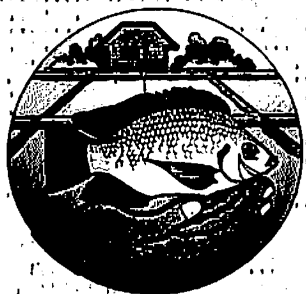
GENETIC IMPROVEMENT OF FARMED TILAPIA (GIFT) RESEARCH METHODOLOGIES

INSTRUCTOR'S GUIDE

(Revised Version)



UNITED NATIONS
DEVELOPMENT PROGRAMME
Sustainable Energy
and Environment Division
GLO/90/016

INTERNATIONAL CENTER FOR LIVING AQUATIC RESOURCES MANAGEMENT

H 292

MANUAL ON

GENETIC IMPROVEMENT OF FARMED TILAPIA (GIFT)

RESEARCH METHODOLOGIES

Instructor's Guide

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MANUAL ON COMPUTER AIDED MONOSCOPIC ANALYSIS (CAMA)

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MANUAL ON GENETIC IMPROVEMENT OF FARMED TILAPIA RESEARCH METHODOLOGIES INSTRUCTOR' S MASTER CHECKLIST

Module No.	Handouts	Progress Test Question	Progress Test Answer	Mastery Test/ Exercise	Visual Aids	Duration
1b		PTQ-1b	PTA-1b		Transparency 1b.1 to 1b.12; slide 1b.1 to 1b.14	4 hrs & 21 mins
2a		PTQ-2a	PTA-2a	Practical exercise 2a	Transparency 2a.7 to 2a.17; Slides 2a.1 to 2a.7	9 hrs & 10 mins
		PTQ-2aA	PTA-2aA			3 hrs & 51 mins.
2b		PTQ-2b	PTA-2b	Practical exercise 2b	Transparency 2b.1-2b.15; slides 2b.1 to 2b.8	7 hrs & 58 mins.
2c	Basic Computer Operation Manual; SAS Operational Manual; GIFT Stat Manual	PTQ-2c	PTA-2c	Practical exercise 2c	Transparency 2c.1 to 2c.10	9 hrs & 30 mins
3a		PTQ-3a	PTA-3a	Practical exercise 3a	Transparency 3a.1-3a.9	3 hrs & 50 mins
4a		PTQ-4b	PTA-4b	Practical exercise 4a	Transparency 4a.1-4a.8	25 hrs & 24 mins
5a	Manual on CAMA	PTQ-5a	PTA-5b	Practical exercise 5a	Transparency 5a.1-5a.16	16 hrs & 16 mins
5b		PTQ-5b	PTA-5b	Practical exercise 5b	Transparency 5b.1-5b.12	13 hrs & 21 mins
6a		PTQ-6a	PTA-6a	Practical exercise 6a	Transparency 6a.1-6a.18	3 hrs & 41 mins
6b	Manual of Operation KRYO 10; Manual on Cryopreservation of Tilapia by K. Rana	PTQ-6b	PTA-6b	Practical exercise 6b	Transparency 6b.1-6b.18	10 hrs & 58 mins
7a		PTQ-7a	PTA-7a	Practical exercise 7a	Transparency 7a.1 to 7a.8	10 hrs & 25 mins
8a	ICES/EIFAC Codes of Practice; Manual of Procedures for Transfer of Fish	PTQ-8a	PTA-8a	Practical exercise 8a	Transparency 8a.1 to 8a.11	4 hrs & 30 mins
8b		PTQ-8b	PTA-8b	Practical exercise 8b	Transparency 8b.1-8b.13	5 hrs & 2 mins
					Total Number of hrs	128 hrs & 28min or 16 days and 30 mins

PRACTICAL GUIDE/ EXERCISE

PRACTICAL GUIDE/EXERCISE
ON
MODULE 2a: SAMPLING AND RECORDING OF PHENOTYPIC TRAITS

INTRODUCTION

Gathering and recording of biological data vital to experiments in a genetic improvement program should be carried out with efficiency, precision and accuracy. This is important in order to avoid marking erroneous conclusion about the results.

The GIFT team has so far standardized the methods of measuring some of the important phenotypic traits in tilapia. These traits are noted and measured during breeding and fry collection, stocking, rearing and harvesting activities.

OBJECTIVES:

Given the necessary materials and sample data forms, the trainee will be able to collect and record biological data according to the standard sampling and recording procedures developed by the GIFT project and with 100% accuracy.

MATERIALS:

- Live fish
  - breeder size (with Floy or PIT tag)
  - fingerling size
  - swim-up fry
- Measuring board (ruler)
- Top loading balance (0.10 g sensitivity)
- Anaesthetic (MS222)
- Plastic tubs, basins, strainers, scoop nets, aerators
- Flat sheet iron
- Paint, paint brush, paint thinner
- hand tally counter
- Data forms w/ clip boards, pencils, ballpen, pentel pen

Breeding and fry collection form

Breeding and fry collection form

Sampling record form of fish transfer to B-net cage/
Sampling record before tagging

Summary form on fish transfer to B-net/ Summary record on sampling
before tagging

Grow out data form

Hapa net cages ($1m^3$)

B-netcage ($1m^3$)

Seine net

Data collector/reader

Data recorder

PROCEDURE:

I. Before Spawning:

1. Prepare/Get breeding and fry Collection Form
2. Collect Sample of breeders from hapa cage
3. Immerse fish sample for few minutes in basin filled with anesthetic solution until sedated
4. Remove fish from anesthetic solution
5. Read and record the code number indicated in the fish tag (Floy or PIT tag)
6. Put the tilapia breeder on the top of loading balance and record the body weight (grams)
7. Put the tilapia breeder on the plastic ruler, read and record the standard length (cm). This is measured from the tip of the upper jaw to the posterior end of the hypural bone or base of the caudal fin.
8. Measure and record the body depth (cm) from the base of origin of the dorsal fin to the base of the origin of the pelvic fin.
9. Count and record the number of caudal fin bars (CFB). Dark ventricular stripes on the caudal fin.
10. Identify the sexual maturity condition whether the fish is ready to spawn (RS), has spawned (HS) and not ready to spawn (NR).

II. After Spawning

1. Repeat procedure 1-4 mentioned in Activity I.
2. Record the hapa number
3. Identify and record the sex
4. Read and record the tag number
5. Read and record the fish body weight (g).
6. Measure and record the standard length (cm)
7. Measure and record the body depth (cm)
8. Count and record the number of Caudal fin bars

III. Fry Collection

1. Prepare/get Breeding and Fry Collection form
2. Record the hapa number
3. Identify and record the batch (number of times the female breeder has spawned)
4. Record the date of fry collection
5. Count and record the number of fry collected, separate the dead and alive collected fry, count record of total number
7. Scoop out the fry collected from each hapa and weight in top loading balance. Get and record the bulk weight in grams.
8. Get and record the mean weigth based on the bulk weight divided by the total number of fry
9. Stock the collected fry and record the hapa size (dimension of hapa net cage). Maintain the stocking density of 150 to 200 per hapa net cage ($1m^3$).

IV. Transfer of Post-fry from nursery hapa to B-net cage

1. Record the hapa number where the family or post-fry harvested
2. Record the size of hapa 1 (refers to the dimension of hapa net cage where post fry are harvested)
3. Get the previous number of fry stocked in hapa 1
4. Count the post fry for each hapa or replicate family to record the survival.
5. Compute and record for the total number of post fry for all replicate families

6. Record size of B-net cage (hapa size 2) this refers to dimension B-net cage where post fry are transferred. Note to maintain the stocking density to 100 per $1m^3$.
7. Collect 30 samples randomly for each replicate family and record for the individual weight (g).

* data are taken from fry collection

V. Before tagging

1. Get and prepare the data form
2. Collect 30 sample of fish for each of the replicate family.
3. Anesthetize the sample
4. Weight and record the individual weight (g)
5. Count and record the total number of fish survived for each replicate family before tagging.

VI. After tagging or during stocking to different test environments for family, group testing or complementary experiments.

1. Get the data form
2. Anaesthetize the fish samples
3. Identify and record the culture system (pond, cage, rice, fish etc.)
4. Record the place of study
5. Record the pond number
6. Record the date of sampling
7. Record the sample number
8. Read and record the tag number
9. Read and record the tag color
10. Identify and record the sex
11. Read and record the body weight (g)
12. Measure and record the standard length (cm)
13. Measure and record the body depth (cm)
14. Remarks (record any observable characters, i.e. deformities, abnormalities etc.)

15. Record the water temperature at stocking and at harvest
16. Record the general observations on water condition
17. Identify and record the name of the person who measure and the recorder.

VII. During regular sampling and harvesting

1. Repeat procedures 1 to 7 in activity VI.
2. Record the special maturity condition of the fish (please refer to Module ___ for details)

PRACTICAL MASTERY TEST:

Given the data forms and the necessary sampling materials, the task is for the trainees to record all pertinent data corresponding to any one of the following activities, depending on the live specimens given.

1. Sampling prior to stocking of tilapia breeders in breeding hapa for spawning.
2. Sampling during fry collection
3. Sampling during transfer of post-fry to B-net cage
4. Sampling prior to distribution of tagged fingerlings to different test stations/environments.

Attachment

PRACTICAL GUIDE / EXERCISE
ON
MODULE 2a : ANESTHETIZATION OF TILAPIA

Introduction:

Fish are sensitive and should be handled with extreme care. The application of an anesthetic eliminates the stress experienced by fish during handling; for example during tagging or sampling.

Objective:

Given the necessary materials and background information on the subject, the trainee will apply anesthetization procedures on a given sample of fish with minimal stress and 100% restoration of fish mobility after a designated period of exposure in recovery basin.

Materials:

. Anesthetic (MS-222)
. Weighing scale
. Spatula
. Petri dish
. Plastic basin
. Tap water
. Stirring rod
. Fish
. Aerator

Procedure:

1. Prepare the materials needed in the application of anesthesia to tilapia.
2. Take a small amount of MS-222 powder with a spatula and weigh this on a weighing scale.
3. Dissolved a known amount of MS-222 powder in water (desired concentration for tilapia is 1 gram of MS-222 powder for every 3 liters tapwater).

4. Immerse the fish in anesthetic solution; after 2-3 minutes or the moment it loses equilibrium, remove fish from solution.
5. Perform the activity desired for the anesthetized fish (e.g. weigh or tag fish).
6. After weighing or performing the activity desired for anesthetized fish, immediately put fish in a recovery basin to regain consciousness.

MASTERY TEST

Actual performance test in the field, the trainee with all the necessary materials will anesthetize tilapia. The anesthetized fish after exposure in recovery water should have minimal stress and should regain 100% mobility after a designated period

INGA PROTOCOL FOR EXCHANGE OF FISH GERMPLASM

Exporting Country:

- Provide information on fish to be exported
- Certify health of fish
- Disinfect fish prior to transport

INGA PROTOCOL FOR EXCHANGE OF FISH GERMPLASM

Importing Country:

- Stocks to be imported should be on early life history stage (e.g fry, fingerling)
- Examine health of newly arrived fish
- Disinfect stocks upon arrival
- Quarantine newly arrived fish (at least 30 days)
- Advise/inform exporting country on status of stocks

Fig. 3. Flow of activities prior to and during transport of live tilapia germplasm from GIFT Project, Muñoz, Nueva Ecija, Philippines to INGA member countries

Collection of fish for transport
↓
Condition of fish in tanks
↓
Treatment with KMnO4
↓ (→ Collection of fish samples for health examination (Fish Health Unit, Bureau of Fisheries and Aquatic Resources) → Issuance of Fish Health Certificate and Certificate of Transport from Fish Health Unit and Office of the BFAR Director → Issuance of Special Permit to Load from the Bureau of Customs)
Preparation of Transport Materials (bags, boxes, oxygen etc.)
↓
Loading of fish in plastic bags
↓
Fish Transport (from GIFT Project-Muñoz to Airport
↓
Water replenishment and repacking of fish in styrofoam boxes for air transport
↓
Air Transport

Fig. 4. Schematic diagram of laboratory procedures on fish health examination

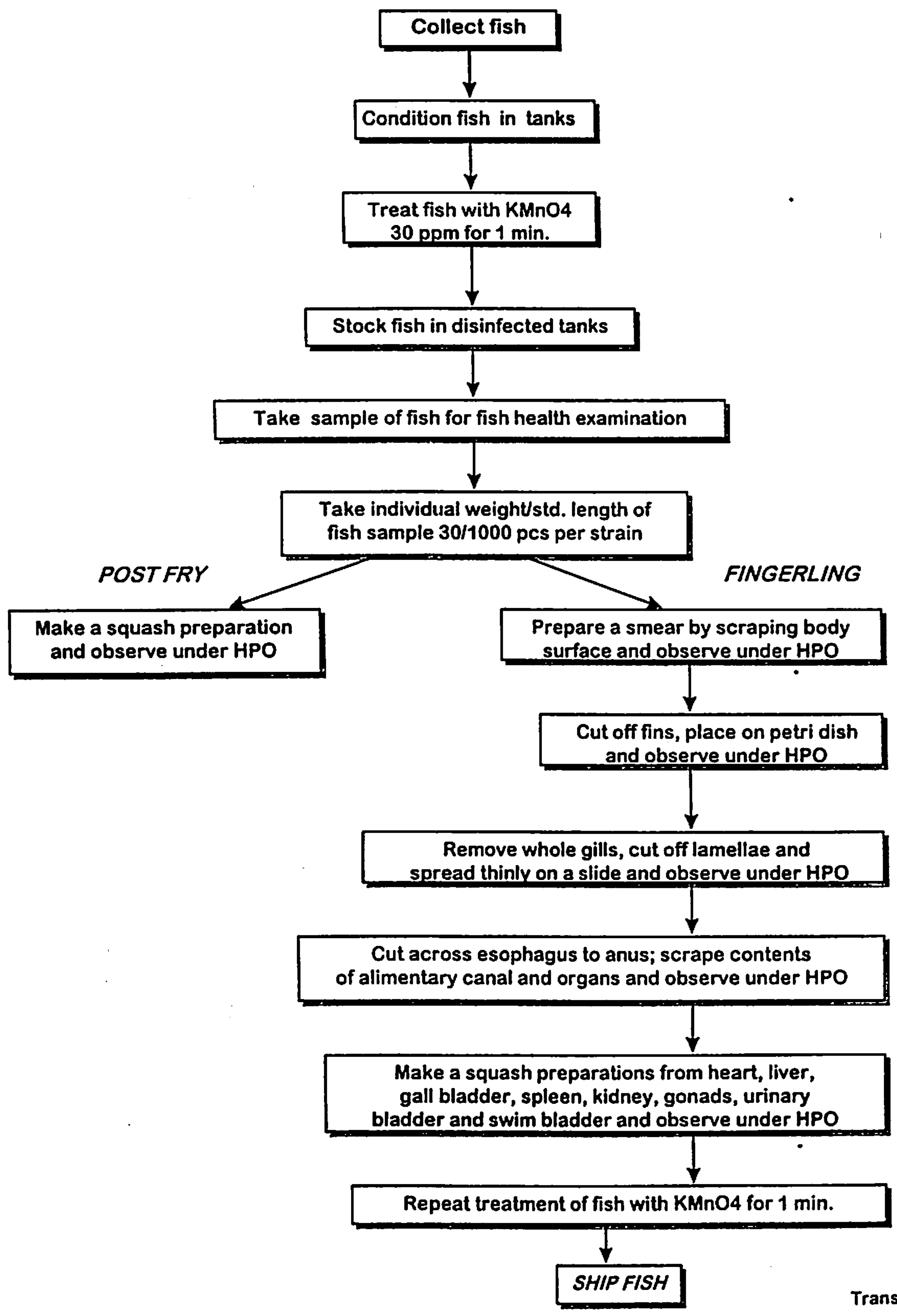

Fig. 5. Steps in packing of tilapia germplasm for transport

1

Fill plastic bag with unchlorinated tapwater (6-8 liters)

2

Transfer fish to plastic bag. Make sure the number of fish per bag is known)

20-25 pcs/bag=tilapia breeder
40-50 pcs/bag=tilapia fingerlings

3

Introduce oxygen to plastic bag

Fill 2/3 of volume of plastic bag with oxygen

Fasten upper end of plastic bag with rubber band

Place in styropore box (2-3 bags per box) for long- distance transport or in buri bag for short-distance transport

SUMMARY

- Policies/Regulations on Transfer of fish
- INGA Protocol on Fish Transfer
- Procedures in Packing/ Transport of Fish
- Importance of Fish Health Examination

TRANSPORT OF FISH GERMPLASM

WHY IMPORT TILAPIA ?

The established farmed stocks in Asia do not form the best genetic base for genetic improvement program.

STRATEGIC PLANNING

WHAT: Transport of Tilapia

WHERE: AFRICA

- Senegal - representing extreme west
- Ghana - Volta system
- Egypt - Nortrhern distributions
- Kenya - Lake Turkana

WHEN: 1988 - 1992

HOW: ?

TITLE OF MODULE

QUARANTINE OF INCOMING AND OUTGOING TILAPIA GERMPLASM

OBJECTIVES

- **Specify procedures on how to:**
  - quarantine of incoming and outgoing tilapia germplasm
  - treat infected fish
  - calculate food required during quarantine
  - calculate treatment concentration

- **Apply procedures on:**
  - post-mortem examination of tilapia
  - disinfection of fish by giving 20 ppm $KMNO_4$ bath

WHAT IS QUARANTINE?

process of holding a particular species in a confined system designed to prevent release of species or any other associated organism into the environment

WHY IS QUARANTINE IMPORTANT?

- risk of spreading diseases and parasites
- health status of fish not fully known

Quarantine Facility Is Disinfected According to the Following Procedure:

1) Soak overnight quarantine facility (tank and aquaria) with 3% $KMnO_4$.
2) Prepare 25 ppt formalin solution for soaking siphon, scoop nets etc.
3) Rinse and flush holding facilities and air dry for 1 day.
4) Fill holding facility with water to a depth of 3/4 its volume.

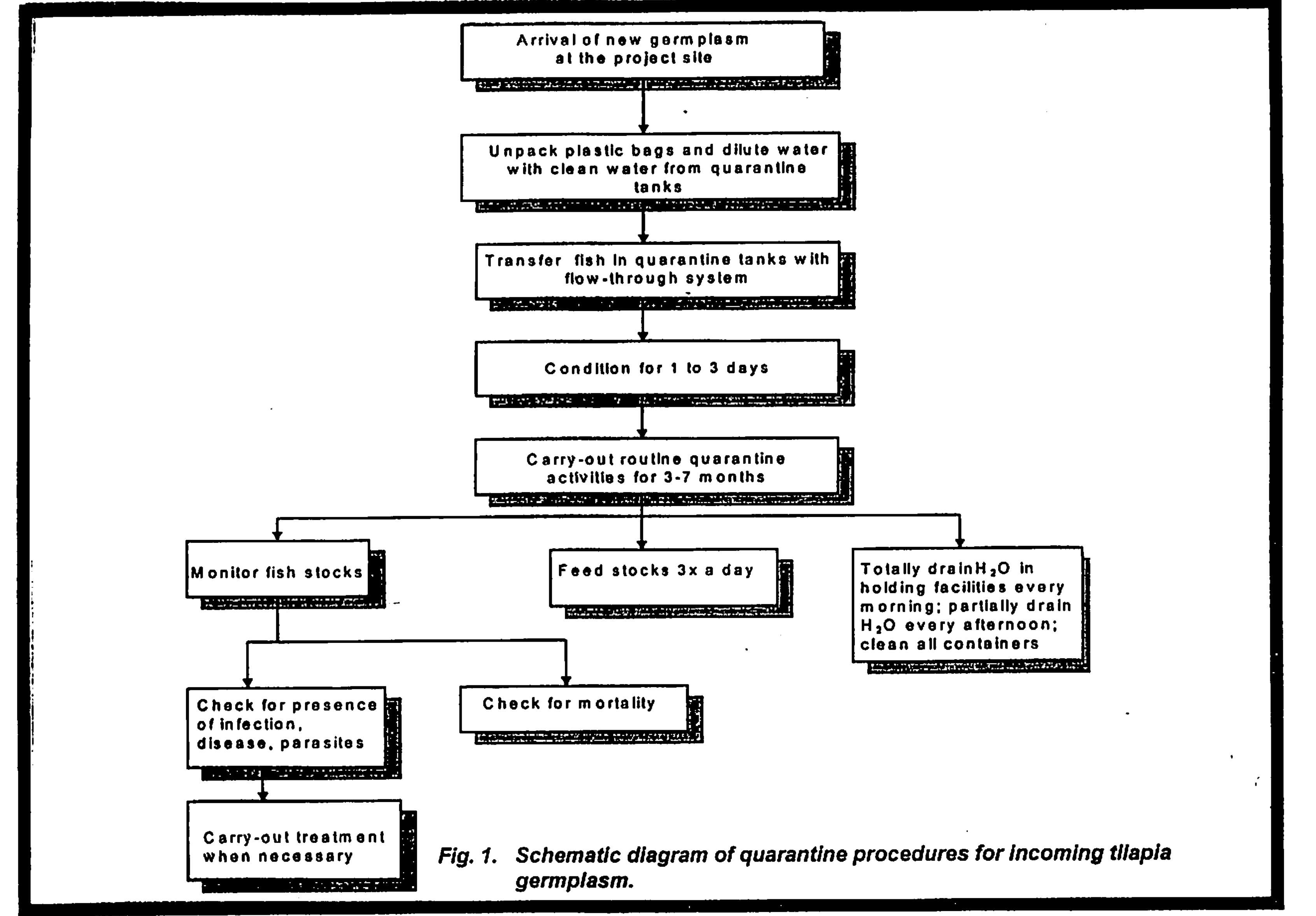

Fig. 1. Schematic diagram of quarantine procedures for incoming tilapia germplasm.

Fig. 2. ***Schematic diagram of quarantine procedures for out-going tilapia germplasm.***

- Harvest fish
- ↓ Condition fish for 1 day
- ↓ Bath fish with 20 ppm KMnO4, Don't feed fish
- ↓ Routine Quarantine Procedure
  - → Feed stocks 3x a day
  - → Totally drain H_2O in holding facility every morning & remove excess food
  - → Monitor fish stock
    - → Check for presence of infection, disease, parasite
    - → Check for mortality
- ↓ After 2 weeks provide sample to Fish Health lab for examination
- ↓ Bath fish with 20 ppm KMnO4
- ↓ 1 day prior to departure, don't feed fish
- ↓ Departure

HOW TO AVOID CONTAMINATION DURING QUARANTINE?

