growth rate
- Delayed sexual maturation
- Disease resistance
- Body composition, texture

Body weight should be recorded at the marketed size, which is 250 - 300 g. As the weight is recorded, sex and sexual maturation should be recorded for each fish.

Challenge test procedures to get estimate of disease resistance are developed for *A. hydrophilla* which is considered to be the most serious disease problem in common carp.

Flesh quality is of importance in common carp, and texture is considered to be the most significant quality trait. Instruments are now developed to measure texture in Atlantic salmon, and an investigation should be carried out to apply the method in common carp.

At the start of the breeding program, the breeding goal should therefore be growth rate, disease resistance and reduced frequency of early sexual maturation. As recording methods are developed for flesh texture, it should be included in the breeding goal.

SELECTION METHOD

The breeding method should be index selection (see Appendix). This will require tagging of all test fish. Family information is required for improvement of frequency of early sexual maturation and disease resistance. The breeding values of the broodstock will be computed based on:

1. The growth performance of the individual and it's full and halfsibs
2. The frequency of early sexual maturation and the survival rate in challenge test with *A. hydrophilla* of the full and halfsibs of the breeding candidates.

The relative weighting of the different traits and sources of information will be determined by the economic importance of the traits for the fish farmers.

The breeding program should be designed to avoid loss of genetic variation and to avoid rapid accumulation of *inbreeding* (see Appendix). This may be done by securing a large effective population size. The proposed breeding plan ensures a large effective population size, accurate recording of pedigree and evaluation of the inbreeding level after each round of selection. Assuming a heritability of about 0.2 for the traits included in the breeding goal, the design of this breeding program is expected to result in a rate of inbreeding of less than 1 % per generation, which will be sufficient to maintain the genetic variation and thus ensure a sustainable genetic progress.

TEST OF GENOTYPE BY ENVIRONMENT INTERACTION AND FORMING OF BASE POPULATION

There are many strains of common carp in Indonesia and several experiments have been carried out to compare and describe these strains. The strains considered to be of particular value and therefore should form a synthetic population to be the basis for a selective breeding program are: Majalaya, Rajadanu, Wildan Cianjur and Sutisna Kuningan.

The base population will be formed by crossing these four strains in a complete diallel cross, see Table 1. Eight to ten pairs of broodstock from each strain should be used in the crossbreeding trial.

This diallel cross makes it possible to estimate the magnitude of non-additive genetic effects for growth rate, age at sexual maturation and disease resistance. In the following it is assumed that the heterosis effect is relatively low and, therefore, standard selection for additive genetic effects is chosen to improve common carp. If, however, the heterosis effect is significant and represents a large part of the total genetic variation, the breeding plan should be revised to combine selection and crossbreeding.

Common carp is farmed under very different farming and environmental conditions in Indonesia. It is therefore important to investigate if genotype by environment interaction (correlation between performance under different environmental conditions for each genetic group) is of importance. This should be done by rearing the different groups in the 4 x 4 diallel cross in at least 3 environments (pond, rice-fish pond and cage). The traits in the breeding goal should be recorded at the marketed size (see below). In order to have about 500 kg of bio-mass in each pond at 300 g size (ca. 1650 fish i.e. ca. 100 from each cross), 350 offspring (assuming 30 % survival) from each cross should be tagged at 5 g average size and stocked into each test environment (for three test environments: 1050 from each cross).

If substantial genotype by environment interaction is found between important farming environments, it should be considered to split the breeding program in two or more programs with different breeding goals. For example, separate breeding programs for cage culture and pond culture.

START OF THE BREEDING PROGRAM

In the first round of selection, one should assure that a majority of the best crosses are represented in the forming of the first generation. This means that only the lowest performing crosses should be discarded as broodstock. This is important in order to ensure broad genetic variation in the future breeding population. Still, selection within the best crosses should be performed.

PRODUCTION OF FAMILIES

The production of families will follow the design described in Figure 1. One testing round is performed in 6 months. The population should be divided in two, and the first half of the population (family 1-100) will be tested the first 6 months, and the other half (family 101-200) will be tested the proceeding 6 months.

Selected and sexually mature broodstock of about one year of age should be used. In due time, hormone treatment should be used to synchronise the spawning. Milt from one male should be used to fertilise eggs from two females to produce both full and halfsib groups. A total of 100 halfsib groups and 200 fullsib groups should be produced.

From each spawning at least 1000 eggs should be transferred into a rearing hapa. Newly hatched larvae from each family should be transferred into one hapa, placed in an earthen pond, and reared there until they attain an average body weight of 5 g. The fingerlings will then be individually tagged, following the method developed by the GIFT project. A total of 160 fingerlings should be tagged per fullsib family, amounting to 32 000 tagged fingerlings per generation. Of these, 100 fingerlings per fullsib family should be communally stocked in a pond at RIFF, Sukamandi, 60 fingerlings per fullsib family should be sent for separate testing in; cage culture (15), pond culture (15) and disease challenge test (30) (Fig. 2).

SELECTION OF BROODSTOCK

Selection of broodstock for the next generation should be based on breeding values estimated on all available data from Sukamandi and the test stations for cage and pond culture together with the results from challenge test against *A. hydrophilla*. Records of fish from the test-stations should be taken prior to those at Sukamandi. Based on records of body weight and frequency of early sexual maturation of the fish at the test-stations and on records from the challenge test, a preliminary ranking of families should be computed.

As fish are recorded at Sukamandi, a first selection of broodstock should be done taking this preliminary ranking of families into account. A sufficient number of top ranked broodstock should be conditioned for mating, while unselected fish may be slaughtered.