- avoid transfer of stocks from one container to another.
- use siphon and scoop nets only to containers/holding facility assigned to it.
- thoroughly wash/disinfect scoop nets after each use.

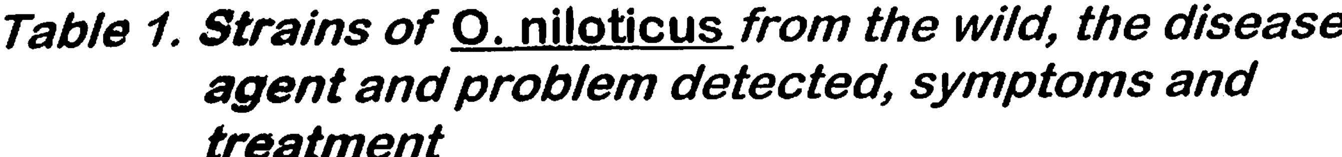

Table 1. Strains of O. niloticus from the wild, the disease agent and problem detected, symptoms and treatment

Strain (Country of Origin)	Disease Agent/Problem	Infected Organ	Symptom	Treatment	Quarantine period (days)[3]
Egypt	injured caudal fin / Fin rot *Gyrodactylus* sp. (monogean parasite) *Gyrodactylus* sp. (monogean parasite) hyperplasia Hyperplasia	caudal fin gills gills	rotten caudal fin / eroded caudal fin eroded gills with white spot reddish and distended opeculum	cicatrin + observe 1.0% silver nitrate and 1.0% potassium dichromate cicatrin + orahesive[1] 1.0% silver nitrate and 1.0% potassium dichromate[2] KMnO4 (20 ppm 10-15 sec.) given as dip with heavy aeration[1] KMnO4 (2.5 ppm, 20-30 mins.) given as bath with heavy aeration[2] 20-30 ppt NaCl, lessen amount of food and flow through water system	249
Ghana	*Cichlidogyrus* sp.	gills	darker color of infected fish	Kmn04 (20 ppm, 10-15 sec.) given as dip with heavy aeration[1] Kmn04 (2.5 ppm, 20-30 min.) given as bath with heavy aeration[2]	105
Sénégal	*Ichthyophthirius* sp.	skin and/or gills	white cysts on the gills or opeculum	cysts were manually removed from the gills or operculum and fish were given bathe of formalin with Malachite green (25 ppm, 0.1 ppm, 2-3 h) and flow through water system	105
Kenya	injured / abrasions	dorsal and caudal fin	rotten fins / eroded fins	1.0% silver nitrate and 1.0% potassium dichromate Oratein on lesion[1] 1.0% silver nitrate and 1.0% potassium dichromate[2]	102

[1] acute stage of infection

[2] early stage of infection

[3] total no. of days in captivity
[1] acute stage of infection
[2] early stage of infection

Pointers to Be Taken Into Consideration When Treating Infected Fish:

- Sort fish by degree of infection
- Before giving any therapeutic or prophylactic treatment, test it first to few fish.
- Calculate dosage carefully and treat fish done early in the morning.
- Repeat treatment when necessary
- Starve fish prior to treatment
- Remove and kill fish which shows sign of diseases

HOW TO CALCULATE AMOUNT OF FOOD REQUIRED BY INFECTED FISH AT A GIVEN PERIOD OF TREATMENT:

HOW TO CALCULATE TREATMENT CONCENTRATION USING TREATMENT CONVERSION CHART:

HOW TO CONDUCT POST MORTEM EXAMINATION:

SUGGESTED READING MATERIALS

LIST OF READING MATERIALS

1. **Diagram on Natural Reproductive Cycle of *Oreochromis sp.***

2. **Establishment of Tilapia Spawning Families Providing a Continuos Supply of Eggs for *in vitro* Fertilization**
   PETER C. PHILIPPS[1] and CHRISTOPHER C. KOHLER[2]

3. **The Ecology and Evolution of Reproductive Synchrony**
   ROLF ANKER IMS

4. **Prospects of Selecting for Late Maturity in Tilapia *(Oreochromis niloticus)* II. Strain Comparisons under Laboratory and Field Conditions**
   W. OLDORF[1], U. KRONERT[1], J. BALARIN[2], R. HALLER[2], G. HÖRSTGEN-SCHWARK[1] and H. J. LANGHOLTZ[1]

5. **Genetic Variation in Quantitative and Selective Breeding in Fish and Shellfish**
   TRYGVE GJEDREM

6. **Improvement of Productivity through Breeding Schemes**
   GJEDREM, TRYGVE, Institute of Aquaculture Research, The Agricultural Research of Norway

7. **Improving Spawning Synchrony in the Nile Tilapia. *Oreochromis niloticus* (L.)**
   D. C. LITTLE, D. J. MACINTOSH and P. EDWARDS

8. **Mass Synchronized Spawning of *Tilapia Guineensis***
   D. CAMPBELL, A. T. MAHATANE, and S. O. ALEEM

9. **Predicted Response to Selection for Early Growth in *Tilapia nilotica***
   DOUGLAS TAVE[1] and R. O. SMITHERMAN

I. NEST BUILDING & COURTSHIP (1-5 days):

II. OVULATION & SPAWNING (<2 hours)

Spawned female leaves lek

III. INCUBATION (6-10 days)*

holding of seed in the mouth continuously

MATERNAL DEPENDENCY

IV. NURSERY (10-30 days)

continued maternal dependency; fry and mother begin to feed

V. FEEDING & RECOVERY (14-30 days)

re entry of recovered female into lek

mature oocytes

Development of ovary after incubation

Speed of ovarian development varies with period of maternal dependency and food availability

1° oocytes

boundary of lek

* Incubation period is T°C dependent

Figure 2. Natural reproductive cycle of *Oreochromis sp*

- also mentioned about resorption of oocytes
- used swollen and reddish urogenital papila as indicator of readiness to spawn.

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Establishment of Tilapia Spawning Families Providing a Continuous Supply of Eggs for *in vitro* Fertilization

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Abstract

Three hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) spawning families were established in three separate culture tanks to provide a continuous supply of fresh gametes. An ovulation event, resulting in a natural spawn, an artificial spawn or oocyte resorption, occurred with a mean of every 1.4 days among 19 females, though only a few females demonstrated periods of evenly spaced 10 to 20 day maturation-ovulation cycles. Ovulation events/female/year were similar among the three spawning families: 13.1, 17.5 and 13.4/female, as were mean interovulation intervals (23.6, 21.9, and 23.9 days). Estimated fertilization rates, both natural and as a result of strip-spawning, were highly variable. Rates from natural spawns ranged from a mean per female of 25.7 to 57.3%, and from artificial spawns from 47.8 to 58.8%. Establishment and active management of spawning families of tilapias proved to be an effective procedure for obtaining a continuous supply of gametes for manipulative genetic research.

Applied genetic research on fishes in areas such as induced polyploidy and gene transfer requires a continuous supply of viable sperm and ova. Tilapias provide an excellent model for assessing the utility of gene transfer technology in fishes because of their prolonged spawning season and frequent ovulations. Recombinant DNA can be microinjected into zygotes to test the optimal form and concentration of exogenous DNA to be used, as well as to test the usefulness of different genes, cloning vectors, and promoter/enhancer constructs (Phillips and Kohler in press; Phillips et al. in press).

Some researchers have successfully stripped tilapia eggs for fertilization and/or artificially hatched fry. Valenti (1975) achieved a maximum success of 90% hatching when he allowed female *O. aureus* to deposit two groups of eggs prior to being stripped. Myers and Hershberger (1990), after strip-spawning *O. niloticus*, *O. mossambicus* and their hybrid, obtained an average hatching rate of 68.6% (mean for all combinations).

To routinely conduct replicated microinjection or ploidy manipulative experiments, it is necessary to artificially, or strip-spawn, tilapia eggs. Most researchers control the natural spawning cycle via environmental manipulation. Much research emphasis has been placed on survival to the yolk-sac stage. Little information is available on numbers of ovulations per individual female tilapia over an extended period of time.

This study was designed to develop a protocol for obtaining a continuous supply of gametes from a population of *O. mossambicus* × *O. niloticus* hybrids. An additional focus was to compare fertilization rates between natural and strip-spawned egg batches.

Materials and Methods

Spawning Families

Four females and one male of a hybrid strain of *O. mossambicus* × *O. niloticus* were obtained from the University of Washington in June 1987. These were the parent

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stock of the two additional spawning families of seven or eight females and one male. The parent broodfish were held in tank 2, and tanks 1 and 3 contained their progeny. The latter were approximately seven months old at the beginning of the spawning management period in January 1988. These three 1,325 L circular tanks were part of a recycled-water system. Each tank was covered with a black plastic tent, and a 25 W incandescent bulb provided a 16 h light : 8 h dark regime throughout the experimental period. Water temperature was maintained at approximately 29 C. An arrangement of 7.6 cm diameter plastic pipes were used to provide refuge. Fish were fed a granular 40% crude protein feed (Zeigler Brothers, Incorporated) daily at a minimum 3% level of body weight or *ad libitum*. Feeding time was irregular.

Obtaining Eggs

Each female was color-tagged (Floy Tag & Manufacturing, Incorporated) to facilitate individual behavioral observations of female : female and male : female interactions. Tilapias exhibit diurnal periodicity in feeding, territorial and spawning behavior. Feeding is mainly concentrated in the morning hours. Territorial and courtship behavior builds to a peak in the afternoon hours when spawning occurs (Munro and Singh 1987). Therefore, observations in the present study commenced at eight hours after the onset of the light cycle. Potential spawning pairs were identified over a period of several days of agonistic, then courting and nesting behavior. A swollen and reddish genital papilla indicated that the female had likely ovulated and was ready to strip-spawn that same day. After approximately 10 hours of light, strip-spawning readiness was confirmed by gently squeezing the ventral region. If discrete non-bloodied eggs were easily extruded, then that female was ready to be stripped.

Eggs were stripped dry (Leitritz and Lewis [illegible]0) into a stainless steel bowl. Sperm was [illegible] suction from the male's genital papilla into a Pasteur pipette and dropped over the eggs. Water was added to the egg and sperm mixture and fertilized eggs were held at 29 C for 10 minutes before rinsing and decanting. The eggs were then cooled on an ice pack over a 15 minute period to approximately 20 C in order to delay the time of first cleavage. This delay was necessary to achieve other research objectives (Phillips and Kohler, in press). Eggs were transferred to Petri dishes by gently drawing them into pipettes with an enlarged orifice in order to minimize mechanical damage to

TABLE 1. *Total number of ovulation events, separated into natural spawnings, artificial spawning and oocyte resorption, per female* Oreochromis mossambicus × O. niloticus *hybrids arranged into three spawning families during one year (January 1988 to January 1989).*

Spawning family	Ovulation events		
	Natural	Artificial	Resorption
1 (7 females : 1 male)	0	5	8
	8	0	1
	9	2	2
	0	4	9
	0	2	13
	0	6	8
	1	0	14
Total	18	19	55
	Grand	total	92
		Mean	13.1
2 (4 females : 1 male)	0	6	9
	3	4	11
	8	6	5
	5	6	7
Total	16	22	32
	Grand	total	70
		Mean	17.5
3 (8 females : 1 male)	9	5	3
	2	7	11
	10	1	2
	2	1	3
	3	0	5
	5	2	8
	1	1	1
	15	8	2
Total	47	25	35
	Grand	total	107
		Mean	13.4

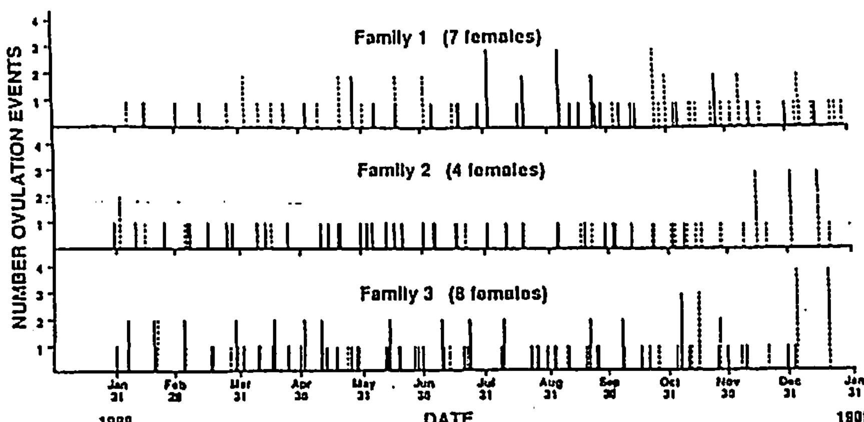

FIGURE 1. *Daily distribution of ovulation events separated into natural and artificial spawnings (solid line) and spawning inhibition (dotted line) per three spawning families of* Oreochromis mossambicus × O. niloticus *during one year (January 1988 to January 1989).*

the chorionic membrane during examination. Fertilization rates were determined microscopically, by estimating the percentage of live eggs among the total batch of eggs released per female after a 24 h artificial incubation period.

In any one reproductive cycle, tilapia either spawned naturally, were artificially stripped, or they resorbed their oocytes. The exact day of spawning could be determined by the stage of embryonic development. By removing eggs from the female's mouth, another oocyte maturation cycle would commence and thus the interval between ovulations would be shortened (Verdegem and McGinty 1987). An artificial spawn refers to manual stripping of both female and male gametes. Resorption of ovulated oocytes or spawning inhibition occurred when a female ovulated, but natural spawning was suppressed or artificial spawning was not attempted. Resorption was identified by the presence of softened, easily broken oocytes while gently stripping the female.

When a female spawned naturally and, during routine inspection, was found to be mouthbrooding, the fertilization rate was estimated and the incubating eggs were discarded. The artificial incubation protocol for maintaining eggs to hatching and subsequent yolk-sac absorption is discussed in Phillips et al. (in press).

Results and Discussion

Among 19 female tilapia in three spawning families, ovulation events were broadly distributed throughout 365 days. Though there was considerable variation in the spawning pattern per female and per family, an ovulation event occurred once every 1.4 days (269 events/365 days, Table 1 and Fig. 1). Female ovulation was asynchronous and a few females accounted for the majority of all natural and artificial spawning events. Within one tank, several females commonly ovulated on the same day. Frequently, one would spawn and the others would resorb their eggs. Occasionally, two females in the same tank would spawn naturally on the same day, and sometimes all ovulated females would resorb their oocytes. The latter was most common in family 1 where 60% of ovulation events ended in oocyte resorption (Table 1). The greatest number of natural spawns occurred in family 3 where 44% of ovulation events resulted in natural spawns (Table 1). When the natural and artificial spawning and oocyte resorption

TABLE 2. *Percent fertilization per female tilapia hybrid (*Oreochromis mossambicus × O. niloticus*) egg batch in natural and artificial spawns when arranged into three spawning families. N refers to the number of natural or artificial spawns per female. Each row refers to one female.*

	Percent fertilization					
	Natural spawn			Artificial spawn		
Spawning family	*N*	Mean	Range	*N*	Mean	Range
1 (7 females : 1 male)	1	5	5	0		
	8	88	50–100	0		
	9	79	0–99	2	95	95
	0			4	28	1–50
	0			2	5	5
	0			6	38	0–90
	0			5	73	60–95
Total	18	57.3	0–100	21	47.8	0–95
2 (4 females : 1 male)	3	1	1	4	46	10–90
	8	65	50–95	6	72	25–99
	5	11	1–50	6	26	0–90
	0			6	91	80–100
Total	16	25.7	1–95	22	58.8	0–100
3 (8 females : 1 male)	9	47	0–95	5	34	10–50
	2	90	90	7	48	0–95
	10	15	0–90	1	10	10
	2	10	10	1	35	35
	5	73	1–99	2	93	90–95
	1	95	95	1	60	60
	15	57	5–99	8	64	20–95
	3	25	10–40	0	0	
Total	47	51.5	0–99	25	49.1	0–95
Overall mean		44.8			51.9	

events were totaled per tank, the mean number of ovulations per female per 365 days was 13.1 in tank 1 for seven females, 17.5 in tank 2 for four females, and 13.4 in tank 3 for eight females.

The range in percentage fertilization rates for both artificial and natural spawning events was extremely broad (Table 2). The mean percentage fertilization rates in family 2 was much higher in artificially-spawned females (59%) compared to those females that naturally spawned (26%). The mean natural and artificial fertilization rates within tanks were comparable among family 1 (57% natural and 48% artificial) and family 3 (52% natural and 49% artificial).

The mean interovulation interval (Burt et al. 1988) per female was 23.6, 21.9, and 23.9 days for families 1 to 3, respectively (Table 3). Several females in each family ovulated regularly every 10 to 20 days during several months of the year-long experimental period. During these periods of regular ovulations, it was possible to accurately predict

TABLE 3. *Mean interovulation interval/female tilapia hybrid (*Oreochromis mossambicus × O. niloticus*) and standard deviation (SD) during one year. There was no significant ($P < 0.05$) difference in mean interovulation interval between spawning families.*

	Interovulation interval (days)	
Spawning family	Days	SD
1 (7 females : 1 male)	23.6	11.5
2 (4 females : 1 male)	21.9	11.2
3 (8 females : 1 male)	23.9	12.2
Overall female	23.1	11.6

the exact day of spawning. Eventually, though, these females became asynchronous and were often replaced by one or more females that would then ovulate regularly. The shortest interovulation interval was 8 days and the longest extended to 5 months. The breadth of these interovulation intervals, taken in the context of the entire year's experiment, account for the large standard deviations (SD) obtained (Table 3). One family 3 female was the only individual to consistently ovulate every 11 to 19 days (SD = 4.9) throughout the year.

Though spawning distribution was uneven, with the management of three spawning families, an ovulated female was obtained on an average of once every 1.4 days. In addition, by removing egg broods from their mouths, the interovulation interval may have been reduced (Lee 1979; Verdegem and McGinty 1987). Removal of eggs from the brooding female appears to accelerate recruitment (Meske 1985) and add to the reproductive potential rather than exploit a fixed gonadal reserve. In addition, reproductively active females continued to feed (when not mouthbrooding) and to maintain growth throughout the spawning season. Therefore, loss of somatic food reserves was made up by feeding; apparently a supply of high quality food to the brooders is essential for successive and frequent spawning of tilapias.

Rothbard and Pruginin (1975) first suggested the establishment of "spawning families." These are established prior to sexual maturity (4–5 mo old or 50–100 g) by high-density stocking of aquaria with 50 2–5 g fry. They are culled until there is one male and seven to ten females left. In the presence of so many females, the male may be maintained in a permanent state of sexual readiness. Upon completing incubation, a female will be ready to mate again. By this method, aggression is reduced between individual family members and frequent spawning is facilitated.

It is difficult to assess which are the key factors that stimulate, inhibit, or exert any regulation on the various stages of the breeding cycles of tilapia. This uncertainty is due, in part, to the paucity of experimental studies dissociating the role of separate environmental and behavioral factors that determine the intensity of tilapia reproductive activity. In indoor aquaria, Katz and Eckstein (1974) found that tilapias became reproductively active at water temperatures above 22 C, whereas below 18 C all activity ceased. Their aquaria were cleaned every two to three weeks and the authors speculated that sexual activity may have been triggered by these water changes. Fishelson (1966) reported that, above 20 C, a female tilapia spawned in aquaria 11 times in a year. In the present study, the maximum number of ovulations for one *O. niloticus* × *O. mossambicus* female was 25—she spawned naturally 15 times, artificially spawned 8 times and resorbed her oocytes twice during one year.

Spawning inhibition or oocyte resorption among these hybrids was likely a result of male-female incompatibility and interruption in the courtship behavior due to interference from the remaining females. This was a common occurrence in the present research. Iles (1973) reported that tilapia ova are competent for fertilization only briefly following ovulation. If spawning doesn't occur, the eggs can be resorbed thereby conserving energy-rich material. Since there is more than one clutch of oocytes present in a tilapia ovary at one time, after egg resorption a new batch of oocytes can rapidly mature. Apparently, oocyte resorption in tilapias is usual, and was not necessarily a result of conditions unique to the breeding facility employed in this study.

It is not possible to make strict comparisons between artificial, or strip-spawning, and natural spawning events. For example, during the time period in which the greatest number of artificial fertilizations were conducted, among families 1 and 2 several successful artificially-spawned oocyte batches would probably have been resorbed if the females had not been stripped. This con-

clusion is reached because far fewer females in those families were ever found mouthbrooding fertilized eggs and fry compared to family 3. Therefore, a successful artificial spawn does not mean that a successful natural spawn was usurped.

Only one published report on egg fertilization rates in tilapia could be found. Watanabe et al. (1989) reported a mean fertilization success (percentage of eggs undergoing embryonic development in a sample of 100 eggs) in *O. urolepis hornorum* × *O. mossambicus* at low salinities in the range of 67–80%. Fertilization decreased to 35% at 36 ppt salinity. A review of survival to hatching will serve to place into perspective the modest 50% fertilization rates of the present study, from both natural and artificial spawning episodes. Myers and Hershberger (In press) reported in laboratory experiments with the same *O. mossambicus* × *O. niloticus* hybrids that hatching success was 65%. Valenti (1975) achieved a maximum successful hatch of 90% (range of 35–90%) with *O. aureus* in aquaria. Cridland (1962) (in Riedel 1965) reported a 61% mean survival-to-hatching rate (range 14–100%) in *O. niger* during buccal incubation in the natural habitat.

An examination of the results of the extreme variability in number of ovulations per female, and in the variation in fertilization rates within families and between families tends to lead to the conclusion that there is no regularity in tilapia spawning. Nevertheless, the overall picture is quite different. By color tagging females for easy identification, careful handling and management permitted familiarization with individual females. Several females ovulated with a consistent and regular pattern during periods of several months. They also produced eggs that gave high fertilization rates. When these females no longer ovulated regularly, other females would often replace them. Therefore, though the mean interovulation intervals for the whole experimental period extended beyond 20 days per female, during periods of several months, it was common to regularly obtain eggs at much shorter and regul[illegible] hours after the onset of the light cycle, a rapid examination for a swollen and reddish urogenital papilla was sufficient to determine spawning readiness. Daily handling did not interrupt courtship and spawning behavior. In addition, the regular timing of stripping eight to ten hours after the onset of the light cycle provided, in the context of the entire year, a reliable supply of high-quality fertilizable tilapia eggs. This allowed for the design of repetitive gene transfer experiments into one-cell stage tilapia zygotes (Phillips and Kohler, in press; Phillips et al., in press).