Final selection of broodstock to produce the next generation in the breeding program should be based on a selection index including the following traits (Figures 2 and 3):

- Survival from challenge test of full and halfsibs
- Body weight and frequency of early sexual maturation of full and halfsibs at the test stations

=> First family-index (growth rate, early sexual maturation and disease resistance)

- Body weight of all individuals and frequency of early sexual maturation recorded at RIFF, Sukamandi

=> A final combined family (early sexual maturation and disease resistance) and individual index (growth rate).

Assortative mating should be avoided, i.e. the mating among selected breeders should be random. Further, there should be a restriction on number of selected breeders from each of the best families. The 2nd best breeders should be kept for mass production of seed for dissemination.

The 5 males with highest breeding values should be stocked and used in the next round of testing. The purpose of this repeated mating is to build genetic bridges or ties between each round of testing and to estimate genetic gain. Selection of offspring from these 2nd time breeders should be restricted.

The routines for production of the next generation of 200 fullsib families will then be repeated as described earlier (Figure 1).

DISSEMINATION OF IMPROVED SEED TO THE FISH FARMERS

In order to maximise the benefit from the breeding program, the genetic improvement should reach the fish farmers without delay. From RIFF, Sukamandi, there are mainly three routes for dissemination, namely trough AIAT, FADC and directly to private hatcheries. In this process of transferring improved stock, both seed and broodstock may be used.

After the production of fullsib families for the breeding program have been completed, the selected parents should be used for mass production of seed for dissemination. The progeny of the selected parents will be top genetic quality seed, followed by the progeny of the second best selected breeders from the final selection (see above). Furthermore, fish from the best 50 % of the fullsib families may be stocked in breeding ponds for mass production of fingerlings that may be disseminated as seed to collaborating operators. The top selected broodstock may also be transferred to other collaborating operators for repeated spawning.

It is very important that the collaborating breeding centres are informed and educated in how to use the new broodstock. The new breed should not be used as the old pure strains since offspring of the selected broodstock are not to be re-entered into breeding. It is also important that the hatcheries understand that it is important to use top quality seed from the breeding system.

ESTIMATION OF GENETIC GAIN

The genetic gain of the breeding program may be estimated by the recurrent use of 5 males with the highest breeding value in each round of testing. At every second testing round, this repeated mating will facilitate evaluation of the genetic gain that is obtained in the previous generation since the males then will be mated to females of different generations.

EXPECTED BENEFIT FROM BREEDING PROGRAMS

When additive genetic variation is present in a trait, there will always be response to selection if efficient selection methods are applied. In the literature there are several estimates of response to selection for increased growth rate in fish, both in large scale breeding experiments and in breeding programs. The following estimates should be mentioned (given as genetic gain in percentage per generation): For coho (Pacific) salmon, 10.1; for rainbow trout, 13; for Atlantic salmon, 10.6-14.2; for channel catfish, 12-20; and for Nile tilapia, 17 %. An average figure of these estimates are *ca* 15 % genetic gain per generation for growth rate. This means that it should be possible to double the growth rate in less than 7 generations. This is a larger genetic gain than usually obtained in farm animals, because fish and shellfish have larger genetic variation in growth rate and have higher fecundity; consequently, it is possible to apply a much higher selection intensity.

The benefits of genetic improvement in growth rate are reduction in both fixed costs and production costs, the latter due to lower energy requirement for maintenance for the entire life span, and often a correlated response can be observed as an improved feed conversion rate.

The Norwegian breeding program, which today supply genetically improved eggs of Atlantic salmon and rainbow trout to more than 70 % of the farming industry, has a cost/benefit ratio of 1/15. Similar estimates are also found from breeding programs in farm animals. This ratio will depend largely on the total production output that benefit from the genetic improvement. In view of the high production of common carp in Indonesia, an even better ratio may be expected for the proposed breeding program.

Table 1. Scheme for crossing of the four strains of common carp

Females from	Males from strain No.			
strain No.	1	2	3	4
1 (Majalaya)	X	X	X	X
2 (Rajadano)	X	X	X	X
3 (Wildan Cianjur)	X	X	X	X
4 (Cangkringan Yogyakarta)	X	X	X	X