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The Ecology and Evolution of Reproductive Synchrony

Rolf Anker Ims

The temporal pattern of breeding in populations is often characterized by a pronounced temporal clustering of births, flowering or seed set. It has long been suspected that this phenomenon is not caused by climatic seasonality alone but that reproductive synchrony represents a strategy that individuals adopt to maximize reproductive success. The classical hypotheses predicting an adaptive advantage of reproductive synchrony incorporate both sociobiological and ecological explanations. However, new theoretical and empirical analyses have shown that the predicted advantage of reproductive synchrony depends on the ecological setting in which populations reproduce, and processes earlier thought to be responsible only for synchrony may under some ecological conditions lead to asynchronous reproduction being the best strategy.

Reproductive synchrony has been defined as the tendency of individuals to carry out some part of the reproductive cycle at the same time as other members of the population[1,2]. This phenomenon is best known from its rather extreme manifestations, for instance in bamboos[3], cicadas[4] and sea turtles[5]. However, besides these famous examples, a pronounced temporal clustering of reproduction is rather widespread in both the animal and the plant kingdoms.

In seasonal environments there is commonly some degree of temporal clustering of reproduction, usually because reproduction at certain times of the year will be best for offspring survival. Hence, what is often termed 'reproductive synchrony' may simply be the consequence of individuals selecting the same favourable time for reproduction in relation to climate. However, reproduction is often far more synchronous than would be expected from environmental seasonality alone. For example, a pronounced temporal clustering of reproduction is quite common in relatively aseasonal, tropical regions[6]. This fact has led biologists to search for explanations other than environmental seasonality (climate) to explain the temporal pattern of reproduction in animal and plant populations, and it is now acknowledged that the temporal pattern of reproduction may be shaped by several ecological and sociobiological processes (Fig. 1).

In this review I will refer to *reproductive synchrony*, or *synchronization of reproduction*, as a phenomenon caused by biological interactions operating to produce a tighter clustering of reproductive events than would have been imposed by environmental seasonality alone.

Fraser Darling[7] suggested in 1938 that the degree of reproductive synchrony in colonial birds may affect nest predation rates. Subsequently, a number of other processes interacting with the temporal pattern of reproduction has been suggested. When such interactions (e.g. predation) affect the reproductive success of individuals, natural selection can modify the temporal pattern of breeding which initially may be determined by environmental seasonality (Fig. 1). Reproductive synchrony will then evolve as a result of a reproductive advantage (e.g. reduced predation on offspring) accrued to individuals capable of synchronizing their reproduction with their neighbours.

Several of the classical hypotheses about the ecological/sociobiological implications and the resulting evolution of reproductive synchrony have long escaped closer theoretical examination and relevant empirical testing. Although recent theoretical and experimental studies lend some support to the older ideas, these new studies also show that the ecology of reproductive synchrony is far more complicated than was earlier believed.

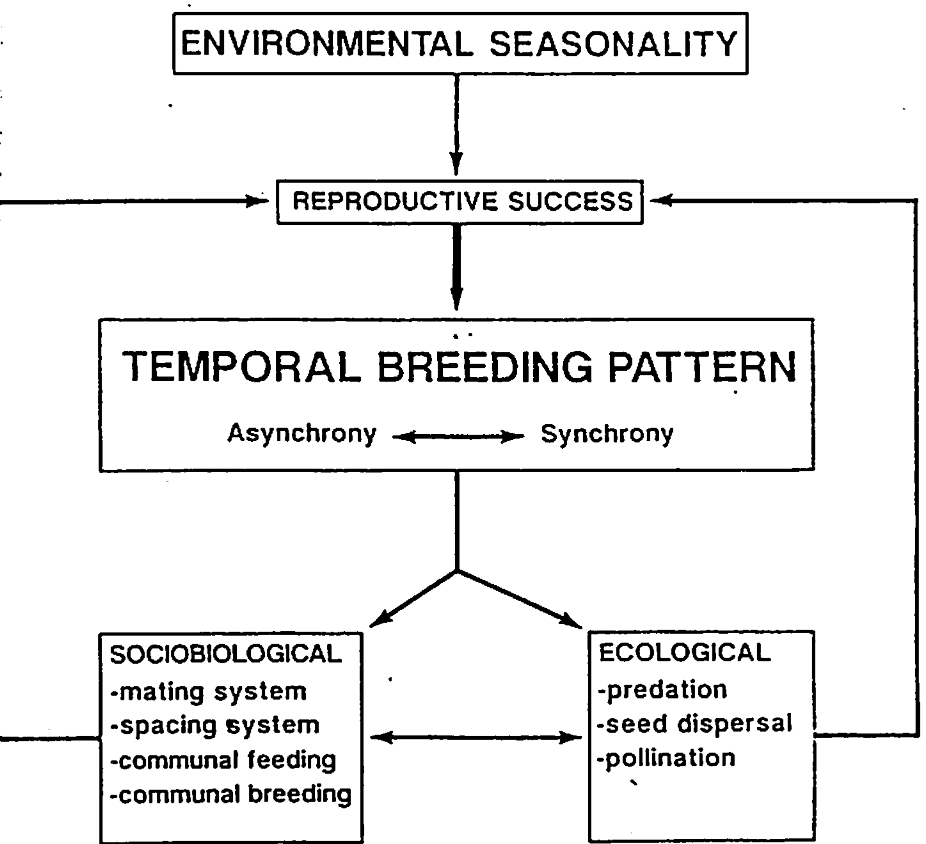

Fig. 1. Environmental (climatic) and biotic (ecological and sociobiological) factors that potentially interact with the temporal pattern of reproduction. The temporal pattern of reproduction may have immediate consequences for ecological processes (e.g. predation) or sociobiological processes (e.g. communal feeding). These processes may in turn affect the relative reproductive success of individuals and ultimately select the degree of reproductive synchrony.

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Fig. 2. Indirect evidence for social stimulation of reproductive synchrony within a breeding colony of the black-headed gull (*Larus ribundus*). The bars show the sum of observed copulations that followed another copulation in < 10 min or > 10 min either in the direct vicinity (radius < 2 m) or at some distance away (radius > 2 m). *Data from Ref. 8.*

Temporal clustering of reproduction in a seasonal environment: climate or biological interactions?

Various environmental factors can function as external cues for organisms to time their reproduction according to climatic seasonality. How can the impact of climatic seasonality be separated from biological determinants of the temporal pattern of reproduction? One clue to this question, which ultimately relates to whether the observed pattern is an adaptation or not, can be provided by the specific cue used by the organism to initiate reproduction.

Three main classes of such cues can be distinguished. The first class represents *environmental* cues that are directly connected to climatic seasonality, such as photoperiod, temperature, humidity and food availability. *Internal* cues from endogenous rhythms in the organisms themselves (e.g. biological clocks) constitute the second class.

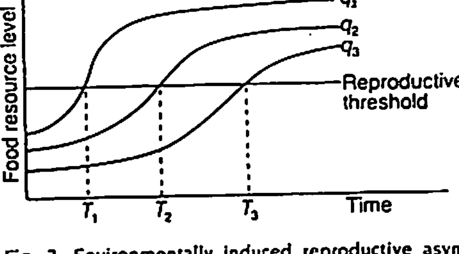

Fig. 3. Environmentally induced reproductive asynchrony among territorial females for which the onset of reproduction is triggered by the amount of food in their territories. The three curves depict the changes in three hypothetical territories of different quality ($q_1>q_2>q_3$) from the start of the plant growing season. The horizontal line represents the minimum food-resource level needed for initiation of reproduction by a single female (reproductive threshold). The intersections between the curves and the reproductive threshold (T_1–T_3) give the times of onset of reproduction in the three territories. *Reproduced with permission from Ref. 12.*

Finally, the third class of cues – signals exchanged between the individuals within a population – can be labeled *social* cues. The last two classes are of particular interest because they can operate to uncouple the temporal pattern of reproduction from environmental seasonality, and can thus indicate that individuals actively synchronize their reproduction and that the temporal clustering of reproduction is not simply a direct consequence of changing climate.

What is the evidence for the occurrence of the different classes of cues? Reproduction-initiating cues have been explored for relatively few organisms. Hence, although some generalizations can be drawn, for example that environmental cues are more predominant among plants than among animals and that social cues are probably more common among vertebrates than among invertebrates, the examples selected below may not reflect the relative occurrence of the different classes of cues in different taxa.

In several species of birds[2] and mammals (including humans)[8], social stimuli exchanged between neighboring females have been shown to be the proximate cause of reproductive synchrony. For mammals, laboratory experiments have shown that oestrus synchrony can be achieved among group-living females through exchange of pheromonal stimulation or as a result of exposure to a novel male[5]. Male-induced reproductive synchrony has also been seen under natural conditions for lions (*Panthera leo*) when the takeover of a pride by a new coalition of males causes birth synchrony within the pride[9].

Although other mechanisms for socially mediated reproductive synchrony are more difficult to discover in the field, evidence other than the mechanism itself may be available to indicate that reproductive synchrony is socially induced. For example, the spatial pattern of reproductive events within a population can be indicative. In particular, the finding that reproduction is significantly more synchronized among neighboring individuals than among more widely separated ones in gulls[10] (Fig. 2) and in ruminants[11], may indicate that synchrony is socially induced within groups of individuals. However, spatial heterogeneity of environmental factors functioning as reproduction-triggering cues may produce similar spatio-temporal patterns of reproduction[12]. In animals with several consecutive litters and young that become sexually mature within the same breeding season, maintenance of reproductive synchrony throughout the season is another indication of socially induced reproductive synchrony[12,13]. Laboratory studies[14] have shown that young maturing golden hamsters (*Mesocricetus auratus*) are able to entrain their oestrus cycles to older, dominant ones by means of olfactorial cues.

The evidence for the existence of internal cues is even more sparse. However, endogenous rhythms may be responsible for some cases of synchronized reproduction at supra-year intervals. In particular, cicadas[2] and bamboos[3] are well known for the long intervals between successive breeding seasons (13 or 17 years in cicadas and up to 120 years in bamboos). Such species have probably evolved a genetically determined internal clock[3] as a response to external selective pressures such as predation (see below). However, these hypothesized clocks can themselves be altered by environmental conditions. Furthermore, within-season reproductive synchrony may be determined by environmental factors: for example, the day of emergence seems to be determined by the ground temperature in periodical cicadas[15].

For species having clustered reproduction on a seasonal basis, and for which the timing of reproduction is determined at least to some degree by environmental factors, it may be more difficult to tell whether individuals actively synchronize their reproduction. In such instances, multivariate statistics may be useful to determine the relative importance of climatic variables and biotic interactions.

Rutberg[10] used multiple regression analysis to examine the importance of climate for birth synchrony in 27 populations of ruminants. He found that climatic seasonality accounted for a significant amount of variation in synchrony in only one of two ecologically distinct groups of species ('hiders') and concluded that birth synchrony of the other

group ('followers') had evolved as an antipredation strategy (see below). Sinsch[17] used the same approach to conclude that breeding synchrony in the natterjack toad (*Bufo calamita*) was uncoupled from environmental seasonality, and that this was probably due to an endogenous mechanism.

In some cases, the temporal pattern of breeding may result from the combined action of environmental and social variables. For example, animals living in an environment with unpredictable food availability often use the amount of food, or some of its properties, as a cue to initiate reproduction[18]. In such species, the temporal pattern of breeding may be determined by the social organization of breeding females together with the spatial distribution of the food resource.

In a spatially homogenous environment – a situation that facilitates space sharing between reproductive females[19] – individual females should be exposed to the same reproduction-triggering cue at the same time, and reproductive synchrony will result[12]. Note that for space-sharing females there will also be rich possibilities for exchange of, for example, pheromonal stimuli facilitating socially mediated reproductive synchrony[8]. In a spatially heterogeneous environment, on the other hand, females will tend to maintain individual territories[19] of different quality, resulting in different onsets of the triggering cue and consequently asynchronous reproduction[12] (Fig. 3). The opportunity for exchange of social stimuli and hence for socially mediated synchrony will probably be less among territorial females than in group-living females[8,12]. The predicted relationship between female social system and temporal pattern of breeding has recently been examined in microtine rodents[12,13], and it seems to hold true for species for which there is information about both spacing system and temporal breeding pattern.

Finding that reproductive events are tightly clustered according to some environmental factor(s) does not necessarily imply that the clustering in itself has no adaptive value. For example, some marine polychaetes use lunar cycles to time their swarming[20], whereas some tropical plants flower immediately after heavy rain showers[6]. Although it has been suggested that the environmental factors in such cases most likely act merely as cues to attain reproductive synchrony, the potential causes are hard to separate. Here, as for all possible cases of reproductive synchrony – irrespective of the type of reproduction-initiating cue – the ultimate test for an adaptive advantage is to compare the reproductive success of asynchronously versus synchronously breeding individuals in a population. However, few critical tests of this kind have been done.

Adaptive hypotheses of reproductive synchrony

Sociobiological causes

Emlen and Demong[21] compared the feeding efficiency and reproductive success of pairs of synchronously breeding, colonial swallows (*Riparia riparia*) that reproduced at the reproductive peak of the colony with pairs that reproduced off-peak. Off-peak breeding pairs fed less efficiently and had lower reproductive success than on-peak pairs. Swallows and many other colonial birds feed communally on unpredictable food patches, and information about such food patches is spread most efficiently among colony members[22] when their reproductive cycles are synchronized.

In addition to enhancing the reproductive success of the individuals, as in the case of communal feeding systems, reproductive synchrony is a necessary condition for communal *breeding* systems. In such systems, groups of breeding females share the parentage of the young and it is necessary for the group members to synchronize their breeding so that the young can be communally nursed (mammals[23,24]) or eggs can be jointly incubated (birds[25]).

For solitary breeding females, it has been argued that reproductive synchrony may have evolved as a means of enforcing monogamy on males[26] when paternal investment in the young increases their probability of survival. Reproductive synchrony is generally expected to decrease the potential for polygamy in animals[27,28] (Fig. 4). Hence, reproductive synchrony may have evolved as a result of sexual selection. However, for females the success of this strategy is not only dependent on the temporal pattern of reproduction and paternal investment, but also on other factors such as the operational sex ratio, the spatial distribution of receptive females, and the mode of mate acquisition in males[28]. For example, if males have the capacity to monopolize spatially aggregated females – either by means of territories or by guarding grouped, mobile females (harem formations) – female reproductive synchrony can result in polygyny rather than monogamy[12,28]. Accordingly, recent genetic evidence for birds indicates that a high degree of reproductive synchrony does not hinder extra-pair copulations in males[29].

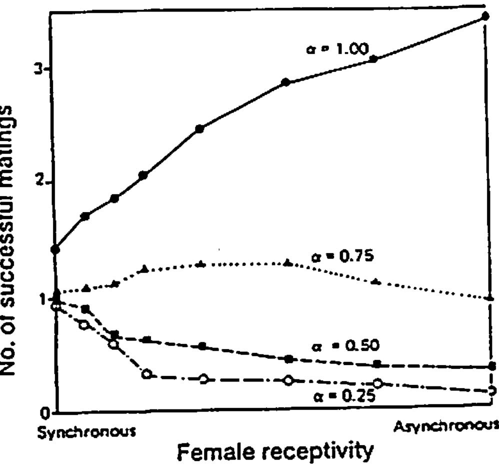

Fig. 4. Predicted number of successful matings for four males of different quality (i.e. ability to find and compete successfully for females) in relation to the degree of synchrony of female sexual receptivity. The prediction is derived from a simulation model assuming a promiscuous mating system characterized by male–male competition and active searching for receptive females. The α values are positively correlated with the males' competitive abilities. The potential for polygyny increases with the degree of asynchrony in the population. *Reproduced with permission from Ref. 28.*

It should also be noted that sexual selection may be responsible for reproductive asynchrony rather than synchrony in species where paternal investment in young is unimportant. Reproductive asynchrony can increase the opportunity for optimal mate choice, since each receptive female can attract more males when no other receptive females are around[13].

The temporal pattern of reproduction can also be an important mating system variable in plants. Although some degree of flowering synchrony is necessary for outcrossing, inbreeding avoidance or sexual selection are probably not the reasons for the widespread occurrence of pronounced flowering synchrony[6]. On the contrary, a slight

asynchrony will promote outcrossing in animal-pollinated plants, since it forces pollinators to move longer distances between individual plants[10].

Recently, McShea[31] has proposed a new hypothesis to explain the widespread occurrence of pronounced reproductive asynchrony in many territorial female microtine rodents[12]. He suggests that reproductive asynchrony may be an adaptation to a territorial spacing system rather than a passive consequence of it[12]. In microtines, female territory size shows a regular oscillation during the course of the reproductive cycle, being smallest around the time of birth and early lactation[31] (Fig. 5). When space is limiting, it can be advantageous for females with oscillating territories to reproduce out of synchrony with their neighboring territory holders, because this will result in the most efficient temporal space partitioning among females (Fig. 5). However, no data are presently available to suggest that females are actively staggering their reproduction.

Ecological causes

Apart from the possibility that reproductive synchrony has evolved as a result of social interactions between individuals within populations, the temporal pattern of reproduction may be the ultimate consequence of interactions with *other* species. In fact, causes of reproductive synchrony are most often sought in interspecific interactions.

Predation is the oldest and probably still the most widely known explanation for reproductive synchrony[7]. In many species, early life stages are particularly vulnerable to predation. Reproductive synchrony can reduce predation on offspring in three ways[16]. First, many individuals breeding at the same time and place have a higher probability of detecting and repelling predators than solitary asynchronously reproducing adults. Collective defence of offspring is common in colonial birds, and so is reproductive synchrony[9,32]. Second, the presence of many offspring can interfere with a predator's ability to pick out a specific target (predator confusion). Third, since there will always be a limit to the number of prey that a predator can catch and handle per time unit, a sudden mass appearance of prey in the vulnerable stage may *swamp* or satiate the predator population, and thereby reduce the fraction of the prey population taken by the predators[7,33].

These three mechanisms can act singly or in symphony to form an antipredation strategy. However, predator swamping is potentially the most general mechanism, since it can explain reproductive synchrony in both animals and plants, as well as the synchrony of other risky life history transitions such as molting in crustaceans[34], metamorphosis in anurans[35] and leaf production in tropical plants[36].

Very long-lived organisms synchronizing their reproduction at supra-year intervals (e.g. cicadas and bamboos) can escape a *numerical* predator response by reproducing at intervals longer than the life span of their offspring's predators[7]. Most organisms, however, reproduce seasonally and if offspring predation is important, their reproductive strategy should be shaped more by the *functional* response of predators than by their numerical response[17].

Several types of frequency-dependent predation (predator functional response) have been identified[18,39]. Although their importance for the ecological stability of predator–prey interactions has been analysed in detail[39], the interaction between different functional responses by predators and the temporal pattern of breeding of the prey population has not until very recently[17] received a formal theoretical analysis. It can be shown that, under different degrees of reproductive synchrony, offspring predation rates are very dependent on the type of predator functional response (Fig. 6), the total reproductive output of the prey in relation to the density at which predators become satiated[17], and the spatial structure of the prey population[17].

In contrast to conventional wisdom, highly asynchronous reproduction may be the best reproductive strategy when predators have a typical generalist-type functional response[38] (Type III; Fig. 6). This is particularly so when prey switching occurs at high offspring densities (Fig. 6) and/or the satiation density of the predator is high relative to the total reproductive output of the prey population[17]. Furthermore, spatially clumped prey populations are expected to experience a dramatically increased predation rate if reproduction is synchronized *within* but not *between* clumps[17]. Hence, mechanisms that promote within-group reproductive synchrony are unlikely to have evolved as a predator swamping strategy in patchily distributed populations.

Although the predator swamping hypothesis was developed originally with animal prey (birds[7]) in mind, few empirical studies have actually tested this hypothesis for animals. Karban[13], studying periodical cicadas, found that reproductive synchrony increased as adult density increased, that avian predator density was independent of cicada density (indicating the lack of numerical response), and that as

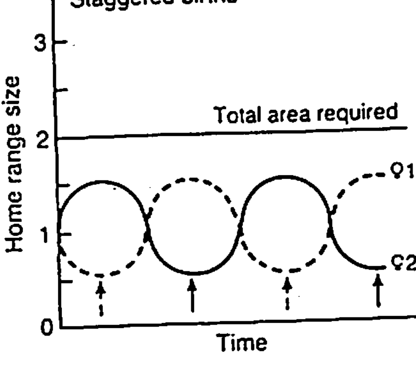

Fig. 5. A graphical model illustrating how birth synchrony may affect the space requirement for two territorial females with home range oscillations coupled to the reproductive cycle. The arrows represent the parturition dates (broken arrows, female 1; solid arrows, female 2). The degree of staggering of births (reproductive asynchrony) will be positively correlated with the amplitude of the home range oscillations and the total area required. *Based on Ref. 31 and W. McShea, pers. commun.*

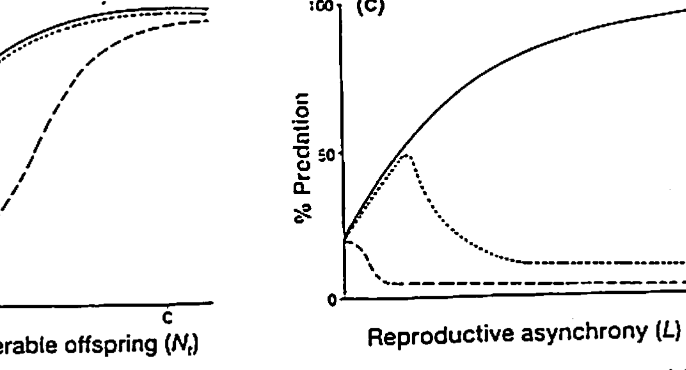
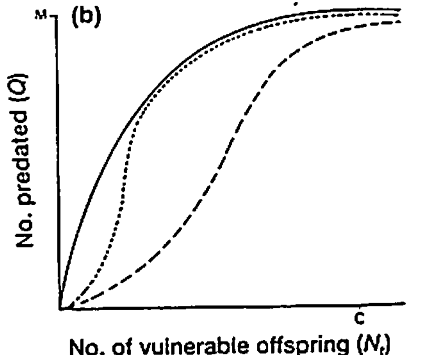
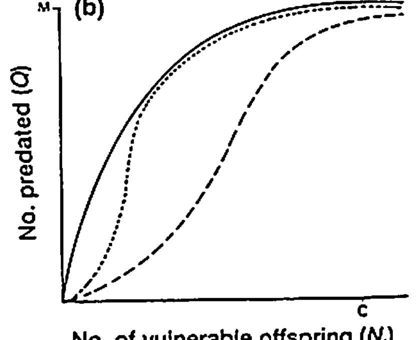
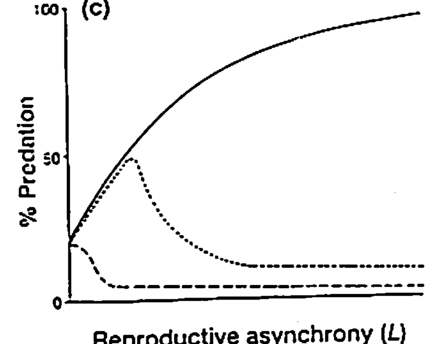

Fig. 6. Expected offspring predation rates imposed by predators with different functional responses to varying degrees of reproductive synchrony of the prey population. For simplicity it is assumed that the offspring are vulnerable to predation only during the day of their birth, that the distribution of births within the breeding season is rectangular (uniform), and that the total reproductive output (C) is constant such that the degree of synchrony and hence the number of young born per day (N_t) is a monotonically decreasing function of the length of the breeding season (L) (part a). Three predator functional response curves are considered (part b): solid curve. Type II response[36] (specialist predator); dashed curves. Type III responses[36] (generalist predators) with different prey-switching points (inflection points). All predators have the same satiation density (M) such that $Q(M) = C/5$ where $Q(M)$ is the daily predation rate of the predator population when $N_t \geq M$. The total loss of offspring due to predation resulting from the different functional responses can be calculated as $P = C^{-1} \sum_{t=1}^{L} Q(N_t)$ where $Q(N_t)$ is the daily predation rate for a given degree of reproductive synchrony. In this model a specialist predator will cause highest predation rates when reproduction is completely asynchronous (part c), whereas the predation peak will be realized at a high degree of reproductive synchrony when the predators show prey switching. Note the effect of different prey-switching points (the two dashed curves in part c) on the location of the predation peak on the reproductive asynchrony axis. *Reproduced with permission from Ref. 37.*

cicada density increased the number of cicadas taken per predator did not increase (suggesting that predators were satiated). Studies on ungulates support the predictions that predator functional responses and the spatial structure of the population are crucial factors[11,16].

For plants, a number of recent studies have examined the relationship between reproductive synchrony and predation. Furthermore, in plants there are two types of functional relationship with animals in addition to predation – seed dispersal and pollination – to which the conventional 'swamping logic' has been applied. For animal-dispersed and/or pollinated plants it is essential *not* to swamp dispersal agents or pollinators, and reproductive asynchrony rather than synchrony has been predicted to be the best strategy for maximizing visitation rates of pollinators and seed dispersers.

Recent studies[10–14] on pollination, seed predation and seed dispersal have challenged the conventional 'swamping logic' by showing that synchronous fruiting or flowering often does not swamp seed predators/dispersal agents or pollinators. Consistent with the expected effects of different functional responses[17], specialist dispersal agents/predators are more likely to be swamped by a synchronized seed set than are generalists[12,15]. Note that for plants interacting with different animals with different functional responses, conflicting selective pressures on the temporal pattern of reproduction may occur. For example, this will be the case if the pollinator behaves as a generalist while the major seed disperser behaves as a specialist. In such cases the strategy (the degree of reproductive synchrony) that provides the best compromise between the opposite selection pressures, in terms of reproductive success, is expected to evolve.

There are empirical results also indicating that the spatial pattern of plant reproduction may determine the success of reproductive synchrony as a strategy for avoiding predators or attracting dispersal agents or pollinators. Reproductive synchrony within trees or patches of plant individuals that are asynchronous with the rest of the population often causes local aggregations of mobile non-territorial seed predators/dispersers, resulting in especially high removal rates[13,10].

Conclusion

There now exists a rich array of hypotheses explaining the adaptive significance of reproductive synchrony, and it is clear that it is at least theoretically possible that synchrony can have various causes including processes both intrinsic and extrinsic to the population. However, the many factors that potentially may be involved in the timing of reproduction complicate the analysis, and it is important to be aware that any observed pattern of reproduction may reflect the combined selective pressures from multiple factors. The recent theoretical studies reviewed above, showing that the same process (e.g. predation) may select for or against reproductive synchrony depending on the ecological setting, demonstrate that even single-factor hypotheses may not always lead to straightforward predictions. Obviously, more theoretical work is needed to evaluate the different

Fig. 7. Example of an experimental manipulation of the temporal pattern of flowering in a naturally synchronous-flowering shrub[45]. Flowering times of individual flowers (horizontal lines) are shown for three naturally synchronous populations (1,2,3), one experimentally induced synchronous population (C) and two experimentally induced asynchronous populations (A,B). Flowering in this species is induced naturally by heavy rain showers in the dry season. However, flowering can be induced at an earlier time by artificial watering. *Reproduced with permission from Ref. 45.*

hypotheses on reproductive synchrony. In particular, it is important to understand how reproductive synchrony at different spatial scales may affect ecological and sociobiological interactions.

However, there is an even stronger need for empirical tests. Although observational studies followed by multivariate statistical analyses may be valuable in assessing the relative importance of different variables for the temporal pattern of reproduction[16,17], an experimental approach is likely to be more rewarding. Recent experimental studies on plants[42-45] have shown that it is possible to manipulate the temporal pattern of reproduction within populations (Fig. 7) and subsequently compare the reproductive success of individuals in populations with more or less temporally clustered reproduction. In particular, experiments performed in different ecological settings, for instance on populations with different spatial structuring and predators, will be essential to advance further our understanding of the ecology and evolution of reproductive synchrony.

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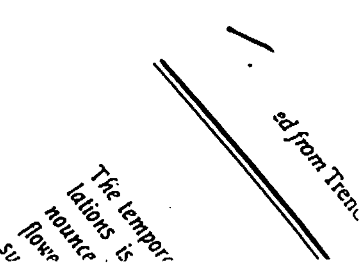

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Brief Technical Note

SEXUAL DIMORPHISM IN TILAPIAS

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(Accepted 13 February 1983)

Sexual dimorphism is known in many tilapias. The male grows larger than the female even in fish from the same spawn. During sexual activity the male, but usually not the female, develops a breeding dress. Morphological changes usually take place in the male during periods of sexual activity. In *Oreochromis aureus* male (formerly known as *Tilapia aurea* or *Sarotherodon aureus*) the dorsal fin thickens and becomes continuous instead of notched (Fishelson, 1966). In *O. mossambicus* there is enlargement of the upper jaw in large males but not in females. The male has one opening, urinogenital, on

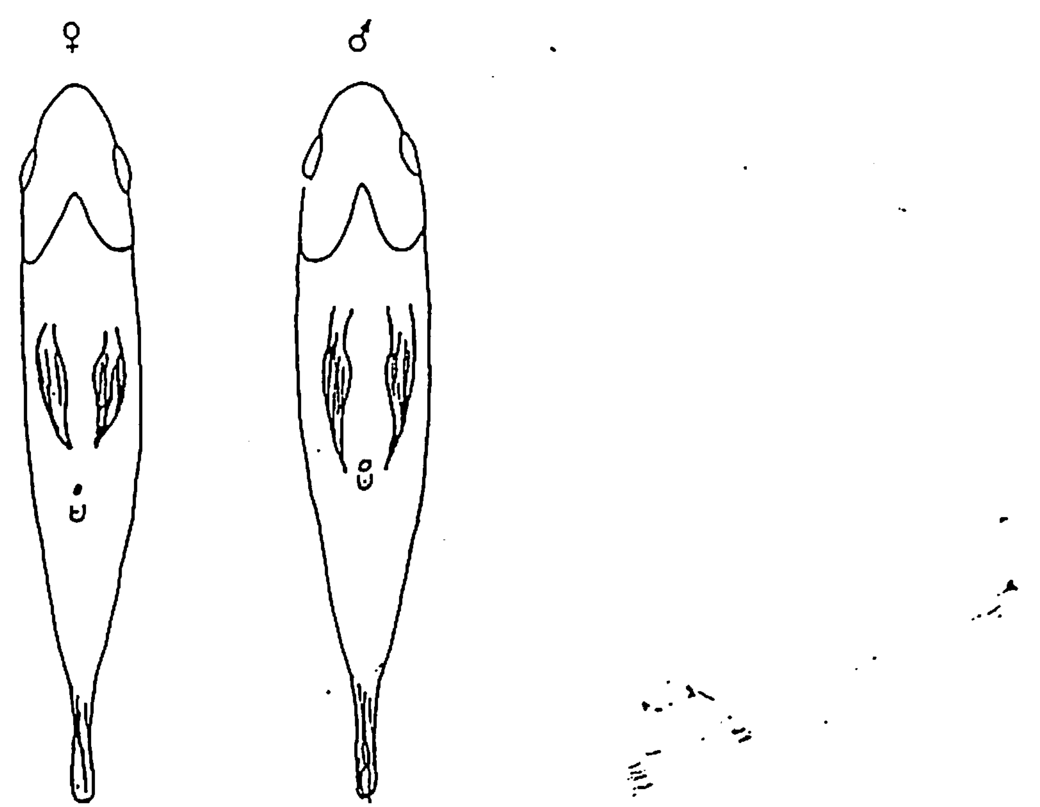

Fig. 1. Schematic drawing of the pelvic fin in males and females of *Oreochromis aureus* and *Sarotherodon galilaeus*.

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the apex of the genital papilla. The female has two openings on the genital papilla. The urinary tract empties through a small pore, whereas the genital opening is a breadth-wise slit in front of the urinary pore. In *S. galilaeus* and *O. aureus* the dorsal and anal fins are pointed in males and rounded in females (Chervinski, 1965).

Working with *O. aureus*, *S. galilaeus* and *T. zillii* it was found that sexing can be accomplished either according to genital papilla or according to the pelvic fin size.

In fish of 60 g the pelvic fin in males is usually longer than that of the females. In males the pelvic fin reaches the anus or the genital papilla, whereas in females the pelvic fin does not usually reach the anus (Fig. 1). In larger fish, 200 g, these differences are more distinct. These secondary sexual characteristics may be helpful in sexing tilapias for monosex culture.

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Prospects of Selecting for Late Maturity in Tilapia (*Oreochromis niloticus*)
II. Strain Comparisons under Laboratory and Field Conditions

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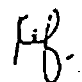

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(Accepted 6 September 1988)

ABSTRACT

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

This investigation deals with the question about the extent to which results on the genetic control of maturity with one population of *Oreochromis niloticus* (Lake Manzala) under laboratory conditions can be generalized. In particular the following two main problems were studied:
(1) The validity of the genetic parameters measured under laboratory conditions for commercial production conditions in the tropics.
(2) The range of strain variability in gonadal development and growth performance.
A representative outbred sample of the reference strain *O. niloticus* Lake Manzala tested under laboratory conditions was subjected to a full-sib family test under commercial farming conditions at the Baobab Farm, Mombasa, Kenya. The testing procedure was organized in an analogous way to that under laboratory conditions, with slight adaptations to local conditions. Despite significantly slower growth under tropical conditions, there was no indication of greater differences in the genetic parameters. Genetic variability turned out to be as high under tropical as under laboratory conditions. Other populations (strains) tested under either laboratory or field conditions also showed very similar genetic structure in the two main traits of concern.

Under both laboratory and field conditions, significant differences between strains were evident, particularly in gonadal development. Some of the strains included matured so late that the variability of the onset of maturity could not be sufficiently analysed by the standard testing procedure applied.

According to findings up to now, there is no indication that breeding results obtained under laboratory conditions cannot be directly transferred to commercial production conditions in the tropics. Furthermore, the results of the strain comparison clearly show the need for a very careful selection of the basic population when starting up breeding programmes for late-maturing tilapia with high growth potential.

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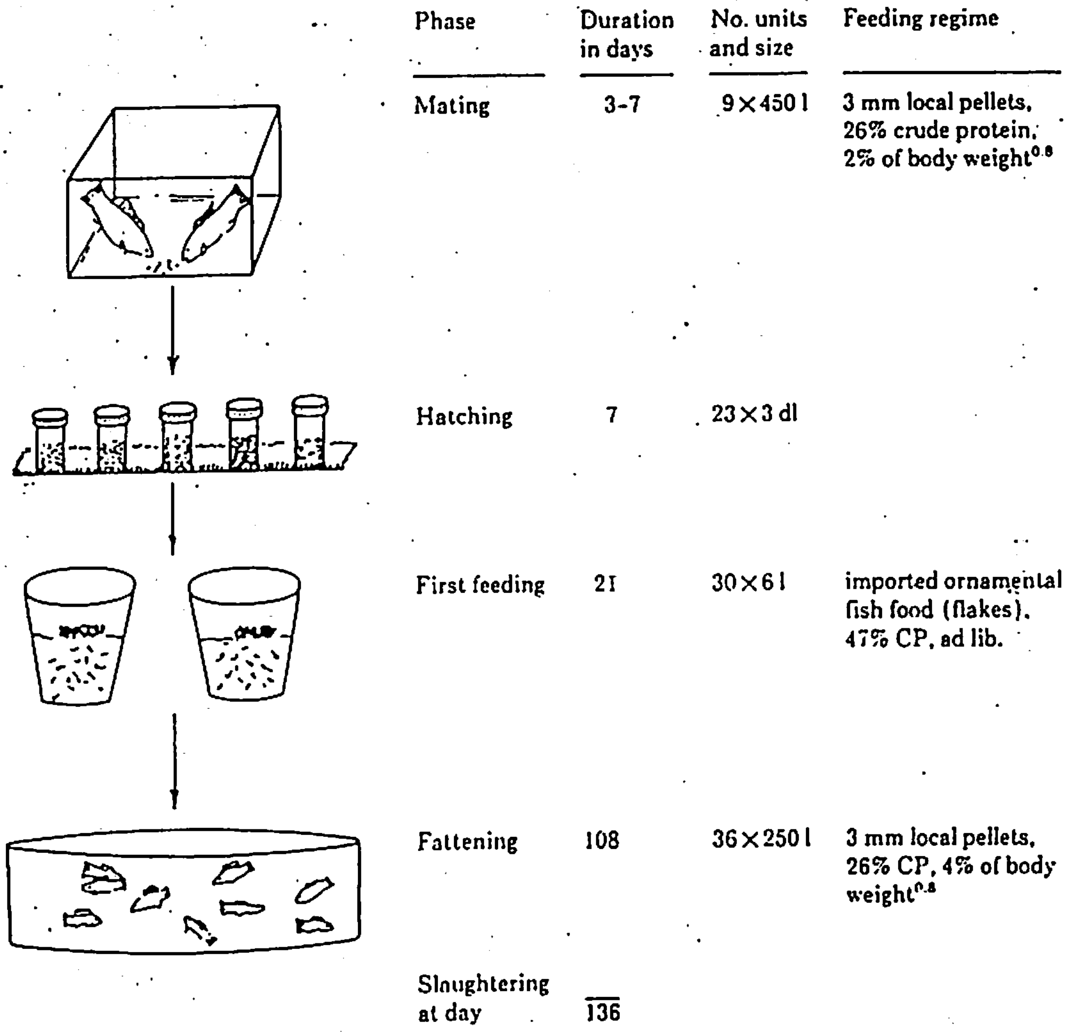


Fig. 1. Testing procedure applied under field conditions.

Despite these necessary adaptations in the testing techniques, no fundamental changes of the testing procedure were made and the length of the test period was maintained. Even though growth under the examined field conditions was to some extent retarded at day 136, a certain frequency of overripe females in the reference strain of *O. niloticus* was observed.

A classification of gonadal development was done for both sexes. Despite the known difficulties in classifying testes, the assessment of sexual maturity stages in male gonads was carried out according to the method described in Table 1, because this technique may simplify recording under field conditions.

The testing period had to be prolonged to 210 days for only one population (*O. niloticus* El Molo, see Material), because the first occurrence of overripe females was delayed to this age.

Continuous recording of weather parameters (temperature, humidity, rainfall, radiation) at the project site, the Baobab Farm near Mombasa, Kenya,

TABLE 1

Classification of gonadal development in males (modified after Babiker and Ibrahim, 1979)

Maturity stage	Appearance of testis
1. immature	thread like; colourless
2. inactive	translucent; wider than above
3. inactive-active	flesh colour; still thin
4. active	white/yellowish; thickened; no milt apparent when cut
5. active-ripe	cream coloured; thick and enlarged
6. ripe	distended fully over length of visceral cavity; milt evident if testis cut
7. ripe-running	white/silvery; milt runs freely under pressure

TABLE 2

Means and standard deviations of the water quality parameters at the project site in Kenya

Parameter	$\bar{x}$	s
Temperature (°C)	26	1.0
Salinity (%)	0.2	0.1
O_2 (%)	85.0	1.0
NH_4^+ (mg/l)	0.0	0.0
NO_2^- (mg/l)	0.0	0.0
NO_3^- (mg/l)	0.0	0.0
pH	7.5	0.1
Total hardness (mg/l)	510	0.2
Alkalinity (mg/l)	180	0.3

revealed nearly no climatic influence on water quality, as can be seen in Table 2. Thus the seasonal effect of the quality of water on the test results should be neglected. However, to ensure that all environmental bias is ruled out, the weekly test batches were composed of families representing all populations simultaneously, as a rule. More details on testing environment and the experimental implementation are given by Oldorf (1987).

Material

In addition to the reference strain, two additional populations were tested under field as well as under laboratory conditions. The stock for the repeated test of the reference strain under field conditions was taken from 10 full-sib families bred under laboratory conditions in Göttingen, which were transferred as fry to the Baobab Farm and raised there. In total 424 of these fish formed the parental stock.

The populations additionally tested under the two test environments were the following:

Laboratory test Göttingen:
O. niloticus originating from fish ponds near Bangkok, Thailand. This population was obtained from the Agricultural Research Organisation, Israel. The parental stock consisted of 400 fish derived from eight full-sib families.
O. niloticus originating from the Central African Republic and Zaire (Mélard and Philippart, 1980). This population was obtained from the Institute de Zoologie de l'Université de Liège. A sample of 60 fish of unknown pedigree was used as parents.

Field test Mombasa:
O. niloticus originating from Lake Baringo, Kenya. A sample of this population was caught in 1976 in the wilderness and since then kept at Baobab Farm. A random sample of 429 fish derived from eight families constituted the parental stock.
O. niloticus originating from El Molo Bay at Lake Turkana. Staff from the Baobab Farm collected this population in the wilderness in 1978. Some 650 fish from 11 full-sib families were randomly taken to serve as parents.

All spawners were individually marked so as to have controlled mating on a single-pair basis and to avoid sib mating. The total numbers of full-sib families for all populations tested, as well as the average group size of full-sib families and their sex ratio, are given in Table 3.

Statistical analysis

As in part one of this study, statistical evaluation was based on LSQ procedures (Harvey, 1977). Each population and each sex was analysed separately.

TABLE 3

Numbers and average size of full-sib families, and sex ratio for populations tested

	Full-sib families		Sex ratio
	Number	Average size	
Field test Mombasa:			
O. niloticus Manzala (reference strain)	23	105	1:1.08
O. niloticus Baringo	18	82	1:1.11
O. niloticus El Molo	16	111	1:1.17
Laboratory test Göttingen:			
O. niloticus Bangkok	25	74	1:1.28
O. niloticus CAR/Zaire	13	75	1:0.53

For calculation of heritabilities and genetic correlations, which in this case was restricted to a full-sib analysis, all data had to be transformed (logarithm) to assure a normal distribution.

RESULTS

Field test of reference strain

Contrary to the laboratory test, the field test did not show an unbalanced sex ratio for the reference strain as can be seen from Table 3.

For a direct comparison between the performance of the reference strain under field and under laboratory conditions, overall means, standard deviations and coefficients of variation for all traits studied are given in Table 4.

Under field conditions in both sexes growth was significantly retarded, whereas variability in all traits turned out to be of the same scale. Growth reduction was much more pronounced for the females, which attained only 55% of the male weights under field conditions as compared to 70% under laboratory conditions.

Reduction in gonad weight corresponded directly to the reduction in body weight, as can be seen from the GSI mean values, which do not show any difference between the two test sites. As already indicated, with reduced gonad weight the average maturity stage in females is significantly lower. Slightly more than 50% of the females did not enter the maturing phase at all, even though the incidence of overripe females was at least equal to that under the laboratory test conditions (Table 5). In males the visual assessment of gonadal

TABLE 4

Number of observations (N), overall means ($\bar{x}$), standard deviations (s) and coefficients of variation (CV) for all traits studied in the reference strain at both experimental sites

Trait	Body weight			Gonad weight			Gonadosomatic index*			Maturity stage		
	$\bar{x}$	s	CV	$\bar{x}$	s	CV	$\bar{x}$	s	CV	$\bar{x}$	s	CV
Males												
Laboratory (N=4028)	28.8	10.5	37	0.18	0.20	11	0.56	0.53	95	-	-	-
Field (N=1165)	20.3	7.8	38	0.11	0.11	100	0.56	0.50	89	5.30	1.50	28
Females												
Laboratory (N=2541)	22.4	6.8	31	0.21	0.30	143	0.88	1.15	131	2.87	1.37	48
Field (N=1254)	12.3	5.1	42	0.10	0.15	150	0.80	0.89	111	1.75	1.10	63

*$GSI = \frac{\text{gonad weight}}{\text{body weight}} \times 100$.

TABLE 5

Distribution of females (%) according to stage of gonadal development for all populations studied

Maturity stage	Population				
	Field conditions			Laboratory conditions	
	Reference strain	Baringo	El Molo	Bangkok	CAR/Zaire
1	53.6	43.6	74.4	71.8	41.5
2	31.5	33.6	20.4	15.6	16.1
3	5.8	9.6	2.3	8.6	17.8
4	4.9	6.7	0.7	3.4	13.6
5	3.3	4.4	2.1	0.3	8.5
6	0.9	2.1	0.1	0.3	2.5

TABLE 6

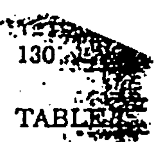
Distribution of males (%) according to their stage of gonadal development

Maturity stage	Population		
	Reference strain	Baringo	El Molo
1	2.7	2.3	3.9
2	2.7	4.9	27.2
3	7.9	11.2	24.2
4	24.1	23.9	26.1
5	18.2	16.9	10.2
6	13.6	13.8	4.5
7	33.4	27.1	3.9

development indicated that close to 50% of the fish were sexually mature (Table 6).