X: Mating of 8-10 pairs for each reciprocal cross

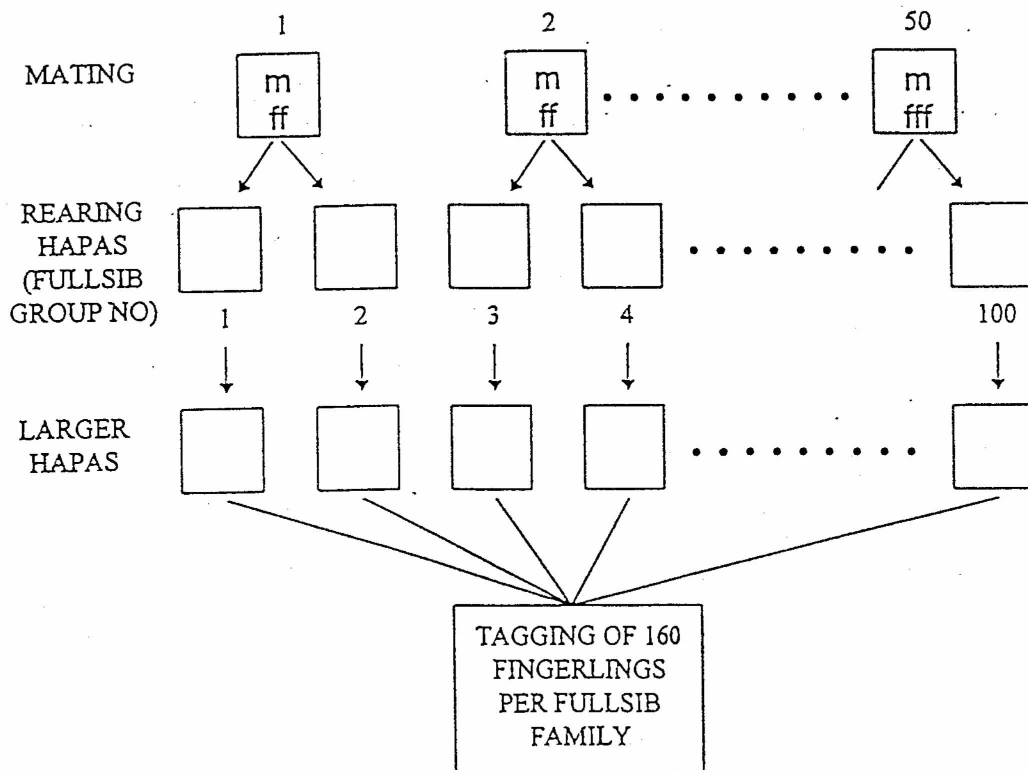


FIGURE 1. MATING AND REARING DESIGN FOR ONE TESTING ROUND

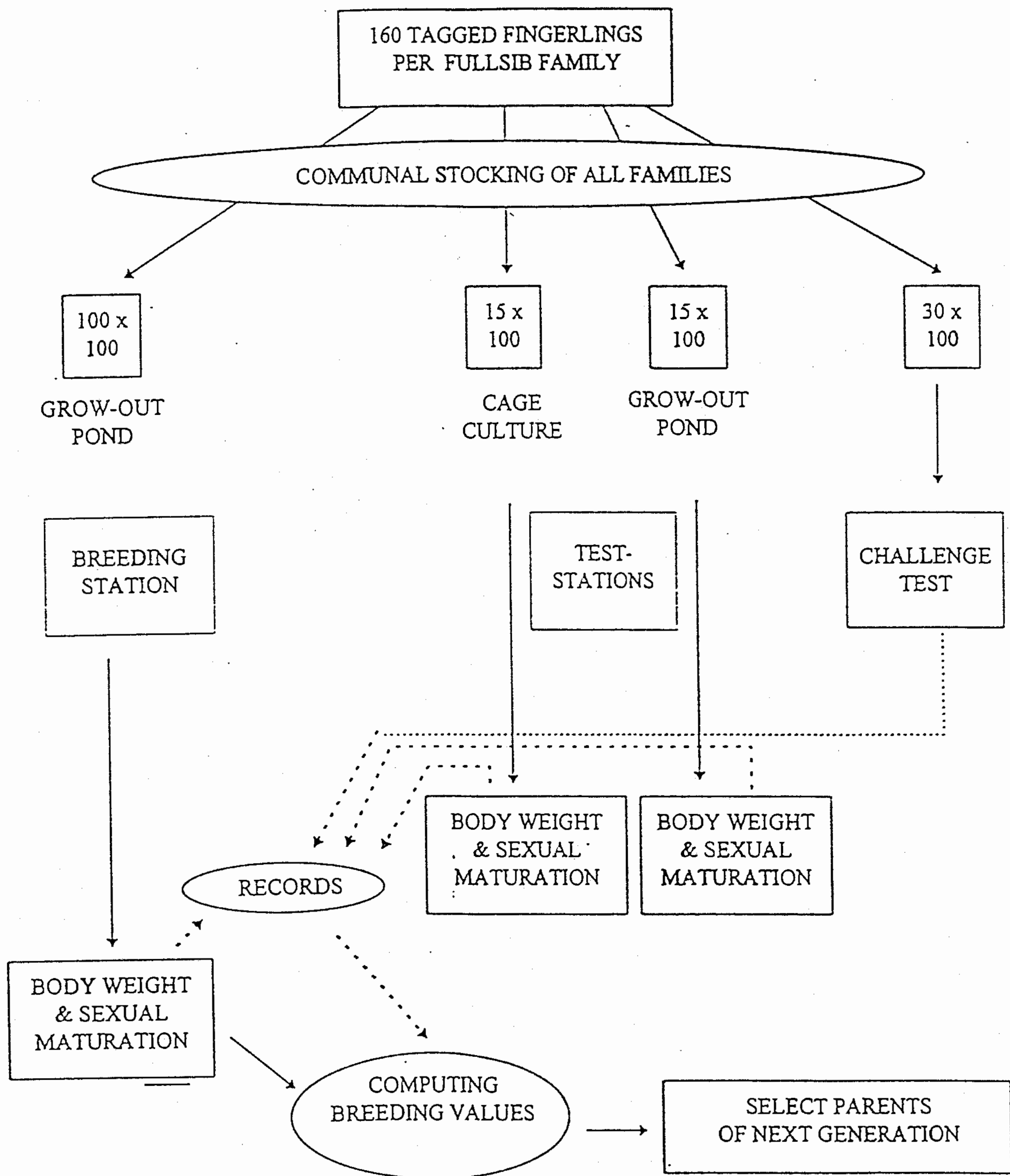


FIGURE 2. TESTING, RECORDING AND COMPUTING OF BREEDING VALUES

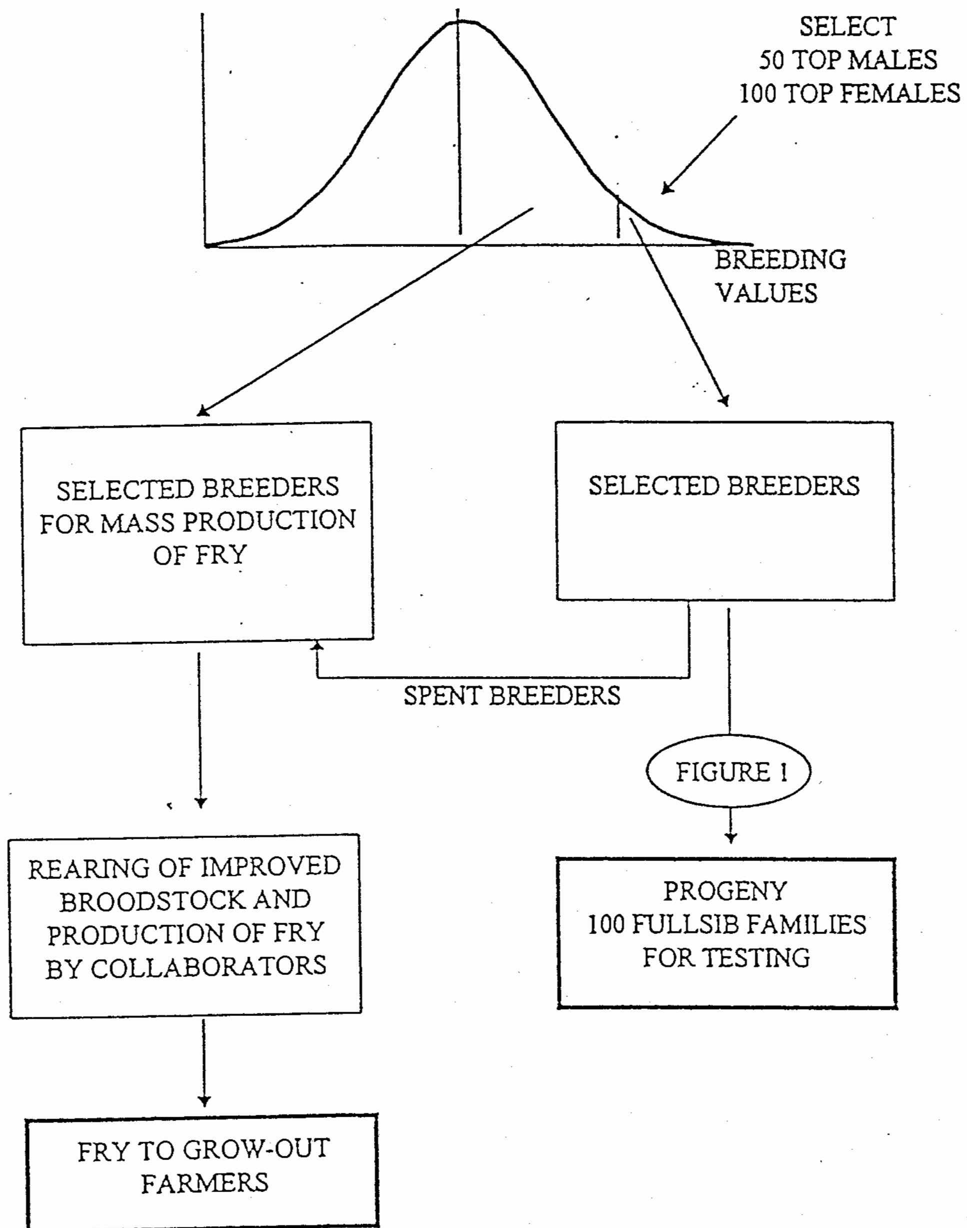


FIGURE 3. SELECTION OF BREEDERS AND DISSEMINATION OF GENETICALLY IMPROVED COMMON CARP TO FARMERS, IN EACH ROUND OF TESTING

Appendix

Some definitions of common expressions from quantitative genetics and selective breeding plans:

Base population: The initial random-mating population that form the base for the selection experiment or selection program. It is customary to assume that the inbreeding coefficient is zero in the base population, and this is therefore the reference for estimation of inbreeding in later generations. This requirement may not always be met, but all efforts should be made to establish a base population of unrelated individuals.

Family selection: Selection based on information from fullsibs and/or halfsibs to estimate the breeding value (may also include information from other relatives). The selection is among families and not within family since no information is available to distinguish between family members (it can, however, be combined with other information in a multiple-trait index which enables us to select the best overall breeding candidate within family). The method requires that information of relatives are recorded, which means that fullsib groups must be reared separately until the fish have reached a size for which a marking system can be applied.

Fullsibs: Offspring from the same sire and dam, i.e. same pair of parents.

Dam: Female parent.

Generation interval: The average age of the parents at the birth of their selected offspring.