Heritability values in Table 7 indicate that also under field conditions there is a clear genetic control of growth and gonadal development. Heritabilities of gonadal development correspond very closely to those observed under laboratory conditions, the heritability estimates for maturity stage being slightly lower under field conditions. Heritability estimates for growth are clearly lower, but still statistically highly significant.

Phenotypic and genetic correlations were estimated but, because of their high standard errors, the actual values are not tabulated. A tendency towards the same relationship between body weight and the traits of gonadal development as seen under laboratory conditions can be observed. While the genetic



TABLE

Heritabilities for all traits studied in the reference strain at both experimental sites

		Gonad weight		GSI		Maturity stage		Body weight	
		h^2	s	h^2	s	h^2	s	h^2	s
Laboratory conditions (pooled full-sib analysis)	♂	0.23	0.06	0.35	0.08	-	-	0.60	0.11
Field conditions (full-sib analysis)	♂	0.17	0.06	0.42	0.11	0.10	0.04	0.39	0.10
Laboratory conditions (pooled full-sib analysis)	♀	0.34	0.09	0.42	0.11	0.57	0.13	0.70	0.14
Field conditions (full-sib analysis)	♀	0.36	0.10	0.50	0.12	0.35	0.10	0.34	0.09

correlation between body weight and maturity stage in females is zero or slightly negative, there seems to be a clear positive genetic correlation between body weight and maturity stage in males.

Strain comparisons under field and laboratory conditions

Differences between populations in patterns of gonadal development have been observed in both tested environments, as illustrated in Table 5. Under field conditions the El Molo population and under laboratory conditions the Bangkok population reach maturity distinctly later, showing more than 70% of females with no gonadal development at all at the time of test slaughter, even though for the El Molo population the testing period was prolonged by 69 days. In males a corresponding diversity of gonadal development can be observed between populations, proving El Molo to be a very late maturing population.

For the additional populations, mean performance and its variation are listed in Table 8. For all populations tested and in all environments, the absolute gonadal weights are low and very similar. This means that the differences in the GSI values, which can be observed to some extent between populations, are predominantly due to differences in body weight.

Estimates of heritability for the additional populations are tabulated in Table 9. There is no indication of great differences in heritability between populations. However, lower heritabilities for growth under field conditions, as observed in the reference strain, were not found. Due to limited numbers of observations the actual figures for heritability estimates are of limited reliability.

Estimates of phenotypic and genetic correlations confirm the pattern in the relationship between body weight and gonadal development described earlier, with one exception. The correlation between body weight and gonadal devel-

TABLE 8

Overall means ($\bar{x}$), standard deviations (s) and coefficients of variation (CV) for all traits studied in additional populations tested under field or laboratory conditions

Population		N	Gonad weight (g)			GSI			Maturity stage			Body weight (g)		
			$\bar{x}$	s	CV (%)	$\bar{x}$	s	CV (%)	$\bar{x}$	s	CV (%)	$\bar{x}$	s	CV (%)
Field conditions														
Baringo	♂	698	0.10	0.11	110	0.73	0.59	81	5.0	1.7	34	16.6	11.1	67
El Molo	♂	823	0.10	0.05	50	0.23	0.14	61	3.4	1.4	41	33.0	16.9	51
Lab. conditions														
Bangkok	♂	815	0.09	0.12	133	0.27	0.29	107	-	-	-	27.0	12.1	45
CAR/Zaire	♂	641	0.10	0.09	90	0.29	0.24	83	-	-	-	31.1	11.2	36
Field conditions														
Baringo	♀	778	0.10	0.14	140	1.31	1.23	90	2.0	1.2	60	9.0	5.9	66
El Molo	♀	963	0.10	0.11	110	0.41	0.43	105	1.4	0.8	55	17.7	7.6	43
Lab. conditions														
Bangkok	♀	1040	0.09	0.18	200	0.34	0.56	164	1.7	1.1	65	23.8	10.9	46
CAR/Zaire	♀	337	0.11	0.16	146	0.41	0.58	142	2.3	1.3	56	25.4	8.8	35

TABLE 9

Heritabilities (h^2) and standard errors (s) for all traits studied in additional populations tested under field or laboratory conditions

Population		Gonad weight		GSI		Maturity stage		Body weight	
		♂	♀	♂	♀	♂	♀	♂	♀
Field conditions									
Baringo	h^2	0.36	0.40	0.44	0.90	0.21	0.43	0.62	0.93
	s	0.11	0.12	0.13	0.18	0.08	0.13	0.16	0.18
El Molo	h^2	0.06	0.04	0.44	0.43	0.48	0.23	0.66	0.58
	s	0.04	0.03	0.13	0.13	0.14	0.08	0.17	0.16
Lab. conditions									
Bangkok	h^2	0.02	0.05	0.01	0.04	n.r.	0.09	0.38	0.26
	s	0.04	0.05	0.04	0.05	-	0.07	0.19	0.14
CAR/Zaire	h^2	1.08	+	0.86	+	n.r.	+	0.75	+
	s	0.36		0.36		-		0.34	

n.r. = not recorded.
+ = no calculation possible due to insufficient numbers of females within families.

opment in females of the late-maturing El Molo populations tended to be as positive as in males. Again, because of the high standard errors, no absolute figures are given.

DISCUSSION AND CONCLUSION

The successful repetition of the testing procedure under commercial conditions in the tropics has shown that the concept of family breeding can also be applied under the less favourable conditions of developing countries, even though a certain reduction in accuracy of testing has to be expected due to necessary adaptation and simplification of the testing procedure. Since the main objective was to obtain sufficiently reliable estimates of the genetic variability in growth and gonadal development, a testing procedure as similar as possible to the one applied under laboratory conditions was employed for the repeated test of the reference strain under field conditions.

Under field conditions genetic variability of the strains studied was as high as under laboratory conditions. The heritability values also remained within a medium range. Therefore a similar pattern of genetic control can be assumed. Thus, selection results obtained under laboratory conditions can most likely be directly transferred to field conditions in the tropics, although a direct proof cannot be drawn from this investigation. The lower heritability values estimated for the body weight of the reference strain under field conditions have to be seen in connection with the lower growth performance. The poorer growth performance under tropical field conditions was most likely caused by the low quality of the pelleted food and the unavoidable denaturalisation processes in storage. These environmental factors should make up for genetic differences in growth potential to some extent. The more pronounced sexual dimorphism under field conditions might be due to the lower stocking density and the special tank shape with a proportionately larger surface at the bottom, both of which favour territorial behaviour of the males. Furthermore, the retarded growth resulted in a corresponding retardation of gonadal development. Thus, at day 136 the fish examined were generally at an earlier stage of maturity than those examined under laboratory conditions. However, even at this stage the full genetic variability could be observed. The results indicate that the testing period should be prolonged to ensure a higher body weight in females and a higher proportion of mature animals. Further investigations should clarify at what stage, with regard to weight and age, the testing period should be terminated in order to obtain a balanced variation in gonadal development without having a higher proportion of overripe females.

The results of the family studies with different strains in the two testing environments do not indicate any major differences in the pattern of the quantitative genetic control of growth and maturation. Thus, in setting up breeding

programmes for a given population, genetic parameters which have been estimated with sufficient accuracy in other populations can be used. For actual breeding programmes, the genetic parameters obtained from half-sib analyses of the reference strain under laboratory conditions should be applied until more specific estimates on genetic parameters of corresponding accuracy are available.

The much more important result from the strain comparison is the observed strain difference in growth and especially in gonadal development. Obviously strains can be found which are much later maturing, e.g. the El Molo strain. Further investigations will have to prove the overall potential of this strain under technically improved environmental conditions. However, even at this stage one can conclude that the selection of the foundation stock deserves our highest attention when establishing breeding programmes.

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GENETIC VARIATION IN QUANTITATIVE TRAITS AND SELECTIVE BREEDING IN FISH AND SHELLFISH

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ABSTRACT

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Before selection programmes can be started, breeding objectives must be defined exactly. In most species, traits of high economic importance are: growth rate, food conversion efficiency, resistance to disease, meat quality and age at maturation.

Estimates of standard deviation and heritability for important traits in several species are listed. Rather few estimates are based on extensive data, and many estimates are missing. Nevertheless, it is concluded that the possibilities for improvement through selection are particularly good for growth rate and age at maturation, because of large genetic variance and because high fecundity makes strong selection possible. For meat quality and disease resistance genetic variance is quite low, and therefore slower selection response is expected.

Sixteen selection experiments are reviewed. Selection was practised for growth rate and disease resistance in 12 and four experiments, respectively. Positive response was obtained in 15 of these experiments. These responses to selection strongly support the conclusion that possibilities for genetic improvement are good in fish.

Selection programmes based on combined individual and family merit are shown to be most efficient. It is recommended to establish breeding stations to carry out selection programmes. High priority should be given to the estimation of phenotypic and genetic parameters necessary to construct selection indexes.

There is a strong need to initiate and develop national selection programmes for fish and shellfish to raise productivity within the industry.

INTRODUCTION

In farm animals and plants selection and hybridization have played important roles in domestication, increasing yield, survival rate and improving product quality. Many examples could be given to show large improvements in production traits in a number of species. Selection for additive gene effects aims at increasing frequencies of favourable genes for the characters in question. Reciprocal recurrent selection aims to in-

crease heterozygosity and the number of favourable gene combinations which can be utilized through crossbreeding. During the last decades selection has been made more efficient by new technology and better selection methods. Thus the genetic gain obtained is much larger today compared with only a few years ago.

In aquaculture, selection programmes are not commonly used by the industry. This is surprising because of the economic importance which aquaculture has reached in many countries. There is no obvious reason for this. Fish and shellfish seem to be little different from farm animals and plants in response to selection and hybridization effects. One reason for selective breeding not being commonly used in aquaculture may be found in the education of researchers, advisory personnel and fish farmers. Education in fish biology involves little attention to selective breeding and quantitative genetics. The majority of published papers discussing genetics in aquaculture deal with genetic variation in single gene characters such as blood groups and protein polymorphisms (see for example Wilkins, 1981; Gosling, 1982). Most frequently researchers do not even try to investigate what relation such characters have to economically important traits. On the other side, aquaculture and agriculture have a close connection in many countries and research is frequently carried out by the same personnel. This approach should make the transfer of knowledge quick and easy.

In the following, a summary is given of estimates of genetic variation in quantitative traits of economic importance and response to selection for some of the species most commonly used in aquaculture. In addition, principles of breeding and selection plans are discussed. Hybridization to utilize non-additive genetic variance will not be particularly discussed here, because this topic is covered by others. However, a combination of selection and crossbreeding is frequently the optimal solution in practice.

BREEDING OBJECTIVES

Breeding objectives should be set by the industry and the consumer and should be exactly defined. Everybody involved in a coordinate selection programme should use the same objectives to avoid working in different directions. Breeding objectives are frequently different from one species to another, from one country to another and even within countries (see Mahon, 1983, this volume). In the following, only traits of common importance for most of the species under cultivation are discussed (Table I).

Food conversion efficiency

When production is based on using artificial food, the primary aim in a selection programme should usually be to improve food conversion effi-

TABLE I

Traits and their relative economic importance for some species used in aquaculture

Traits	Salmonids	Warm water fish	Bivalves	Prawns
Growth rate	+++	+++	+++	+++
Meat quality	++	+	+	++
Mortality, early	+	+	+	+
Mortality, late	++	++	++	++
Age at maturation*	++	?	?	?
Fecundity	0	0	0	0

* Production of large fish.

ciency. In carnivorous species, food costs may be a very large part of the total production cost while it is of less magnitude for herbivorous animals or detritus feeders.

Being poikilothermic, fish use less energy for maintenance compared to domestic animals. It is therefore likely that genetic variation in basic metabolic rate will be less in fish than in mammals. On the other hand, most of the fish species that are at present under domestication may be under continuous stress which would raise the maintenance requirement.

Until recently, there has been no population information on food conversion efficiency suitable for genetic analysis, because of the difficulty in direct measurement of food consumption on a large scale. Kinghorn (1981) developed a system to estimate food consumption in 33 tanks at a time through consideration of energy metabolised (via oxygen consumed) and the energy component of growth. It is, however, concluded that net food conversion efficiency in young rainbow trout is heritable ($h^2 = 0.31 \pm 0.11$) and with a low coefficient of variation (CV = 6%).

Edwards et al. (1977) and Refstie and Austreng (1981) showed no significant family X diet interaction in trials involving rainbow trout on feed of various carbohydrate levels. This indicates that selection for the specific ability to utilize a cheaper, high carbohydrate food would meet with little success.

Although food conversion efficiency usually is the most important economic trait in fish farming, it can not yet be included in a selection programme because of insufficient methods of measuring it. Further development of systems for direct and/or indirect measurement of food consumed should be given high priority.

Growth rate

Growth rate is of economic importance for all species used in aquaculture. Usually it is easy to estimate through measurement of weight or body length.

Rapid growth speeds up the turnover of production and frequently large animals demand a higher price per kilogram compared with smaller ones. In farm animals, there is a high genetic correlation between growth rate and food conversion so selection for high growth rate results in a rapid correlated improvement in food conversion. Kinghorn (1981) concluded that young rainbow trout which consume more food, grow faster but are not better net converters of food.

The genetic correlation between body weight and length in salmonids is close to unity (Refstie and Steine, 1978; Gunnes and Gjedrem, 1978, 1981; Refstie, 1980) suggesting that one should use the trait with the highest heritability to measure growth rate, although body weight must be considered as the breeding objective.

Mortality/resistance to disease

Mortality in fish and shellfish is of considerable economic importance to the farmer. Since egg and young individuals are very cheap, mortality is of particular importance for ongrowing fish and shellfish. Mortality is, however, a very complex trait. The cause may be different from one farm to another, and from one year to another. Mortality is therefore a rather difficult trait to work with and heritability is commonly very low (Table II). Death rate is, however, usually the only way to measure this economically important trait.

Measuring resistance to specific diseases may give more scope for improvements (Gjedrem and Aulstad, 1974; McIntyre and Amend, 1978). A possible way of measuring resistance is to expose fish (family members) to specific disease agents and count number of deaths as done by McIntyre and Amend (1978). It is, however, a rather expensive method to practice. Indirect measure of resistance to certain diseases may be obtained by applying immunological methods such as antibody levels after vaccination.

It is believed that fish under stress are more susceptible to disease. Therefore, if one could measure the reaction to stress one could possibly speed up the domestication rate through selecting fish which better tolerate stress. Estimates of cortisol levels in the blood after a standard stress are considered to be an accurate measure of stress response (Fagerlund, 1967).

Fecundity

Fish and shellfish have usually a very high reproductive rate (salmonids have more than 1000 eggs/kg body weight, common carp and channel catfish more than 100 000/kg, tilapia 1000/kg, oysters millions/spawn). This makes the expense of brood stocks quite low. In salmonids, for example, eggs account for less than 1% of the total cost of producing large fish and less than 5% for small-portion size fish. Therefore, it is not important to consider egg number as an economically important trait in a

selection scheme. An exception could be a production system where the egg is the end product, for example caviar. In addition there seems to be a high genetic correlation between egg number and body weight (Gall and Gross, 1978a).

Meat quality

In general, aquaculture produces food for human consumption. The quality of the meat is of great importance particularly in species of luxury food. It is, however, difficult to define quality because it varies from one species to another and from one market to another. For fish it is usually a question of size, meatiness, fat percentage, meat colour, taste, shape, dressing percentage, and so forth. For some of these traits it is difficult to make objective measurements. This is true for meat colour, taste, meatiness and shape. Development of exact measurements for these traits should be considered.

Age at maturation

Several fish species must be marketed prior to sexual maturation because of reduced meat quality and high frequencies of mortalities. Particularly when producing a large fish, age at sexual maturity becomes a very important economic trait.

Recapture frequency

In sea-ranching systems, recapture frequencies are of great importance. This is, however, a complex trait where survival is an important component. In the literature very few attempts have been made to study genetic variance for this trait. Carlin (1969) found significant difference in recapture percentage after release of 17 full-sib families in the Baltic sea. Ryman (1970) analysed the data statistically.

GENETIC VARIATION IN QUANTITATIVE TRAITS OF ECONOMIC IMPORTANCE

A parameter of great importance when discussing selective breeding is the additive genetic variance (σ^2_G). The magnitude of genetic variance determines response to selection. It may be calculated by multiplying heritabilities (h^2) by the phenotypic variance (σ^2_p). Heritabilities based on sire components and regression of offspring on parent usually give unbiased estimates of additive gene effects. The difference between heritabilities estimated from dam and sire components may be taken as an estimate of non-additive genetic variance and possibly maternal effects. Heritabilities based on full-sib analysis are of less value in this connection because they contain genetic variance in the broad sense, together with maternal and common environmental effects.

TABLE II

Phenotypic and genetic parameters for quantitative traits in fish and shellfish. ($\overline{X}$ = mean, σ = standard deviation, CV = coefficient of variation, S.E. = standard error. Heritability (h^2) as estimated from sire (S), dam (D) and family (F) component of variance)

Species/traits	$\overline{X}$	σ	CV	$h^2_S \pm$ S.E.	$h^2_D \pm$ S.E. $h^2_F \pm$ S.E.	Number of families	Authors
Rainbow trout:							
150 day weight (g)	13.6	4.12	30	0.09 ± 0.10	0.06_D ± 0.09	14_S; 28_D	Aulstad et al. (1972)
150 day length (cm)	9.8	1.14	12	0.16 ± 0.14	0.06_D ± 0.09	14_S; 28_D	Aulstad et al. (1972)
280 day weight (g)	13.6	4.37	33	0.29 ± 0.20	0.01_D ± 0.05	14_S; 28_D	Aulstad et al. (1972)
280 day length (cm)	10.1	1.26	12	0.37 ± 0.23	0.03_D ± 0.04	14_S; 28_D	Aulstad et al. (1972)
180 day weight (g)		16.5			0.48_F	8_F	Møller and Naevdal (1973)
2 year weight (kg)	1.1	0.26	23		0.21_F ± 0.05	39_F	Gall (1975)
300 day weight (g)	51.0	16	32		0.13_F	19_F	Chevassus (1976)
Dead eyed eggs				0.15 ± 0.04	0.27_D ± 0.04	143_D	Kanis et al. (1976)
Dead alevins				0.14 ± 0.03	0.06_D ± 0.01	143_D	Kanis et al. (1976)
147 day weight (g)				0.06		Realized h^2	Kincaid et al. (1977)
175 day weight (g)	7.0	2.41	34		0.60_F ± 0.10	54_F	Gall and Gross (1978a)
150 day weight (g)	2.8	0.82	29		0.32_F ± 0.08	46_F	Gall and Gross (1978a)
610 day weight (kg)	0.8	0.12	15		0.74_F ± 0.10	54_F	Gall and Gross (1978a)
630 day weight (kg)	0.6	0.13	22		1.04_F ± 0.12	46_F	Gall and Gross (1978a)
2 year weight, female (kg)			17		0.50_F^{**}	126_F	Gall and Gross (1978b)
2 year weight, male (kg)			20		0.31_F^{**}	126_F	Gall and Gross (1978b)
Survival of eyed eggs			20		0.23_F	126_F	Gall and Gross (1978b)
125 day weight (g)					1.06_F ± 0.49	6_F	Klupp (1979)
334 day weight (kg)					0.82_F ± 0.38	6_F	Klupp (1979)
140 day weight (g)	29.7	16.6	56	0.06	1.04_D^{**}	34_S; 41_D	Refstie (1980)
140 day length (cm)	12.5	2.4	19	0.20	0.63_D	34_S; 41_D	Refstie (1980)
2½ year weight (kg)	3.0	0.9	30	0.17*	0.24_D^{**}	34_S; 115_D	Gunnes and Gjedrem (1981)
2½ year length (cm)	56.3	5.6	10	0.23*	0.20_D^{**}	58_S; 115_D	Gunnes and Gjedrem (1981)
Growth rate (%/day)	1.63	0.31	19	0.26 ± 0.12		34_S	Kinghorn (1981)
Food consumed (%/day)	1.37	0.27	20	0.41 ± 0.13		34_S	Kinghorn (1981)
Net food conversion	1.46	0.08	6	0.31 ± 0.11		34_S	Kinghorn (1981)

TABLE II (continued)

Species/traits	$\overline{X}$	σ	CV	$h^2_S \pm$ S.E.	$h^2_D \pm$ S.E. $h^2_F \pm$ S.E.	Number of families	Authors
Fat %	9.71	1.00	10	0.47 ± 0.34		13_S	Kinghorn (1981)
Oxygen consumption	3.73	0.68	18	0.51 ± 0.12		34_S	Kinghorn (1981)
2½ year weight (kg)	2.8	0.7	26	0.17 ± 0.11	0.39_D ± 0.11	56_S; 108_D	Gjerde and Gjedrem (1983)
2½ year gutted weight (kg)	2.3	0.6	26	0.19 ± 0.12	0.40_D ± 0.11	56_S; 108_D	Gjerde and Gjedrem (1983)
2½ year length (cm)	54.6	4.6	8	0.11 ± 0.11	0.44_D ± 0.12	56_S; 108_D	Gjerde and Gjedrem (1983)
Dressing percent	82.3	4.5	6	0.01 ± 0.05	0.14_D ± 0.07	56_S; 108_D	Gjerde and Gjedrem (1983)
Carcass quality score	3.6	0.7	20	0.14 ± 0.06	0.07_D ± 0.05	56_S; 108_D	Gjerde and Gjedrem (1983)
Flesh colour, score	3.4	0.8	23	0.06 ± 0.08	0.28_D ± 0.09	56_S; 108_D	Gjerde and Gjedrem (1983)
Age at maturity				0.18 ± 0.07	0.09_D ± 0.05	56_S; 108_D	Gjerde and Gjedrem (1983)
Atlantic salmon							
Tolerance to vibriosis				0.12 ± 0.05	0.07_D ± 0.05	42_S; 140_D	Gjedrem and Aulstad (1974)
180 day weight (g)				0.15 ± 1.00	0.21_D ± 1.00_D		Naevdal et al. (1975)
2 year length (cm)				0.27 ± 0.36	0.07_D ± 0.36_D		Naevdal et al. (1975)
Dead eyed eggs				0.05 ± 0.04	0.62_D ± 0.05	361_D	Kanis et al. (1976)
Dead alevins				0.04 ± 0.01	0.15_D ± 0.01	361_D	Kanis et al. (1976)
Dead fry				−0.02 ± 0.01	0.11_D ± 0.02	144_D	Kanis et al. (1976)
3½ year weight (kg)				0.34	0.34_D		Naevdal et al. (1976)
3½ year length (cm)				0.76	0.84_D		Naevdal et al. (1976)
Age at maturity				1.00	0.67_D		Naevdal et al. (1976)
190 days weight (g)	7.8	5.9	76	0.08**	0.15_D	28_S; 29_D	Refstie and Steine (1978)
190 day length (cm)	8.2	1.9	23	0.12*	0.17_D	28_S; 29_D	Refstie and Steine (1978)
3½ year weight (kg)	5.6	1.6	28	0.31**	0.31_D^{**}	107_S; 214_D	Gunnes and Gjedrem (1978)
3½ year length (cm)	78	6.9	9	0.28*	0.30_D^{**}	107_S; 214_D	Gunnes and Gjedrem (1978)
3½ year weight (kg)	5.7	1.4	25	0.44 ± 0.11	0.54_D ± 0.08	105_S; 248_D	Gjerde and Gjedrem (1983)
3½ year gutted weight (kg)	5.0	1.2	25	0.44 ± 0.11	0.53_D ± 0.07	105_S; 248_D	Gjerde and Gjedrem (1983)
3½ year length (cm)	80	5.7	7	0.35 ± 0.10	0.60_D ± 0.08	105_S; 248_D	Gjerde and Gjedrem (1983)
Dressing percent	90	3.1	4	0.03 ± 0.02	0.02_D ± 0.02	105_S; 248_D	Gjerde and Gjedrem (1983)
Carcass quality score	3.8	0.7	19	0.16 ± 0.05	0.14_D ± 0.03	105_S; 248_D	Gjerde and Gjedrem (1983)
Flesh colour score	3.6	0.6	16	0.01 ± 0.03	0.17_D ± 0.04	105_S; 248_D	Gjerde and Gjedrem (1983)
Age at maturity				0.42 ± 0.08	0.15_D ± 0.03	105_S; 248_D	Gjerde and Gjedrem (1983)