Halfsibs: Offspring from one sire (i.e. paternal halfsibs) or one dam (i.e. maternal halfsibs). This means that within a halfsib family some of the fish may also be fullsibs, e.g. when one sire is mated to two dams.

Heritability expresses the extent to which phenotypes, i.e. the observed value of that trait, are determined by the genes transmitted from the parents. It is given by the ratio: (additive genetic variance) / (phenotypic variance)

Hierarchical mating structure: A mating structure where for instance one male is mated with two females. Simulation studies have shown that this structure gives maximised genetic gain.

Inbreeding means the mating together of individuals that are related to each other by ancestry. This is unfavourable for two reasons: 1) it leads to inbreeding depression (low performance) in many traits, especially fitness traits (e.g. survival and fertility), and 2) it leads to decreased genetic variation.

Index selection: Selection based on a combination of sources of information to estimate the breeding value, from the individual itself, and from relatives, especially fullsib or halfsib-information. The method requires that genetic relationship among the individuals must be recorded, which means that fullsib groups must be reared separately until the fish have reached a size for which a marking system can be applied.

Individual selection: Selection based on the performance of the individual itself only, to be distinguished from *Family* and *Index selection*. The method do not require a marking system, but the number of offspring from each family that are allowed to contribute their genes to the next generation must be restricted in order to control *inbreeding*.

Sire: Male parent.

Attachment 15.

Record of visitors received by the GIFT Project

Records of Visitors Received by the GIFT Project

DATE	NAME OF VISITORS	INSTITUTIONS AFFILIATED
8 February 1994	Dr. David Dixon Journalist/broadcaster	British Broadcasting Corporation (BBC), UK
13 April 1994	ICLARM Board of Trustees	International Center for Living Aquatic Resources (ICLARM)
24-30 April 1994	Dr. P.V.G.K. Reddy Senior Scientist	Central Institute of Freshwater Aquaculture (CIFA), India
	Sri S.D. Gupta Senior Scientist	-do-
	Mrs. Kanta Das Mahapatra Senior Scientist	-do-
7 May 1994	Dr. John E. Thorpe Senior Scientist Department of Zoology	University of Glasgow, UK
7-9 November 1994	Dr. Erling Fimland Executive Director	AKVAFORSK (Institute of Aquaculture Research), Norway
	Dr. Hans Bentsen Senior Scientist	-do-
27 November - 2 December 1994	Dr. Graham Gall Professor Applied Genetics and Breeding	University of California, Davis
	Dr. Brian Davy Executive Coordinator	Strategy for International Fisheries Research, Ottawa, Canada
	Dr. Vo Tong Xuan Vice Rector	Cantho University, Cantho, Haugiang, Vietnam
	Dr. Stein Bie Director	NORAGRIC Norway

DATE	NAME OF VISITORS	INSTITUTIONS AFFILIATED
	Phil Reynolds	UNDP/STAPS
10 February 1995	Prof. Jong Wha Lu Rector	College of Natural Sciences and College of Physical Education, Soonchunhyang University, South Korea
13-14 February 1995	Dr. K.V. Devaraj Vice Chancellor	University of Agricultural Sciences, Bangalore, India
3 March 1995	Ms. Gabriela del Valle Ph.D. student	Kochi University, Japan
7 April - 8 May 1995	Dr. Trygve Gjedrem Senior Scientist	AKVAFORSK (Institute of Aquaculture Research), Norway
26 April 1995	Mid-Term Review Team:	
	i) Mr. Said Hanif	UNDP/STAPS, New York, USA
	ii) Dr. Sakuntala Kadirgamar Rajasingham	UNDP/STAPS, New York, USA
2 May 1995	Dr. Yousep S. Al Hafedh Assistant Professor	King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia
2 May 1995	Dr. Faustino Rodriguez-Romero	Instituto de Ciencas del Mar Y Limnologia, Universidad Nacional, Autonoma de Mexico, Mexico
9 June 1995	Dr. J.F. Prinsloo Director of Aquaculture Research Unit	University of North South Africa
29-30 June 1995	Mr. Satya Nand Lal Senior Scientist	Ministry of Fisheries, Suva
	Mr. O. Selvaraj Research Fellow	University of Malaya, Malaysia

DATE	NAME OF VISITORS	INSTITUTIONS AFFILIATED
12-15 September 1995	Dr. Terje Refstie Scientist	AKVAFORSK (Institute of Aquaculture), Norway
6 October 1995	Dr. Tabrez Nazar (IIRR) with Integrated farming Trainees from Bangladesh	
12 October 1995	Dr. Md. Shuhadal Ali Professor	Department of Zoology, University of Dhaka, Bangladesh
16-20 November 1995	External Advisory Panel Review of the GIFT Project. The composition of the Review Panel are: I) Dr. Graham Gall Professor;	 Department of Animal Science University of California
	II) Dr. Brian Davy Executive Secretary	Strategy for International Fisheries Research (SIFR) c/o IDRC
18 November 1995	Participants of the Intensive[1] Training Program on the Application of Quantitative Genetics to Aquaculture	
21 November 1995	CGIAR-System wide Review of Genebanks. The composition of the Review panel are: i) Dr. N.L. Innes Consultant	 c/o Scottish Crop Research Institute, Dundee, U.K.
	ii) Dr. N.M. Anishetty Senior Officer;	Plant Genetic Resources, AGPS, FAO, Rome
	iii) Dr. E.P. Cunningham Professor of Animal Genetics	University of Dublin, Ireland
	iv) Dr. Tereso Abella Director	Freshwater Aquaculture Center/Central Luzon State University, Munoz, N.E.

1 See Appendix 1 of Report on "Intensive Training Program on the Application of Quantitative Genetics to Aquaculture" for names of participants and institution affiliations.

DATE	NAME OF VISITORS	INSTITUTIONS AFFILIATED
5 December 1995	Dr. Y. Basavaraju Associate Professor	Fisheries Research Station University of Agricultural Sciences, Hesaraghatta, Bangalore, India
5 December 1995	Dr. Chindi Vasudevappa Fisheries Research Officer	-do-
2 February 1996	Dr. Chris Barlow Principal Fish Biologist	Queensland Department, Primary Industries
	Dr. Cam Mcphee Principal Geneticist	-do-
21 March 1996	Dr. Christine Bergmark Science Advisor	USAID, Office of Agriculture and Food Security, Washington, DC, USA
	Dr. Frans Newman Advisor, Agricultural Research	CGIAR, Netherlands
31 May 1996	Bangladesh Officials MADECOR Group	Asian Institute of Developmental Studies
26 August 1996	Dr. Per Bovberg Pedersen Aquaculture Specialist	DANIDA, Denmark
21 September 1996	Dr. Nyawira Muthiga ICLARM Board of Trustees	Kenya Wildlife Services Mombassa Marine Park & Reserves Mombassa, Kenya
9 October 1996	Dr. Heinz Leger Science News Editor	Austrian Broadcasting Corporation
6 December 1996	Amb. Karleen Kwint Ambassador of Netherlands to Philippines	Makati City, Philippines
16-17 December 1996	Dr. Graham E. Gall Dr. Brian Davy Dr. Bernard Chevassus Mr. Phil Reynolds Dr. Erling Fimland Dr. Hans Bentsen	University of California, Davis, USA SIFR, Canada INRA, France UNDP, New York AVKAFORSK, Norway AVKAFORSK, Norway

DATE	NAME OF VISITORS	INSTITUTIONS AFFILIATED
16 August 1997	Effendi Hatchery Manager	Kiatgaya, Indonesia
30 April 1997	Le Nhu Xuan Le Xuan Sinh Duong Nhut Long Tuan Thi Tien Lecturers/Researchers	Cantho University Vietnam
6 May 1997	Njock Jean Calvin Director	Ministry of Fisheries, Cameroon
	Thomas Maembe Director	Ministry of Fisheries, Tangsonia
17 October 1997	Michael Pickstock Director	WREN Media, UK
20 November 1997	Dr. Rex Dunham Program Leader Germplasm Enhancement and Breeding Program	ICLARM

Attachment 16.