TABLE II (continued)

Species/traits	$\bar{X}$	σ	CV	$h^2_S \pm$ S.E.	$h^2_D \pm$ S.E. $h^2_F \pm$ S.E.	Number of families	Authors
Pacific salmon							
Tolerance to IHN				0.32			McIntyre and Amend (1978)
Carp							
Weight of fingerlings				0.10 ± 0.20			Kirpichnikov (1972)
Body weight				0.25			Smisek (1979)
Dry matter				0.15 ± 0.18			Smisek (1979)
Fat content				0.14 ± 0.15			Smisek (1979)
N in dry matter				0.15 ± 0.17			Smisek (1979)
4 month weight (g)	71	17	23		0.46_F	9_F	Nagy et al. (1980)
4 month weight in lab (g)					0.12_F	9_F	Nagy et al. (1980)
Tolerance to hypoxia	179	50	28		0.51_F	9_F	Nagy et al. (1980)
Weight gain (g)	366	81	22	0.47 (b)		17 off/ parents	Brody et al. (1981)
Catfish							
5 months weight (g)				0.61 ± 0.35		17_S; 34_D	Reagan et al. (1976)
5 months length (cm)				0.12 ± 0.30		17_S; 34_D	Reagan et al. (1976)
15 months weight (g)				0.75 ± 0.53		17_S; 34_D	Reagan et al. (1976)
15 months length (cm)				0.67 ± 0.57		17_S; 34_D	Reagan et al. (1976)
48 week weight, female (g)	237	56	24	0.52 ± 0.42	0.51_D ± 0.37	13_S; 26_D	El-Ibiary and Joyce (1978)
48 week length, female (cm)	31	2.3	7	0.81 ± 0.46	0.25_D ± 0.27	13_S; 26_D	El-Ibiary and Joyce (1978)
Dressing %, female	69	1.5	2	0.0 ± 0.20	0.47_D ± 0.36	13_S; 26_D	El-Ibiary and Joyce (1978)
Lipids %	42	3.8	9	0.08 ± 0.25	0.50_D ± 0.32	13_S; 26_D	El-Ibiary and Joyce (1978)
48 week weight, male (g)	328	90	27	0.27 ± 0.37	0.67_D ± 0.41	13_S; 26_D	El-Ibiary and Joyce (1978)
48 week length, male (cm)	33	2.6	8	0.37 ± 0.39	0.60_D ± 0.38	13_S; 26_D	El-Ibiary and Joyce (1978)
Dressing %, male	68	1.2	2	0.00 ± 0.27	0.84_D ± 0.44	13_S; 26_D	El-Ibiary and Joyce (1978)
Lipids %, male	43	3.1	7	0.00 ± 0.23	0.65_D ± 0.37	13_S; 26_D	El-Ibiary and Joyce (1978)
42 week weight (g)	53	2.4	46				Bondari (1980)
60 week weight (g)	375	11.2	30				Bondari (1980)
150 day weight (g) (1975)				0.28 ± 0.85		10_S; 20_D	Reagan (1980)
150 day weight (g) (1976)				0.0		10_S; 20_D	Reagan (1980)

TABLE II (continued)

Species/traits	$\overline{X}$	σ	CV	h^2_S ± S.E.	h^2_D ± S.E. h^2_F ± S.E.	Number of families	Authors
150 day weight (g) (1977)				0.81 ± 0.95		10_S; 20_D	Reagan (1980)
18 months weight (g)				0.41 ± 0.69		10_S; 20_D	Reagan (1980)
Fat %				0.61 ± 0.78		10_S; 20_D	Reagan (1980)
Tilapia							
90 day weight (g)	19	5.1	27	0.04 ± 0.06	0.04_D ± 0.08	16_S; 32_D	Tave and Smitherman (1980)
90 day length (cm)	99	0.8	8	0.06 ± 0.06	-0.02_D ± 0.07	16_S; 32_D	Tave and Smitherman (1980)
42 week weight, male (g)	187	52	28				Bondari (1980)
42 week weight, female (g)	116	37	32				Bondari (1980)
60 week weight, male (g)	676	148	22				Bondari (1980)
60 week weight, female (g)	363	75	21				Bondari (1980)
Oysters							
18 months weight (g)	30				0.33_F ± 0.19	11_F	Lannan (1972)
18 months meat weight (g)	63				0.37_F ± 0.06	11_F	Lannan (1972)
Larval survival (%)	35				0.31_F ± 0.06	11_F	Lannan (1972)
Setting success %	45				0.09_F ± 0.08	11_F	Lannan (1972)
Larval growth				0.24			Longwell (1976)
6 day growth				0.33	0.43_F	8_S	Newkirk et al. (1977)
16 day growth				0.50	0.60_F	8_S	Newkirk et al. (1977)
7 day length				0.44 ± 0.21		8_S	Losee (1978)
6 weeks length				0.50 ± 0.30		5_S	Losee (1978)
Blue mussel							
16 day length				0.16*	0.29_F	6_S; 36_F	Innes and Haley (1977)
Growth rate				0.12	0.62_F		Newkirk (1980)
Length of larvae	169	8	5	0.43			Newkirk (1980)
Fresh water prawn							
311 day weight unsexed (g)	2.3			0.16 ± 0.14	0.15_D ± 0.15	16_S; 14_D	Malecha (1983, personal commun.)
311 day weight, male (g)	2.2			-0.14 ± 0.25	0.19_D ± 0.19	16_S; 14_D	Malecha (1983, personal commun.)
311 day weight, female (g)	2.4			0.35 ± 0.30	0.35_D ± 0.28	16_S; 14_D	Malecha (1983, personal commun.)
Lobster							
100 day weight (g)					0.33_F	12_F	Hedgecock et al. (1976)
90 day weight (g)					0.38_F	9_F	Hedgecock and Nelson (1978)

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

TABLE III

Average values for coefficients of variation (CV) and heritabilities based on sire component calculated from figures given in Table II for economically important traits. Superscripts give number of estimates involved

Economically important trait	Rainbow trout		Atlantic salmon		Common carp		Channel catfish		Tilapia		Oysters		Prawns	
	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2
Body weight, juveniles	33^{7}	0.12^{4}	78^{1}	0.08^{1}		0.15^{1}	46^{1}	0.42^{4}	26^{1}	0.04^{1}		0.36^{3}		0.12^{3}
Body weight, adults	22^{7}	0.17^{3}	27^{3}	0.36^{3}	22^{1}	0.36^{2}	27^{3}	0.49^{4}						
Body length, juveniles	14^{3}	0.24^{3}	23^{1}	0.14^{2}				0.12^{1}	8^{1}	0.06^{1}		0.47^{2}		
Body length, adults	9^{2}	0.17^{2}	8^{2}	0.41^{4}			8^{2}	0.61^{3}						
Mortality/ resistance		0.14^{1}		0.11^{4}	28^{1}									
Carcass traits:														
Meatiness	20^{1}	0.14^{1}	19^{1}	0.16^{1}										
Meat colour	23^{1}	0.06^{1}	16^{1}	0.01^{1}										
Fat-%	10^{1}	0.47^{1}				0.14^{1}	8^{2}	0.23^{3}						
Dressing-%	6^{1}	0.01^{1}	4^{1}	0.03^{1}			2^{2}	0.00^{3}						
Age at maturation		0.18^{1}		0.71^{2}										

The coefficient of variation (CV) is a useful parameter for judging the magnitude of variance. It expresses the ratio of phenotypic standard deviation to the mean of the trait in question (CV = $(\sigma_p/\bar{x})$ 100). CV may be misleading for traits recorded as percentage because it is so dependent on the average frequency.

In Table II some estimates of phenotypic and genetic parameters found in the literature are listed. The estimates are grouped by species. It has not been possible to fill in all the columns because of lack of information. It is surprising how many authors do not give simple averages and standard deviations for the traits studied. Heritability estimates based on less than five sires or five full-sib groups were considered to be of little value and are not included. It would have been desirable to set the limit much higher, but then very few estimates would have been left, except for salmonids.

A few general comments can be made about the estimates in Table II. Many of them are based on few families and have large errors. Since so few estimates for each trait per species are available, the actual estimates given by authors have been listed. Few attempts have been made to estimate the magnitude of non-additive genetic variance. However, looking at body weight, there are five estimates from sire components which are higher than estimates based on dam components, three estimates are equal and nine dam estimates are higher than the corresponding sire estimate. It may be concluded that there is some non-additive genetic variance or maternal and common environment variance in growth rate of fish.

In Table III average values are summarized for CV and heritabilities estimated from the sire component of variation. It would have been more correct to weight each estimate by the inverse of its standard error, but since several of the estimates do not have standard errors this could not be done. It is not advisable to use the estimates presented in Table III for practical purposes, because parameters should be estimated from data recorded under similar environmental conditions where selection shall take place. However, if no estimate is available for the population in question, the estimates given in Table III provide a good guide to the likely relative values.

From Table III the following conclusions can be drawn:

(a) Only a few estimates of CV and heritability are available for each trait. Atlantic salmon and rainbow trout are the only species which have estimates for most of the traits in question. For Pacific salmon only one estimate of heritability is given, namely for disease resistance (Table II).

(b) Coefficients of variation are very high for body weight for all species, and higher for young than for older animals. CV for body length is quite low.

(c) Heritability for body weight of juveniles is rather low with the exception of channel catfish and oysters. Heritability for body weight of adults is of medium size and higher than for young animals.

(d) Body length shows varying heritability from low to medium in large Atlantic salmon and small oysters, and high in channel catfish.

(e) The high phenotypic variance of body weight of adults together with medium size heritability means that there are large genetic effects for this trait for all species with estimates.

(f) Mortality shows low heritability while resistance to specific diseases shows medium to high heritability.

(g) Meat quality traits show some genetic variation. An exception is dressing percentage which shows low genetic variance.

(h) Age at sexual maturation shows low heritability in rainbow trout compared with Atlantic salmon.

RESPONSE TO SELECTION

Response to selection usually gives the most valuable information about the possibility for genetic improvement. However, many selection experiments utilize very limited numbers of animals resulting in inbreeding and thus ruin the experiment.

Common carp

Common carp have been farmed for thousands of years and have become well adapted to the pond environment. Schäperclaus (1961) reports that farmers of Western Europe have been selecting carp for growth rate for many years and a lot of distinct breeds have been developed through inbreeding. Soviet fish breeders have followed the same procedures to improve growth rate and resistance to disease (Kirpichnikov, 1972).

Israeli scientists have for several years worked with selection experiments. Moav and Wohlfarth (1973) summarise the results from several small experiments as follows:

(a) Single brother—sister mating is followed by a high degree of inbreeding depression.

(b) Selecting for slower growth rate yields a response.

(c) Selection for fast growth rate does not yield positive response, in fact, a slight negative response is indicated.

In 1965 a new selection experiment was started (Moav and Wohlfarth, 1976). Mass selection for slow growth and fast growth rate to 7 months of age was done for five generations followed by two generations of family selection in the high lines. The selection was practiced on fish grown in earthen ponds. During the last three generations of mass selection, replicated groups were held in cages and in ponds. As reported by Moav and Wohlfarth (1976) selection for slow growth rate yielded strong response for the first three generations while the high growth rate groups showed no response to selection.

However, the three generations of the high selection line reared in cages

icant additive genetic variance. It is not possible to get a correlated response when there is no additive genetic variance for the selected trait in the selection line. Family selection also showed significant increase in growth rate in the high line. This also means that there must have been additive genetic variance for growth rate in the high line.

Kirpichnikov et al. (1976) report a moderate response when selecting against dropsy disease in common carp. Hines et al. (1974) showed that while testing five inbred strains and nine crossbred strains of common carp, one inbred strain was found to be infected by a swimbladder inflammation and another by epidermal epithelioma disease. Crossbreds between these two strains, as well as between them and other strains did not show a single fish infected by either disease. It was concluded that susceptibility to both of these diseases is controlled by recessive genetic factors.

Salmonids

Lewis (1944) selected rainbow trout for fast growth rate and large number of eggs at 2 years of age. He reports large gains in egg number, growth rate and the frequency of females spawning at 2 years of age. No control line was used.

In 1949 Donaldson initiated a selection programme with chinook salmon in a sea-ranching programme. He found large gains in growth rate and fecundity (Donaldson and Menasveta, 1961; Donaldson, 1970). Donaldson and Olson (1957) and Donaldson (1970) report remarkable progress for selection in rainbow trout. The selected stock excelled in almost every character of importance compared to cultured rainbow trout. However, Donaldson did not have a proper control group in either of his experiments.

Kincaid et al. (1977) selected for increased weight at 147 days post-fertilization. The genetic gain during three generations of selection was 0.98 g or 5% gain per year. A correlated response in percentage hatch and fry survival was obtained.

From a selection programme at the Nikko research station, Japan, Kato (1978) reported response in lines selected for number of eggs, egg diameter, growth rate and in one of the lines they succeeded in getting one which spawned twice a year.

Response to one generation of selection in 190 day weight in Atlantic salmon was 2 g or 7% per year (Gjedrem, 1979). Genetic change in body weight after keeping the fish for 2 years in sea cages was found to be 3.6% and 2.7% per year compared with wild control and control groups, respectively. This response is of the expected magnitude when estimating genetic change based on genetic parameters.

Channel catfish

Bondari (1980) selecting for fast growth rate in channel catfish, reported

ward lines after one generation of selection. A very high response to selection was observed by Reagan (1980): 90-day weight was increased by 59 g per generation.

Oysters

Logie et al. (1961) reported that over a period of many years the oysters of Malpeque Bay slowly but surely became resistant to the Malpeque disease through natural selection. Selection progress has also been obtained for resistance against MSX (*Minchina nelsoni*) disease of the American oyster. Artificial selection for resistance to MSX in Delaware Bay was carried out on a number of hatchery-reared lines (Haskin and Ford, 1978). After four generations of selection the percentage survival rate was nine times the average of unselected stocks. These unselected stocks were imported and not previously exposed to MSX. Offspring of native Delaware Bay oysters had two to three times higher survival than the susceptible lines.

Haley et al. (1975) reported that mass selection of adult oysters gave an apparent strong response to selection for growth rate. They concluded that because of large environmental variability, a combination of family and mass-selection would be required to achieve maximum response.

Newkirk (1980) obtained considerable selection response in growth rate of oysters after one generation of selection. He concluded that 10—20% gain per generation in growth rate is a reasonable expectation.

In conclusion, 11 selection experiments for improving growth rate and four for improving disease resistance reported here, have given quite high responses. For growth rate there is one exception, namely the early Israeli experiment with common carp.

BREEDING PLANS

It should be stressed that selection in general should be based on traits taken close to the time of marketing the animal. Records taken at younger ages can only be used as correlated traits. Further, animals should be tested in the same type of environment as the production takes place to avoid possible genotype—environmental interaction.

Methods of selection

It has been shown that investment in selection programmes may give very high returns expressed as interest on capital spent (Hill, 1971) and there is no reason to believe that fish and shellfish are exceptions in this respect.

What selection methods should be used with fish and shellfish is quite easy to determine by studying Figs. 1 and 2 (taken from Falconer, 1960).

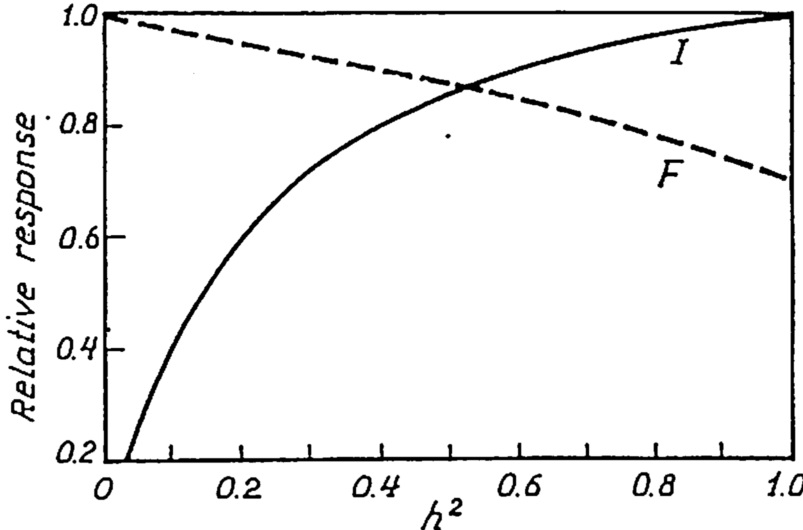

Fig. 1. Relative merit of fullsib family (F) selection compared with individual (I) selection. Number per family is infinite and there is no variance due to common environment (Falconer, 1960).

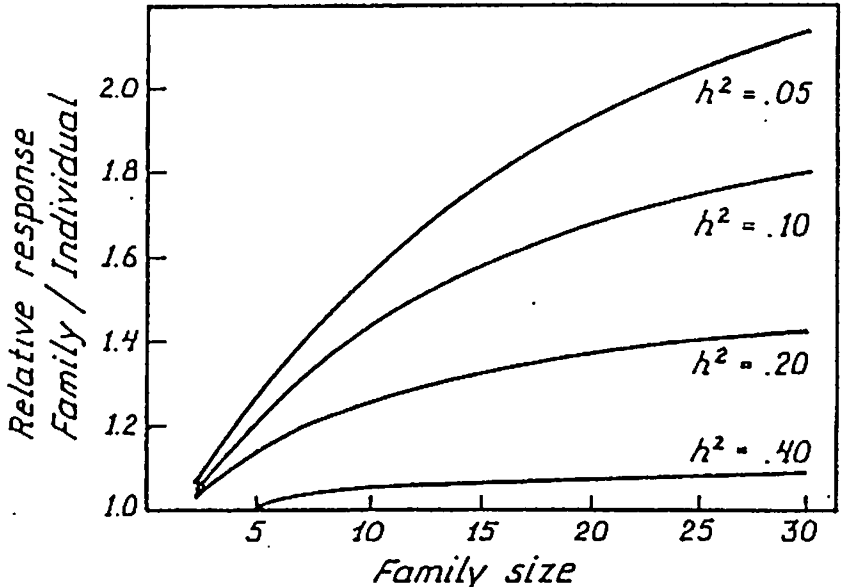

Fig. 2. Responses expected under family selection relative to that for individual selection, plotted against family size. It is considered that there is no variance due to common environment (Falconer, 1960).

The following conclusions can be drawn:

(a) A combination of individual and family selection is always most efficient (Fig. 1).

(b) Family and individual selection are of equal efficiency when the heritability is approximately 0.5. When the heritability is lower, family selection is more efficient. When h^2 is higher than 0.5, individual selection is more efficient than family selection. If there are common environmental

effects involved, the above relationships are true at lower values of heritability.

(c) The efficiency of family selection compared with individual selection increases markedly as the number per family increases (Fig. 2) whenever the heritability is below 0.4.

Progeny-testing is widely used in farm animals. In fish and shellfish it is of much less advantage since family selection can be applied. Progeny-testing will increase the generation interval markedly, frequently it will be doubled. Besides, the fact that several fish species spawn only once or a few times would make progeny-testing difficult. Deep freezing of milt is, however, now possible in salmonids (Stoss and Holtz, 1981). Brody et al. (1976) strongly recommend progeny-testing in common carp although they admit its limitations.

Individual selection can only be applied when the traits in question can be recorded directly on the individual. Individual selection is rarely effcient for all-or-none traits. An exception here could be when the desired trait has a very low frequency and is highly heritable.

With fish and shellfish, selection should be based on a combination of individual and family merit. Individual selection alone is only of interest when growth rate is the only trait of economic importance and is highly heritable.

A breeding programme involving crossbreeding will need a reciprocal recurrent selection scheme which will be more complicated and is likely to be more expensive to run than a selection programme aiming at improving additivity.

Marking fish and shellfish

Testing of families requires a marking system. Except for shellfish, where a label or code in the shell can be used (Newkirk, 1980), this is a real problem. Attached marks harm the fish and should thus be avoided (Refstie and Aulstad, 1975). Tatooing has given varying results (Hill et al., 1970; Refstie and Aulstad, 1975). A combination of finclipping and cold branding has given satisfactory results (Gunnes and Refstie, 1980), and Brody et al. (1976) and Joyce and El-Ibiary (1977) used brand marking. Nose-tags have many advantages and are widely used in sea-ranching. One can mark small fish, a large number of fish can be marked per hour and there are few losses. The disadvantage is that the small metal tag must be dissected out of the nose before it can be read. This makes nose-tagging impractical for a breeding programme where selection of brood stock must take place. It is a pity that nobody has made a "nosetag" that can be read on live fish on a large scale. The present technology should make this possible.