Selected media coverage on GIFT

Selected Media Coverage on GIFT Project

Agribusiness Weekly. Improving our Nile tilapia stocks. 7-13 August 1990.

Biotechnology and Development Monitor. ICLARM: The world's leading aquatic center. June 1991. No. 7.

The Indonesia Times. Aquatic chicken breeding:an improved freshwater fish could boost tropical fish farming. 27 February 1992.

Philippine Daily Inquirer. RP diplomat tells UN of super tilapia. 4 April 1992.

Manila Bulletin. Philippine record on food production. 23 August 1992.

Daily Globe. Feeding RP's growing population. 28 August 1992, p.6.

The Business Star. The Philippines can feed its growing population. 1 September 1992.

The SEARCA Diary. 1993. Here comes super tilapia. 22 (3):4.

The Philippine Star. The breaks sesame opens synthetic tilapia strain. 4 July 1993.

The Business Star. BFAR develops better tilapia strain. 7 July 1993.

Business World. FVR distributed super tilapia. 19 July 1993.

Manila Bulletin. Super tilapia fish distributed. 22 July 1993.

Philippine Times. RP is world's biggest producer of tilapia. 25 July 1993.

Greenfields. And now a super tilapia. 23 August 1993. 21(8).

Malaya. Higher yielding tilapia strain to be introduced in October. 21 September 1993.

The Philippine Journal. Laguna fishers stage 'blue revolution' in Sto Domingo. 3 January 1994.

Philippine Times Journal. Super tilapia invades Asian tables. 14 February 1994.

Malaya. Genetically improved tilapia available. 27 June 1994.

Triba, D.E. 1994. Feeding and greening the world: the role of international agricultural research. CAB International, London

The Freeman. Better fish species to meet growing demand. R. Fernandez. 4 December 1994.

We Forum. Better fish species developed to meet growing demand. R. Fernandez. 18-24 November 1994.

Daily News. Better fish species developed to meet growing demand. R. Fernandez. 22 November 1994.

The Indonesia Times. Better fish species developed to meet growing demand. R. Fernandez. 24 November 1994.

Philippine Daily Inquirer. Tilapia breeding makes new waves. Jose G Burgos Jr. 17 August 1995.

Philippine Star. Tilapia to emerge as 'super fish'. 28 May 1996: 6.

Akhbar. New Kinds of Nile tilapia. (In Arabic). 9 July 1996.

Manila Bulletin. Tilapia is our best bet for sufficiency in fish. Z.B. Sarian. 2 August 1996: 44

Philippine Daily Inquirer. Filipino fishpond farmers urged to breed 'super' tilapia. 12 September 1996: C7.

Philippine Star. Foundation for super tilapia. R.A. Fernandez. 29 September 1996: 28.

Today. New home for 'super tilapia' set up. 1 October 1996: 15.

Manila Bulletin. Foundation to assure the super tilapia availability. Z.B. Sarian. 4 October 1996: 3

Manila Bulletin. Foundation for super fish soon to be launched. 5 October 1996: 14.

Philippine Daily Inquirer. Tilapia foundation to be launched. 3 October 1996.

Philippine Star. Escudero sites need to sustain growth in agriculture sector. M. Galvez. 14 October 1996: 6.

The Manila Times. Aquaculture to save Pinoys from hunger. A. Galang. 14 October 1996

Business World. Frankenstein's GIFT. K. C. Yao. 15 October 1996: 5.

Philippine Daily Inquirer. Super tilapia, a gift to the world. 15 October 1996: D5-6.

The Manila Bulletin. There's no need to reverse the sex of super tilapia. 18 October 1996.

Philippine Star. Dr. Ambekar Eknath inspects a tilapia breeder at BFAR-FAC research station. 20 October 1996: 26.

Philippine Recorder. Tilapia Foundation to be launched. 21-27 October 1996.

Manila Bulletin. Super tilapia growth in forum. C. Chavez. 21 November 1996: 12.

Fishing Chimes. October 1996. 16(7):11

Attachment 17.

List of meetings attended/papers presented by GIFT Staff

Meetings Attended, Papers Presented

4th International Symposium on Genetics in Aquaculture, Wuhan, China, 29 April-3 May 1991. (B.O. Acosta, J.B. Capili, M.P. de Vera, A.E. Eknath, R.S.V. Pullin).

Papers presented:

Agustin, L.Q., J.M. Macaranas and A.E. Eknath. Use of RNA:DNA ratio as index of nutritional status of six Nile tilapia *(Oreochromis niloticus)* strains under different environments.

Bolivar, R., A.E. Eknath, H. Bolivar and T. Abella. Phenotypic correlations between growth and reproductive traits in individually tagged Nile tilapia *(Oreochromis niloticus)* of different strains: implications for planning selection programs.

De Vera, M.P. and A.E. Eknath. Predictability of individual growth rates in tilapia.

Eknath, A.E., M.M. Tayamen, M.P. de Vera, J.C. Danting, R. Reyes, E. Dionisio, J.B. Capili, H.C. Bolivar, T. Abella, A. Circa, H. Bentsen, B. Gjedre, T. Gjedrem and R.S.V. Pullin. Genetic improvement of farmed tilapias: the growth performance of eight strains of *Oreochromis niloticus* tested in different farm environments.

Macaranas, J.M., A.E. Eknath, L..Q. Agustin, R.R. Velasco, M.C. Ablan and R.S.V. Pullin. Genetic improvement of farmed tilapias. Documentation and genetic characterization of strains.

Reyes, R. and A.E. Eknath. Environmental effects on expression of genetic potential for growth in seven strains of Nile tilapia *(Oreochromis niloticus)* and their implications for applied breeding programs.

Third International Symposium on Tilapia in Aquaculture (ISTA III), Abidjan, Cote d' Ivoire, 11-16 November 1991. (A.E. Eknath, B.O. Acosta, J.B. Capili, R.S.V. Pullin and R.R. Velasco).

Papers presented:

Acosta, B.O., E. Dionisio and A.E. Eknath. Growth and food conversion of wild and domesticated strains of *Oreochromis niloticus* fry. (poster)

Capili, J.B. Mitochondrial DNA restriction endonuclease and Isozyme analysis in three strains of *Oreochromis niloticus*.

Eknath, A.E., J.C. Danting, M.P. de Vera, E. Dionisio and R. Reyes. A practical quantitative methods of estimate relative reproductive performance during the routine production cycles in *Oreochromis niloticus*.

Velasco, R.R. Morphometric characterization of eight Philippine and African *Oreochromis niloticus* strains.

Technical Consultation on "Genetical Methods for the Discrimination of Fish Populations, both in Aquaculture and Conservation, Hamburg, Germany, 20-22 February 1992 (A.E. Eknath)

Paper Presented:

Eknath, A.E. Morphometric and biochemical approaches to characterize tilapias.

SAS Users Groups International Conference, Honolulu, Hawaii, 5-8 April 1992. (C. Janagap)

First Meeting for Establishing International Collaborative Linkage for Fish Genetics, Manila, Philippines, 30 May 1992. (B.O. Acosta, A.E. Eknath, C. Janagap, R.S.V. Pullin, R. Velasco)

First Meeting for Establishing International Collaborative Linkage for Fish Genetics, Manila, Philippines, 30 May 1992. (B.O. Acosta, A.E. Eknath, C. Janagap, R.S.V. Pullin, J. Rius; R. Velasco)

Workshop on International Concerns in the Use of Aquatic Germplasm, Caylabne, Cavite, Philippines, 1-5 June 1992. (B.O. Acosta, A.E. Eknath; R. Froese, C. Janagap; R.S.V. Pullin; R. Velasco)

International Workshop on Genetics in Aquaculture and Fisheries Management,. University of Stirling, UK, 31 August - 4 September 1992. (L. Agustin ?)
Paper Presented:
Agustin, L.R. Froese, A. Eknath and R.S.V. Pullin. Documentation of genetic resources for aquaculture - the role of FISHBASE

The Third Asian Fisheries Forum, World Trade Centre, Singapore, 26-30 October 1992. (H. Bolivar, A.E. Eknath, R. Reyes)
Paper presented:
Bolivar, H. M. de Vera, R. Reyes, R.B. Bolivar, H.B. Bentsen and A.E. Eknath. Early growth and survival of eight strains of Nile tilapia (O. niloticus) and their crosses.

Eknath, A.E., J.B. Capili, J.C. Danting, E.E. Dionisio, R.A. Reyes, N.D. Gerundo, M.M. Tayamen and R.S.V. Pullin. Experiences with the importation and quarantine germplasm for developing a national tilapia breeding program in the Philippines.

FAO Expert Consultation on Utilization and Conservation of Aquatic Genetic Resources, Grottaferata, Italy, 9-13 November 1992. (A.E. Eknath, R.S.V. Pullin)

Sixth Genetic Improvement of Farmed Tilapia (GIFT) Training Workshop: Formulating Strategies for the Establishment of Philippine National Tilapia Breeding Program, NFFTRC/BFAR, Muñoz, Nueva Ecija, Philippines, 10-13 May 1993. (B. O. Acosta, H. Bolivar, A. E. Eknath, J. Rius, D. Rosales, R. Velasco, M. de Vera)

International Network on Genetics in Aquaculture - Network Planning Workshop, ICLARM HQ, Makati, Metro Manila, Philippines, 17 - 22 July 1993. (B.O. Acosta, A.E. Eknath, C. Janagap, J. Rius, D. Rosales).
Paper presented:
Eknath, A. E. Highlights of GIFT Project experiences.

Second National Conference of SAS Users in the Philippines, Shangrila EDSA Plaza Hotel, Manila, Philippines, 26 August 1993. (C. Janagap, J. Rius, D. Rosales)

UNDP/DGIP Tripartite Review Meeting for the GIFT Project, ICLARM HQ, Manila, Philippines, 10 November 1993. (B.O. Acosta, H. Bolivar, A.E. Eknath, C. Janagap, R.S.V. Pullin, J. Rius, D. Rosales)

Third National Symposium on Tilapia Farming, University of the Philippines in the Visayas, Iloilo City, Philippines, 25-27 November 1993. (B.O. Acosta, G. Bimbao, H. Bolivar, A.E. Eknath, C. Janagap, J. Rius, D. Rosales, P. Virly)
Paper presented:
Eknath, A. E. Genetic Improvement of Farmed Tilapia Project (GIFT) project: from modest beginnings to an international network.

18-21 May 1994. First INGA Steering Committee Meeting, Bangkok, Thailand (A.E. Eknath, B.O. Acosta and C. Janagap)
Papers presented:
Eknath A.E. Progress of GIFT Project activities/Genetics Research at ICLARM.

Acosta, B.O. Procedures for Evaluation of Fish Genetic Materials.

Janagap, C. GIFT Database.

19-25 June 1994. Fifth International Symposium on Genetics in Aquaculture, Dalhousie University, Halifax, Canada. (A.E. Eknath and M.P. de Vera)
Papers presented:
Bentsen, H.B., A.E. Eknath, M.P. de Vera, M.J.C. Danting, H.L. Bolivar, R.A. Reyes, E.E. Dionisio, F.M. Longalong, M.M. Tayamen and B. Gjerde. Genetic improvement of farmed tilapias: growth performance in a complete diallele cross experiment with eight strains of Nile tilapia *(Oreochromis niloticus)*.