Electrophoretic (y) detectable genetic markers have been proposed for use by several authors and this is strongly recommended by Moav et al. (1976). However, they admit the method is time-consuming, difficult

and expensive. The advantage of electrophoretic markers is that one can avoid common environmental effects both in hatcheries and later on. Newkirk (1980) states that the expense of using electrophoretic markers to keep track of individuals hardly seems justifiable at least for mollusks.

For breeding schemes with large numbers of families and several thousand individuals, electrophoretic markers are not practical because:

(a) Selection of brood stock must be carried out on the spot.

(b) The marking system would reduce selection intensity for economic traits in order to select for markers that would distinguish the progeny by their electrophoretic phenotype.

(c) Rather a high frequency of the animals may not be identifiable. Brody et al. (1980) report that 3/11 of the groups had to be abandoned because the progeny marker phenotype did not match that expected from the known parental phenotype.

Thus we are left with finclipping, cold-branding and hot-branding as methods of marking fish in large scale selection programmes. Since these methods also have some drawbacks, one can only hope for a readable "nose-tag".

Selection indexes

In most cases selection should be based on several economic traits in each species. The traits may vary from one species to another and from one market to another. In addition information from individual and families must also be combined before selection can be applied. This situation has been discussed in many papers and the general conclusion reached by Hazel and Lush (1942) has been confirmed. *Their conclusion was that when selecting for several traits it is most efficient to do this simultaneously based on a selection index.*

Multibreed selection

For most species there are a large number of strains available for farming. This expands the possibilities for genetic improvement in two ways:

(a) Selection between strains. Selecting the best strain or strains gives an immediate improvement in genetic value.

(b) Crossbreeding among strains. This exploits heterotic effects and is probably best carried out in practice by forming a new synthetic population by mixing the best strains. A large proportion $(n - 1)/n$ (Dickerson, 1969) of the heterosis is maintained when n breeds are mixed with equal participation.

Both these policies must compete with the selection policy within strains. However, the three policies can be merged into one by using a multibreed selection index (Kinghorn, 1982). This will give the optimum rate of genetic improvement from the resource of fish stocks available. Only when two

strains are notably better than all the other strains available, will it be worthwhile to form an F_1 cross in a system with maintenance of parental lines.

Breeding station

Because of high fecundity of fish and shellfish, it is possible to produce large numbers of eggs and sperm from only a few selected animals. This means that only one or a couple of breeding units can supply a national market even if it is large. It is therefore of interest to discuss the possibility of developing breeding stations for fish and shellfish as are widely used in plant and poultry breeding.

A breeding station could be organised on a cooperative basis among breeders, or private firms could establish their own stations.

A breeding station should be able to test a large number of half- and full-sib families each year. Large efforts should be made to standardize environmental conditions in order to reduce errors in estimating breeding values. The breeding station must be able to control the whole life-cycle of the species in question. Testing should be done under similar environmental conditions as in the farming industry. All economically important traits should be recorded close to the time of marketing. Strong selection should be practiced at both the family and individual level. However, one should not allow much increase in inbreeding. By practicing strong selection there is always the risk of loosing desirable genes. Small founder population sizes and bottlenecks during selection should be avoided. Therefore, the testing capacity should be as large as economically feasible. As an example, let us consider a breeding station with a capacity to hatch and rear 100 full-sib families per year. In order to avoid inbreeding, brood stock should at least be selected from ten families yielding a selection intensity between families of only 10%. It is therefore recommended that a breeding station should at least have a testing capacity of 100 families per year-class.

The weakness of concentrating on one or a few national breeding stations is the risk of infectious diseases which could quickly be spread to the whole industry. Therefore steps should be taken to reduce the possibility for infections. One should also try to find ways to store the latest achievements in the selection programme, by deep freezing or by starting parallel isolated programmes. This would, in the case of an infectious disease, make a new start possible without being forced to start from wild populations.

A breeding station should have a control population to measure genetic response.

CONCLUSION

The outlook for obtaining genetic progress in fish and shellfish is very

(a) It is possible to practice a very high selection intensity in all species discussed.

(b) There is considerable genetic variance in growth rate and age at maturation for all species (Tables II and III). For mortality and meat quality the genetic variance is much lower.

Since there are several economically important traits in each species and since combined individual and family selection should be practiced, selection indexes should be used to rank animals. However, looking at Table III, there are not sufficient genetic and phenotypic parameters available to construct an index for all species. High priority should therefore be given to estimating the necessary phenotypic and genetic parameters to be able to construct selection indexes. The necessary parameters for each trait are, mean, standard deviation, economic value and heritability. Genetic and phenotypic correlations between all traits involved should be estimated.

So far there are only a few selection programmes aimed at developing more productive animals for the industry. It is our duty to try to initiate and stimulate development of breeding schemes. This will be very important in order that aquaculture may become competitive with other meat products in the future. A selection programme will, however, cost money but it is likely that it will give higher interest than other investments in the industry.

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Improvement of Productivity through Breeding Schemes

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Abstract: The possibility to improve productivity in aquaculture by applying selective breeding and hybridization is very good. This is due particularly to large genetic variability in most economically important traits and high fecundity in species of interest. To increase efficiency, individual selection should be combined with family selection. It is concluded that if additive genetic variance is present in economically important traits, selection should be practiced, and if non-additive genetic variance is considerable, selection should be combined with crossbreeding. It is recommended that breeding stations be established to carry out selection programmes. Breeding programmes need considerable investments and operating funds. However, they have been shown to produce very favourable benefit/cost relationships.

In an appendix a recording scheme for rainbow trout and a description of how selection is practiced are given.

Introduction

There is a long tradition to increase yield and product quality of farm animals and plants through breeding and selection. The rate of change has been particularly rapid in the last 2–3 decades and today one cannot really imagine animal husbandry and plant production without such selection programs.

In aquaculture, efficient breeding and selection programs are rarely practiced even though the tradition of fish farming goes back thousands of years, in some regions. There are several reasons for the lack of selective breeding, the main one being that control of the reproduction cycle is a requisite for it. In fact it is one of the overall obstacles to aquaculture development that artificial reproduction has not been achieved in some farmed species, because hatching and feeding of larvae or fry has not been possible or has been difficult to perform. This has been the case, for instance, in eel and yellowtail farming. Gjedrem (1983) speculates that another reason for the lack of attention to breeding in aquaculture could lie in the education of fish biologists which pays little attention to quantitative genetics and breeding plans. Yet breeding and selection programs will give aquatic animal husbandry a new dimension since it is possible to make the animals conform to existing environmental conditions.

The purpose of this paper is to discuss the potential of breeding and selection in aquaculture and how to develop such programs for fish, and the appendix describes a recording scheme in rainbow trout and how selection is practiced in this species.

Possibilities for Genetic Change

Varying results have been reported in a large number of selection experiments but with some plant and animal species remarkable progress has been obtained. For example Enfield (1974) accounted that after 85 generations of selection the pupa weight of *Tribolium* was increased by more than 3000 microgram or 14 phenotypic standard deviations and 26 additive genetic standard deviations. The base population had an average pupa weight of 2450 microgram with a phenotypic standard deviation of 207 microgram and an additive genetic standard deviation of 115 microgram.

Dudley (1977) reported the progress of 76 generations of selection for high and low oil and protein content in corn to be more than 20 additive genetic standard deviations in the high direction for both oil and protein. The selection had not exhausted genetic variation for either trait in either direction. Dudley (1977) concluded that this experiment suggested the potential for improvement of random mating corn population may well be beyond what is commonly considered possible.

These two selection experiments show no sign of a selection plateau. However, many of the reported experiments have reached selection limits in 30 generations or less. The main reason for reaching a selection limit after few generations is small population size and involvement of few genes (Falconer 1981).

Gjedrem (1983) reviewed the results of selection experiments which had been carried out in fish and shellfish. In 11 experiments selection for growth rate, and in 4, selection for disease resistance had been practiced. The responses have been fairly high in all experiments except in one selecting for high growth rate in carp which had given no response. Bondary (1983) and Dunham and Smitherman (1983) found positive response to selection for growth rate for channel catfish. In European oysters, selection in the second generation was efficient for survival but not for growth rate (Newkirk and Haley 1983). Busack (1983) found selection responses in growth rate in both sexes of mosquitofish but only the male responded significantly.

Gjedrem (1975, 1976) discussed the possibilities for genetic change in salmonids on a general basis. Gains are particularly dependent on selection differentials, genetic variance of the traits in question and they are inversely related to length of the generation interval. He concluded that the possibilities for a large genetic gain was good, particularly because of the high fecundity of salmon and trout – and for that matter of many other fishes – which makes a strong selection possible. At that time very few estimates of heritability and genetic variance for economic important traits were published but lately several such estimates have been added. In Tab 1 average estimates of heritability and coefficient of variation ($CV = (\sigma_p/\bar{x}) \cdot 100$) are given for different traits and for several species referred by Gjedrem (1983). The information given in Tab 1 can be summarized as follows.

a) Coefficients of variation are very high for body weight in all species and higher for younger than for older animals.
b) Heritability for body weight of juveniles is rather low with the exception of channel catfish and oysters. Heritability for body weight of adults is not pronounced but it is higher than for young animals.
c) The high phenotypic variance of body weight of adults together with medium size heritability means that there is large genetic variance for this trait for the species listed.
d) Mortality shows low heritability while resistance to specific diseases shows medium to high heritability.
e) Meat quality traits show some genetic variation. An exception is the dressing percentage which shows low genetic variance.
f) Age at sexual maturation being an all or none trait shows medium size heritability in rainbow trout but a high one in Atlantic salmon.

The generation interval in fish is variable; in the most important cultured species in the temperate zone it is relatively long with sexual maturity only occurring in the third year of life, or later. This will reduce the yearly genetic gains that may be attained.

In this paper special breeding programs for invertebrates will not be discussed. The principles are of course the same, but the magnitude of the genetic variance for the

Tab 1 Average Values for Coefficients of Variation (CV) and Heritabilities (h^2) Based on Sire Component Calculated from Figures Given in Tab 2 (of source) for Economically Important Traits (superscripts give number of estimates involved). Source: Gjedrem, 1983.

Economically important trait	Rainbow trout		Atlantic salmon		Common carp		Channel catfish		Tilapia		Oysters		Prawns	
	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2	CV	h_s^2
Body weight, juveniles	33^7	0.12^4	78^1	0.08^1		0.15^1	46^1	0.42^4	26^5	0.04^1		0.36^3		0.12^3
Body weight, adults	22^7	0.17^2	27^2	0.36^3	22^1	0.36^2	27^3	0.49^4						
Body length, juveniles	14^3	0.24^3	23^1	0.14^2				0.12^1	8^1	0.06^1		0.47^2		
Body length, adults	9^2	0.17^2	8^2	0.41^4			8^2	0.61^3						
Mortality/ resistance		0.14^1		0.11^4	28^1									
Carcass traits:														
Meatiness	20^1	0.14^1	19^1	0.16^1										
Meat color	23^1	0.06^1	16^1	0.01^1										
Fat-%	10^1	0.47^1				0.14^1	8^2	0.23^3						
Dressing-%	6^1	0.01^1	4^1	0.03^1			2^2	0.00^2						
Age at maturation		0.18^1		0.41^2										

economic traits in the different species are not yet well documented (Gjedrem 1983). Other things, such as simplicity of life cycles being equal, (which they rarely are), the advantage of invertebrates is that they usually have short generation intervals. Based on experimental results, Newkirk (1980) concludes that one may expect genetic gains of 10–20 percent per generation in bivalves. Some tropical food fishes also breed every year. Tilapia is a prime example here; its tolerance of a variety of environmental conditions and the relative ease with which it can be propagated have already given it the name of the "aquatic chicken" of the future (ICLARM 1984).

In conclusion, the possibility for genetic gains from selection programs in aquatic animals is very high.

Breeding Goals

Definition of the breeding goal is an important prerequisite of a selection program; all traits of economic importance which show genetic variance, should be included in it. As discussed by Gjedrem (1983) the breeding objectives should be set, cooperatively by the industry and by consumers. Each selected trait should be defined precisely so that it can be measured and/or judged in the most accurate way.

Some traits — *growth rate, food conversion efficiency* and *survival* — are of economic importance in most production systems and species. Growth rate is easy to record and can be measured exactly either as body weight or as body length. Growth rate seems to have a heritability of moderate magnitude (Tab 1 and 1a) and a high phenotypic (individual) variance. Food conversion efficiency is, however, more difficult to deal with. Under practical conditions — the subjects live in water — individual measurements cannot be taken on many animals. Food conversion efficiency is also difficult to record even on a family basis of young animals. Methods are laborious and thus expensive as it is difficult to record food not eaten once given; furthermore some of the nutrients will dissolve in the water.

In farm animals relatively high genetic correlations are found between growth rate and food conversion efficiency (Andersen (1977); $r_G = 0.95$ in cattle and Vangen (1984); $r_G = 0.93$ in pigs). Kinghorn (1983) found a high positive genetic correlation in young rainbow trout between growth rate and gross food conversion (Growth/Food consumed) and a negative genetic correlation between growth rate and net food conversion (Growth/Food consumed less the maintenance ration). The gross food conversion had a low heritability ($h^2 = 0.03 \pm 0.10$) while net food conversion had moderate heritability ($h^2 = 0.31 \pm 0.11$). More information and more accurate recording systems should be developed before it is justifiable to include food conversion efficiency in a selection program. After all selection for growth rate is likely to improve food conversion efficiency through a correlated response.

Survival rate is of great economic importance but of rather low heritability, as shown in Tab 1. It is presently recorded as number of dead or alive subjects and it is confounded by other factors. Recording the frequency of survival is not a satisfactory observation because one should also be able to measure degree of resistance to disease. Immunological studies may create new ways of recording degrees of resistance to disease and thus increase efficiency in selection.

Other traits may be of different economic importance in one market compared to another and may vary considerably according to production systems. This is certainly true for *meat quality*. Fish quality is usually a question of size, meatiness, fat percentage, meat color, taste, shape of body, dressing percentage and so on. It is difficult to find exact definitions and make objective measurements for some of these traits. Heritability seems to be low for them except for fat percentage traits (Tab 1).

When producing large salmonids *age at sexual maturation* is of great importance and early maturation is a disadvantage because food is diverted from meat to gonads. According to the estimates presented in Tab 1, the heritability for age at maturation seems to be higher for Atlantic salmon than for rainbow trout.

The *fecundity* in fish is usually very high and need not be improved. However, *egg size* could be of importance as a selection trait, if it is correlated with survival and early growth rate.

Traits under selection may be genetically correlated with other traits not considered. It is therefore important to take records also on other traits, not necessarily of economic importance today and to study whether they are changing or not.

Selection

The efficiency of selection is partly dependent on how accurate the *breeding value* of individual animals are evaluated. In genetic parlance the breeding value of an individual is the mean value of its progeny (Falconer 1981). Breeding value can also be defined in terms of the sum of the average effects of the genes of an individual, the summation being made over the pair of alleles at each locus and over all loci. Falconer (1981) adds that one cannot speak of an individual's breeding value without specifying the population within which it is to be mated.

In order to estimate breeding values and make a ranking list of individual animals, it is necessary to obtain information about its production capacity. This requires having a recording system throughout the whole life span of the animals. An example of a recording system for rainbow trout is given in the appendix.

Selection based on individual merit or phenotypic selection (also called, mass selection) is the most widely used selection method in fish. Such *individual selection* can only be used on traits possible to record on live animals and it is not efficient for traits with low heritability. Therefore in fish it is best applied to growth rate and to some extent to selection for age at maturity. For the other economic traits mentioned earlier other selection methods must be chosen.

Family selection is of particular interest in fish because of their high fecundity; both full- and halfsibs are used. Family selection compared with individual selection is particularly efficient when the heritability is low. Thus it will be efficient for survival, meat quality and age at maturation. One of the advantages of using full- and halfsib families in a selection program is that the generation interval will not be increased compared to individual selection.

Because it is impossible to mark newly hatched larvae or fry, each family must be re:r:d in separate tanks for the first months of their life, before they can be marked. This type of rearing will introduce some environmental or tank effects between families. Refstie and Steine (1978) estimated the tank effect on body weight in Atlantic salmon fingerlings to be 4.5% while Aulstad et al. (1972) estimated the tank effect in 150 and 280 days weight in rainbow trout to be 6.2% and 4.3%, respectively.

For most traits family selection is more efficient than individual selection (Falconer 1981) but generally a *combination of individual and family selection* will be more efficient than using only one of them.

Testing of families requires a *marking system*. Gjedrem (1983) discussed several types of marks and concluded that at present we can choose from finclipping or cold- and/or hotbranding as methods of marking fish in large scale selection programs. Since these methods have several disadvantages one should try hard to develop a "nosetag" readable on live fish. Molluscs can be marked quite easily once their shells are formed but crustaceans pose great problems, because they moult.

Progeny testing, i.e. assessment of breeding results in the next generation, is widely used in farm animals. In fish where both sexes could have been progeny tested, it is of much less advantage since family selection can be applied. Progeny testing will increase the generation interval markedly, frequently doubling it. Besides, the fact that several fish species spawn only once or a few times in their lives would make progeny testing difficult to practice.

Selection Indices are much used in selection schemes; they are the most efficient method to combine information from several traits as well as information from an individual and its relatives (Hazel and Lush 1942). In the case of individual selection for body weight simple indices can be used to evaluate the breeding value of each animal and to make ranking lists[1].

Economic aspects of selection programs have been investigated by several authors for most of the domestic animal species. In general, investment in selection programs gives very high returns expressed as interest on capital spent. Full investment appraisals have recently been worked out by Barlow (1982) and Mitchell et al. (1982). They covered sheep, pigs and beef cattle and showed net benefit/cost ratios ranging from 5/1 to 50/1.

Gjedrem (1983a) considered a national production of 25,000 t of Atlantic salmon and a yearly genetic gain in growth rate of 3%, which has been obtained in practice (Gjedrem 1983). He calculated that farmers cooperatives could invest 7.5% of the yearly production value into a selection program and get 20% interest on the investment. The actual expense entailed in obtaining the genetic gain is likely to be below 1% of the value of the fish marketed.

One or More Breeds

A breed or a strain is a genetically different population of a species, and the basic question to be asked when planning a breeding program is, whether or not there are genotype-environment interactions? If there is no such interaction, a selection program can concentrate on the best breed only or combine the best breeds into one breeding population. On the other hand, if significant genotype-environment interaction exists, one certain breed should be kept in each environment.

Moav et al. (1975) and Wohlfart et al. (1983) found genotype-environment interaction in carp. Sneed (1971) estimated considerable genotype-rearing system interaction in catfish, and Gunnes and Gjedrem (1978) found significant interaction for growth rate between Atlantic salmon strains and certain sea water farms. However, the component of variance accounted for only 1.2% to 3.7% of the total phenotypic variance. Similar results were obtained by Gunnes and Gjedrem (1981) for interaction between full-sib families of rainbow trout and sea water farms. Gunnes and Gjedrem (1978, 1981) concluded that the interaction was so small under these conditions that it could be ignored when planning selection schemes. In another rearing scheme Ayles and Baker (1983) estimated the strain-lake interaction for growth and survival in rainbow trout to account for 2% to 28% of the total phenotypic variance.

Thus it appears that one cannot draw a general conclusion about how many breeds should be used. That question must be investigated for each species and each location. It is, however, likely that genotype-environment interaction will increase with the variability of environmental conditions.

Crossbreeding

In heterosis or hybrid vigor the offspring surpasses the average of its parents for one or more traits; it is the reverse of inbreeding depression which is obtained by mating related animals. The two phenomena are almost universally distributed in plants and animals and are particularly associated with reproductive fitness.

Using inbreeding and crossbreeding in breeding schemes is to try to exploit non-additive genetic variance. In practical breeding work inbreeding is only of interest when inbred lines are to be used in crossbreeding programs.

Gjerde and Refstie (1984) investigated the heterosis effect between crosses of five Norwegian strains of Atlantic salmon. They did not find a significant heterosis effect for either growth rate or survival rate. Likewise, Friars et al. (1979) found no heterosis effect for growth rate of Atlantic salmon fry. But in rainbow trout Gall (1975) and Ayles and Baker (1983) reported significant heterosis for body weight among crosses of rainbow trout strains.

Moav et al. (1976) discussing hybridization between Chinese and European races of the common carp, concluded that hybrids between the two races can be valuable under polyculture rearing systems using feed made of cheap agricultural byproducts. Moav et al. (1975) showed high hybrid vigor in growth rate between races of carp but the best cross did not exceed the best race in growth rate. Brody et al. (1980) reported that hybrids generally grew more rapidly than purebreeds in four 2 × 2 diallel experiments in the Dor-70 line of common carp and Suzuki and Yamaguchi (1980) also found pronounced heterosis for growth rate when crossing different races of common carp.

Chevassus (1979) reviewed the status of hybridization between species of salmonids. He concluded that in most cases the hybrids raised in the same environment as the parental species show intermediary or, at best, equal growth to that of the better of the parents. Better results have been found for survival rate, the hybrid often being similar or even superior to the most hardy species. Kirpichnikow (1981) lists 18 promising species of hybrid combinations and says that many other hybrids are also possible to produce, particularly in salmonids and cyprinids.

The search may become very important for infertile hybrids which do not divert food into gonads and therefore have superior production traits. As unisexual offspring have been obtained in several interspecific crosses in tilapia such monosex male cultures are recognized as the best solution to over-population caused by the very high fecundity of tilapia under almost any pond condition. In addition, males also grow faster than females. Pruginin et al. (1975) list several crosses which have given 100 percent male offspring while Hulata et al. (1983) propose to use progeny testing to ensure getting 100 percent male progeny in tilapia.

Introduction of new genes from strange populations into a local strain are frequently done by crossbreeding. This is usually a simple and very cheap way to improve native races. However, prior to introduction of a new breed or cross it should be tested under the existing local conditions. Milt or fertilized eggs rather than fish should be used when introducing a new breed. This will reduce the possibility of importing new diseases.

After these general remarks on crossbreeding it will now be of interest to describe a crossbreeding scheme for fish farming:

I Test of all possible crosses between different strains or species for the economic traits in question. If the number of strains available is large, one must select the crosses which are most likely to give valuable results. However, it is very difficult to try to predict results of different crosses. It may be useful to try and use strains with very different origin and also to use strains which, in combination, have favorable traits.