Bolivar, R.B., A.E. Eknath, M.P. Hechanova and H.L. Bolivar. Measurement of age at first spawning in Nile tilapia *(Oreochromis niloticus)*.

de Vera, M.S.P. and A.E. Eknath. Growth performance of male and female different strains of Nile Tilapia *(Oreochromis niloticus)* in different culture environments.
Eknath, A.E. 'Formal' and 'informal' breeding programs and conservation of genetic diversity.

Eknath, A.E., R.A. Reyes, H.L. Bolivar, M.P. de Vera, J.C. Danting, E.E. Dionisio and F.M. Longalong. Genetic improvement of farmed tilapias: estimation of genetic variation and heritability for age and size at first spawning of *Oreochromis niloticus.*

Posters presented:
Bolivar, H.L., R.B. Bolivar, C.C. Janagap, R.A. Reyes and A.E. Eknath. Fry production differences between eight strains of Nile tilapia *(Oreochromis niloticus)* and their crosses.

Circa, A.V., A. E. Eknath and A.G. Taduan. Genetic improvement of farmed tilapias: the growth performance of the GIFT strain of Nile tilapia *(Oreochromis niloticus)* in rice-fish environments.

Danting, M.J.C., A.E. Eknath and H.B. Bentsen. Evaluation of growth performance testing methods for strain comparisons of Nile tilapia *(Oreochromis niloticus)*.

Dionisio, E.E., A.E. Eknath and C.C. Janagap. Progeny sex ratio in a complete diallele cross with eight strains of Nile tilapia *(Oreochromis niloticus)*.

Janagap, C.C. and A.E. Eknath. User friendly computer software for aquaculture geneticists: a GIFT application.

Longalong, F.M. and A.E. Eknath. Development of techniques for synchronization of natural spawning in Nile tilapia *(Oreochromis niloticus)*.

Morales, G.A., A.E. Eknath, R.C. Sevilleja, M.M. Tayamen, R.B. Bolivar and R.A. Reyes. An evolving national tilapia breeding program for the Philippines.

Seshu, D.V., A.E. Eknath and R.S.V. Pullin. The International Network on Genetics in Aquaculture (INGA).

Velasco, R.R., C.C. Janagap, M.P. de Vera, L.B. Afan, R.A. Reyes and A.E. Eknath. Genetic improvement of farmed tilapias: estimation of heritability of body and carcass traits of Nile tilapia *(Oreochromis niloticus)*.

7-11 November 1994. International Workshop on Genetics in Aquaculture and Fisheries Management in Asia, Bangkok, Thailand.

Papers presented:
Dionisio, E.D., R.A. Reyes, and A.E. Eknath. Preliminary study on the salt tolerance of difference strains of Nile tilapia *(Oreochromis niloticus* L.*)* fry.

Longalong, F.M., M.J.C. Danting, A.V. dela Cruz, M.C. Tayamen, R.A. Reyes and A.E. Eknath. Combined effects of genetic improvement through selection and sex reversal on survival and growth of Nile tilapia *(Oreochromis niloticus)*.

5-10 December 1994. International Symposium on Biotechnology Applications, Taipei, Taiwan (A.E. Eknath and B.O. Acosta)

24 June 1995. Special Session on Fish Biodiversity: Genetic Resources for Aquaculture. Hyderabad, India. (A. Eknath)

26-27 June 1995. Forward Planning Workshop to Develop Strategies for ICLARM's Genetic Research. Hyderabad, India. (A. Eknath)

16-20 October 1995. Fourth Asian Fisheries Forum. Beijing, China. (A. Eknath) Chaired the Session on Management and Conservation of Aquatic Biodiversity.

20 November 1995. UNDP Third Tripartite Review of the GIFT Project. Makati City, Philippines. (A. Eknath and B. Acosta)

DEGITA (Dissemination and Evaluation of Genetically Improved Tilapia in Asia) Training Workshop (A. Eknath, C. Janagap). Los Baños, Philippines, 12-30 March 1996.

Inauguration Meeting of the research facilities for the Center for Applied Fish Breeding and Genetics Research and Launching of the GIFT Foundation International (A. Eknath, B. Acosta, M.P. de Vera, R. Velasco, H. Bolivar, F. Rius, P. Virly, C. Federigan, F. Lopez, N. Cabrera) Muñoz, Nueva Ecija, Philippines, 9 October 1996.

Workshop on Finalization of the Philippine National Tilapia Breeding Program (A. Eknath, R. Velasco). Manila, Philippines, 26-28 February 1996.

The Third Steering Committee Meeting, International Network on Genetics in Aquaculture: INGA (M.M. Dey, A. Eknath, B. Acosta). Cairo, Egypt, 8-11 July 1996.

16-19 November 1996. External Advisory Panel/UNDP Review of the GIFT Project. NFFTRC/BFAR, Muñoz, Nueva Ecija and ICLARM HQ, Manila, (GIFT Project Staff).

Strategic Planning Workshop for the GIFT Foundation (A. Eknath, M.P. de Vera, R. Velasco, H. Bolivar). Muñoz, Nueva Ecija, Philippines, 16 December 1996.

Final GIFT Project Research Partners Meeting (A. Eknath, R. Sevilleja, T. Abella, R. Reyes, B. Acosta, M. de Vera, J. Danting, R. Velasco, H. Bolivar, J. Rius, F. Longalong, E. Dionisio, T. Gonzales, M. Danting, P. Virly, C. Federigan, M. Igharas). NFFTRC/BFAR, Muñoz, Nueva Ecija and ICLARM HQ, Makati City, Philippines. 24-26 September 1997.