II Develop inbred lines and test the crosses under natural conditions to find the most valuable cross for farming. This breeding system aims particularly at utilizing non-additive genetic variances. One of the practical difficulties here could be to keep the inbred lines running because of high mortality.

III Start a reciprocal, recurrent selection (RRS) program. This is necessary in order to ensure continuous genetic improvement. RRS will aim at utilizing both general and specific combining abilities simultaneously.

The theoretical basis of RRS has been given by Comstock et al. (1949) and Dickerson (1952). Falconer (1981) outlines the procedure as follows: "The start is made from two populations, preferably two already known to give some heterosis when crossed. These two populations, whose combining ability is to be improved, will be refered to as lines A and B. Crosses are made reciprocally, a number of A♂♂ (males) being mated to B♀♀ (females) and a number of B♂♂ to A♀♀. The crossbred progeny is then measured for the traits to be improved and the parents are judged from the performance of their progeny. The best parents are selected and the rest discarded, together with all the crossbred progeny, which are used only to test the combining ability of the parents. The selected individuals must then be remated to members of their own line to produce the next generation of parents to be tested. These are crossed again as before and the cycle is repeated. Deliberate inbreeding is avoided because random changes of gene frequencies are not desired."

According to Falconer (1981) RRS programs are used by commercial breeders of poultry and have given promising results in corn. However, direct comparison with other selection methods has not been encouraging. RRS can only be used for multiple

spawners and can therefore not be applied in Pacific salmon. It would also be very difficult to practice in Atlantic salmon since the majority of males die after the first spawning together with a high number of females. It should work better with tilapia.

Breeding Stations

In species with high fertility in both sexes, the breeding can be concentrated on only a few animals in each generation. In poultry, for example, where the hen lays 200–300 eggs per year, only a low percentage (2–3%) of the animal's needs to be involved in breeding schemes and most of the work takes place at breeding stations.

In fish and shellfish where the fecundity is even higher, with thousands of eggs per female, the breeding can be concentrated on less than one percent of the animals. As described earlier in this paper, family testing and selection will play an important role in fish breeding, mainly because several of the economic important traits cannot be recorded on individuals while alive and secondly because some of the traits have low heritability. Both these arguments make central units or breeding stations a necessary tool in practical fish breeding.

As pointed out by Gjedrem (1983), a breeding station should be large to allow testing of many full- and halfsib families each year. Large efforts should be made to standardize environmental conditions in order to reduce errors in estimating breeding values. The breeding station must be able to control the whole life-cycle of the species in question. Testing of animals should be done under similar environmental conditions as in the farming industry; this will reduce the problem of genotype-environment interactions. All economically important traits should be recorded close to the time of marketing. If records are taken in younger fish, there may be low correlation between records taken early and records taken at marketing.

Since the genetic gain is so closely related to selection intensity, very strong selection should be practiced at both family and individual levels. One should, however, not allow much increase in inbreeding because significant inbreeding depression has been found both in survival and growth rate in rainbow trout (Aulstad et al. 1972; Kincaid 1976a, 1976b; Gjerde et al. 1983) and in growth rate in carp (Moav 1976). Mallet and Haley (1983), however, reported positive effects due to inbreeding for larval survival, but depression for spat size in oysters.

Yet, desirable genes may be lost by practicing strong selection. Therefore small founder populations during selection should be avoided. These difficulties can be reduced using a breeding station with a large testing capacity, by systematic introduction of new genes and by letting the yearclasses overlap each other.

In Fig 1 the curves give the relationship between selection differential, number of families selected and numbers tested. The shape of the curves show that much is to be gained by testing 150 families compared with 100, because selection differentials are increased from 1.75 to 1.96. In practice capital for investments and running costs will usually set the upper limit for testing capacities.

The weakness of concentrating breeding work in one or a couple of breeding stations in a region or a country is the risk of infectious diseases which could quickly spread to the whole industry. Therefore steps should be taken to reduce the possibility of infections at all levels.

Conclusion

A breeding program will have two main objectives: First, to increase productivity and product quality, secondly to develop animals which are better adapted to captivity. When the breeding aims are exactly defined, the objectives are to work out breeding plans which will reach the aims in the shortest time, taking cost into consideration.

In practice there are particularly two breeding methods to choose from for fish, namely crossbreeding and selection or, of course, combinations between them. Crossbreeding has the advantage of simplicity and often an immediate and visible impact on animal performance (Fig 2). To use crossbreeding only as a breeding method over several generations is, however, a static procedure. As illustrated in Fig 2, the advantages of using inbred lines or strain crosses, are reached during the first generation and if no further testing and selection is practiced there will be no more progress. It is, however, often used, and usually with success to change races or strains completely or to partly upgrade existing populations. In some species unisexual offspring have successfully been produced by crossbreeding.

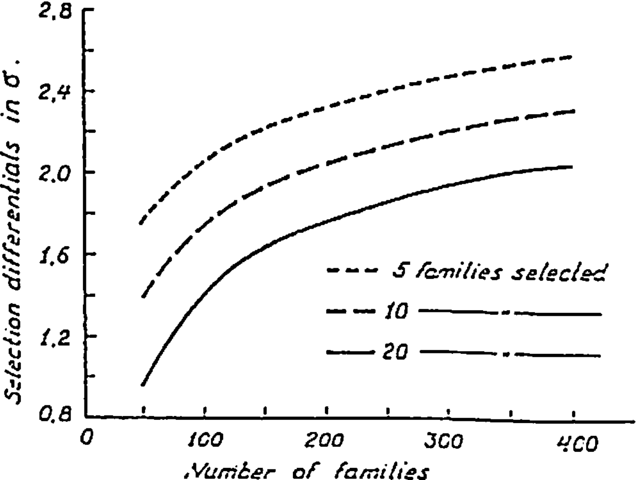

Fig 1 Selection differentials related to number of families tested and number selected.

Reciprocal recurrent selection (RRS) aims at improving both general and specific combining abilities, thus potentially taking advantage of both selection and crossbreeding. There was considerable expectation for RRS which was used with some positive results in poultry and cornbreeding (Falconer 1931). However, investigations by Calhoon and Bohren (1974) and McNew and Bell (1976) who compared RRS with other methods of selection were not encouraging. Also RRS requires a very complex and extensive testing system and it will usually lengthen the generation interval considerably.

More simple crossbreeding systems have been developed and used in farm animals as well as in plants and fish: Two to four races which combine favorably in crossbreeding, are selected on a within races basis, to improve each of them additively. Other alternatives are to develop specialized sire and dam lines for crossbreeding.

Selection schemes based on individuals can be very simple and cheap to practice. However, if selection should be made more efficient and include traits such as meat quality, survival and age at maturation, a more complex system, including family selection should be developed. Because of the very high fertility of fish, it is only necessary to have few breeding stations. This will reduce the expense considerably compared with testing systems for species with low fertility. As illustrated in Fig 2 the gain from selection will compete favorably with that of crossbreeding, already after few generations of selection, but gains will depend on the magnitude of the heterosis effect and the realized selection response.

As pointed out earlier it is not likely that an additive genetic variance will be reduced in the first generations of selection. If the number of parents is kept quite large, in each generation, and inbreeding is avoided, it is likely that the additive variance for the quantitative traits selected

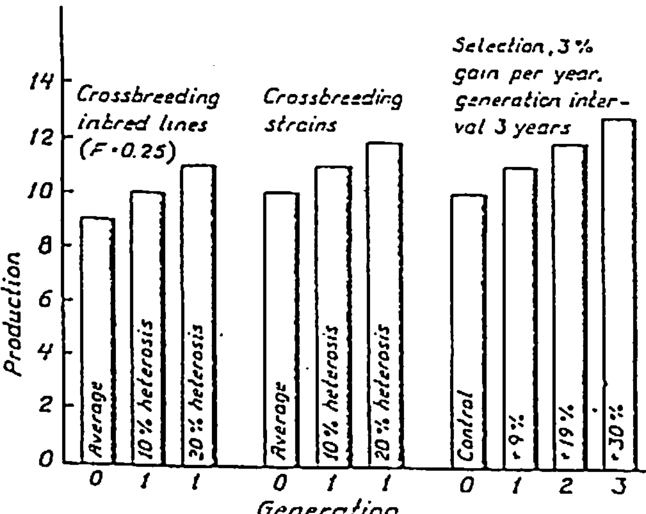

Fig 2 Comparison between two types of crossbreeding and selection, a simplified example; production in arbitrary units.

will last many generations. If there are indications of reduced variance, matters can be restored by introducing genes from unrelated populations or by using crossings between generations. There are several advantages developing synthetic races where new genes are continuously tested and included in the synthetic (Webb and King 1976).

Finally, as long as additive genetic variance is present in the traits of interest, selection should be practiced in any breeding program. If the component of non-additive genetic variance is considerable, selection should be combined with one or another system of crossbreeding.

As regards aquaculture in general, this brief account makes it abundantly clear that the application of standard applied genetic techniques holds much promise for the husbandry of aquatic organisms. Their use soon pays for itself and it promises to raise the potential of aquatic food production by a substantial margin.

Appendix

A testing and selection plan for rainbow trout to utilize additive genetic variance

The Production Cycle

This testing and selection scheme is carried out at the Institute of Aquaculture Research, Sunndalsøra unit (fresh water farm) and Averøy unit (sea water farm), Norway. The production cycle is as follows: Spawning occurs in February and March and during the first year the fingerlings are reared in fresh water at Sunndalsøra and reach 100–150 g. In May the fingerlings are transported to Averøy and reared for 1-1/2 years in sea water cages. In November the selection of brood stock takes place and culled fish are slaughtered. The average body weight at that age is 3.5 kg, with a standard deviation of 1 kg[2]. The selected brood stock are transported to Sunndalsøra and kept in brackish water for the next 3 months as a high proportion would die during this period if they were retained in full sea water.

The Fresh Water Period

Usually 200 dams and 30 sires are used as parents during February and March when full- and halfsib families are made up. Each fullsib family is kept in a separate tray in the hatchery. At the eyed egg stage the number of eggs is reduced to 3000 per family. The number of dead eyed eggs and larvae are recorded. There is usually a variation in hatching data of 4–5 weeks.

Each family is started and reared in separate plastic tanks of 1 m^3. All families are kept in the same barn. Water, feed, treatment and so on is uniform in all units. When the fingerlings have been on feed for 140 days, a sample of about 200 fish is weighed in bulk and some groups are individually weighed to give estimates of variance of weight. The number of dead fish is recorded except during the first 4 weeks on food.

The fish are marked when they reach 15–20 g. Finclipping (adipose and pelvic fins) and cold branding (Refstie and Aulstad 1974) are used. Two hundred and fifty fish are randomly selected from each family and marked with the same mark. One hundred

and fifty fish from each family are put into a concrete pond and reared there until May for later transport to Averøy. The last 100 fish from all families are put into a separate unit and, sold in May, to three private farms with which special agreements have been made. From time of marking until transfer to the sea water farms, the number of dead fish in each family are recorded.

The Sea Water Period

From May on, the fish are kept for 1-1/2 years in 2–3 nylon net cages, each with a volume of 300–500 m^3, both at Averøy and in one cage at each of the three private farms which received samples of all families. During the first winter all fish are examined for approaching maturity. These early maturing fish which are usually males, are slaughtered, after recording family mark and body weight. Further records are not taken during the sea water period except that the number of dead fish is counted each week and sample weights are taken four times a year.

At the private farms marked fish are slaughtered after 1-1/2 years and before October 1. Body weight, sex and gonad development and carcass traits such as fat percentage, flesh color and score for meatiness are recorded on each fish which can be identified. The data are transferred to a computer and family indices are estimated, based on body weight, frequencies of early maturation and carcass traits, also taking halfsib information into account. This family ranking is the basis for selection of broodstock at Averøy.

Selection

A stepwise selection is practiced:

I. *Hatchery survival.* Based on survival during the eyed egg and fry stages, the 150 best families out of 200 are selected. (There are 150 one-m^2 tanks available for testing of rainbow trout families at Sunndalsøra unit.)

II. *Growth rate of fingerlings.* One hundred forty day weight is adjusted for date of hatching and density in the tank at recording, as described by Refstie (1977, 1980). Each family is ranked according to its own average body weight and the average body weight of halfsib groups. The best 120 families are selected, while the other families are discarded. The marking system allows only 120 combinations of coldbranding and finclipping.

III. *Selection at Averøy.* Based on records taken at the private farms, of body weight, frequency of early maturation and carcass traits, indices are calculated for ranking of families. Broodstock are selected with males taken from the best 8–10 and females from the best 15–20 families. Individual selection is practiced within these families. Only males and females are selected with body weights of 2 and 1 standard deviations above the average, respectively. At this time individual weights are recorded and selected fish are individually marked with a metal tag in the gill cover.

IV. *Final selection.* All data are then accumulated and processed. Records are adjusted for sex and environmental factors such as date of hatching, cage, farm and so on. Individual indices are calculated for all fish selected at Averøy taking all available information into account, prominently encompassing individual body weight and information about full- and halfsibs. At the final selection the best 30 males and 200 females are selected.

There will, however, always be a few fish with a higher index in the second best families than in the best. It is therefore necessary to make some exceptions from the ranking list to ensure that a necessary number of families are represented among the selected males and females. To avoid increase in inbreeding, full- and halfsib matings are not practiced.

Footnotes

1) The selection response for one trait over one generation can be predicted by:

$$I_i = h^2 (X_i - \overline{X}) + \overline{X}$$

where h^2 is heritability, X_i is weight of individual fish and $\overline{X}$ is the population average. If breeding values of fish reared in different environments (j) are to be compared the index will be as follows:

$$I_{ij} = b_i (X_i - \overline{X}_i) + b_j (X_j - \overline{X}_j) + \overline{X}$$

where $\overline{X}_j$ is the rearing unit average and the b's are weighting factors (see below).

When several traits (1 to n) are under selection and individual and family information is available, the index will become more complex:

$$I_i = b_1 (X_1 - \overline{X}_1) + b_2 (X_2 - \overline{X}_2) + b_3 (X_3 - \overline{X}_3) + \ldots b_n (X_n - \overline{X}_n)$$

b_i is the weighting factor for each trait and is dependent on heritability, numbers in each family, correlation between traits, economic value of the trait and so on. According to Falconer (1981) it is estimated by finding what value gives the maximum correlation between the index and the breeding value. X_i is the phenotypic value of an individual or average of a full- or halfsib family and $\overline{X}_i$ are averages of trait i.

2) Rainbow trout must spawn in fresh water though they grow well in brackish or full strength sea water; sea-grown trout are often preferred on the market aside from the fact that it may be cheaper to rear them in cages in the sea than in ponds or raceways.

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Improving spawning synchrony in the Nile tilapia, *Oreochromis niloticus* (L.)

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Abstract. The development stage and number of seed (eggs and fry) removed from individual incubating *Oreochromis niloticus* (L.) broodfish at 10-day interharvest intervals (IHI) was compared with the harvest of free-swimming fry from undisturbed fish held in a tank within the same system. Spawning synchrony and productivity were compared for (1) total exchange of females for 'conditioned' females after each harvest, (2) exchange of only spawned females, and (3) return of females back into the spawning tank. Improved synchrony and seed production were positively related to removal of eggs and fry from incubating females and could have important implications for broodstock management in commercial hatcheries. Allowing fish to remain undisturbed in clear water tanks may lead to inhibition of spawning activity related to the formation of hierarchies. Individual weight loss by females during incubation was correlated with stage of seed harvested and thus with timing of spawning during the interharvest interval.

Introduction

The asynchronous breeding habit of maternal mouthbrooding tilapias, *Oreochromis* spp., together with their low fecundity, are major constraints to controlled mass production of seed. Attempts to improve natural breeding synchrony using gonadotropins were ineffective in *O. niloticus* (L.) (Srisakultiew & Wee 1988). However, use of GnRHa with dopamine antagonists has since proved successful to induce ovulation and allow stripping of eggs (Gissis, Levavi-Sivan, Rubin-Kedem, Ofir & Yaron 1991). The economic feasibility of these labour-intensive techniques is doubtful except for research purposes including induction of chromosome manipulation, interspecific hybridization and genetic studies (Mair & Little 1991).

Spawning synchrony does occur in wild populations and has been related to the influence of sunlight, temperature and rainfall (Lowe McConnell 1959). In Taiwan, cultured broodfish tend to spawn most synchronously in spring after inhibition of breeding by low winter temperatures (Liao & Chen 1983). Spawning synchrony and frequency of spawning has been stimulated by using warmwater effluents in temperate climes (Behrends & Smitherman 1983). Guerrerro & Guerrerro (1985) found that synchrony can occur if broodfish are conditioned prior to spawning. Lovshin & Ibrahim (1988) reported that female broodfish

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exchange failed to improve seed output in hapas, but 21-day spawning and conditioning periods exceeded the optimum period (Little, Macintosh & Edwards, in press). Some studies suggest that early removal of seed from mouthbrooding females can improve output (Lee 1979; Verdegem & McGinty 1987), but others believe the effects of broodfish disturbance in larger systems are detrimental (Liao & Chen 1983; Beveridge 1984). This study investigated the effect of disturbance and female broodfish exchange on spawning synchrony in large groups of fish.

Materials and methods

The 155-day study (5 March – 8 August 1986) was conducted on the campus of the Asian Institute of Technology (AIT), near Bangkok, Thailand. Two tank systems were used, consisting of four circular spawning tanks (diameter 4 m) and 32 conditioning tanks (of which eight were used during the trial; diameter 1·5 m), each with a water depth of 1 m. The two groups of tanks were connected to separate but similar recirculation systems supplied continuously with water filtered through horizontal biofilters. Both tank systems were sheltered by a 3-m-high galvanised iron roof. Residence time of water in spawning and conditioning tanks was 7·3 and 1·3 h respectively. Each spawning tank had concrete blocks placed perpendicularly to the wall at intervals of 16 cm to provide nest sites.

Treatments (one per spawning tank) were: (1) daily removal of free-swimming fry from the edge of the tank with minimal disturbance of broodfish (NODIS); (2) replacement of all fish back into the tank after eggs and fry (seed) harvest, i.e. no exchange (NOEXC); (3) exchange of only spawned females (SPEXC); or exchange of all females (ALLEXC) with equal numbers of 'conditioned' fish taken from tanks stocked with only females.

Broodstock (Chitralada strain) were obtained from the final harvest of a free breeding population in a fertilized earthen pond and were 3–18 months of age and ranged from 70 to 300 g. Female broodfish were tagged behind and under the dorsal fin with nylon line after sedation using benzocaine in aerated water. Every female fish was weighed to the nearest gram and total length measured to the nearest 0·1 cm at each interharvest interval (IHI) in treatments NOEXC, SPEXC and ALLEXC. Body weights were recorded before spawning, after spawning (+10 days) and after conditioning (+20 days). Male broodfish were individually weighed and measured at stocking and final harvest and were bulk weighed and counted at every seed harvest. Undisturbed fish (NODIS) were weighed at the beginning and end of the trial. Broodfish density and sex ratio were maintained at 8 fish/m^2 and 1:1 respectively, for the duration of the experiment. Females in conditioning tanks were stocked at the same density (50/m^3) in both treatments (SPEXC and ALLEXC).

At 10-day intervals, the water level was reduced to 20–30 cm in the NOEXC, SPEXC and ALLEXC tanks to facilitate capture of broodfish. Seed were harvested from the mouths of incubating females after carefully catching the fish in a hand-held net; fry already liberated were taken directly from the tank. Individual clutches were held in plastic bowls to allow staging before preservation in 4% formaldehyde (Peters 1983) in sealed glass or plastic bottles. Stages included: (1) no development visible; (2) eyed eggs; (3) pre-hatched; (4) hatched, yolk-sac larvae; (5) free-swimming, yolk-sac absorbed.

Replacements for females in SPEXC and ALLEXC treatments were selected randomly from the conditioning tanks. Male and females mortalities were replaced using fish from the same original broodstock population maintained as single sex groups in seperate tanks. The

undisturbed treatment tank (NODIS) was checked for free swimming fry 5–6 times daily during daylight hours and any fry present were removed immediately.

All broodfish were fed to satiation three times daily with a floating catfish pelleted feed (Chareôn Pokaphand, Bangkok; crude protein content 30%). Feed intake, expressed as % body weight/day, was calculated from summing the daily feed portions and mean total weight of fish in the system.

After the 125-day trial was completed, experimental fish were restocked and management continued for 30 days with a reduced IHI of 5 days. Variance of individual seed production data was greater than the mean and contained zero values, therefore data were transformed using log $(x + 1)$ before comparing means using a t-test to detect significant differences at the 0·05 level of probability. A Spearman rank correlation coefficient (r^s) was used to described the relationship between seed stage and individual weight change of spawned female fish (Fowler & Cohen 1990).

Results

Removal of seed from the mouths of incubating females every 10 days significantly improved yields from 31 seed/kg/day (NODIS treatment) to 106 seed/kg/day (Table 1, $P < 0{\cdot}001$). Exchange of female fish further improved productivity by more than 50%, with exchange of all females resulting in statistically higher yields (274 seed/kg/day) than exchange of only spawned females (160 seed/kg/day). Reducing interharvest interval (IHI) from 10 to 5 days appeared to improve individual productivity (seed/kg female/day), but this was only significant ($P < 0{\cdot}05$) when compared to the non-exchange treatment (NOEXC). There were no significant differences in the productivity of individual females among treatments when comparing seed productivity at 5-day intervals. By using the shorter IHI of 5 days, there was no release of seed into the tank between harvest periods (Table 1).

The amounts of early spawned (stage 4–5) and late spawned (stage 1–2) seed collected indicated that spawning synchrony was positively correlated to female exchange (Fig. 1). Exchange of female broodfish stimulated early spawning. Exchange of all females was more effective than the exchange of spawned females only ($P < 0{\cdot}05$). Thus, relatively few late spawned seed were harvested from exchanged females, whilst more then 50% of seed

Table 1. Yield of seed (eggs and fry) of female broodfish under different management regimes held in spawning tanks before and after a reduction in interharvest interval from 10 to 5 days

	Mean seed number/kg female/day			
	Spawning period (days)			
	10		5	
Treatment	Tank	Mouth	Tank	Mouth
NODIS	31·1	–	7·9	–
NOEXC	23·3	82·6	–	278·5
SPEXC	37·2	122·6	–	231·6
ALLEXC	48·8	225·6	–	321·7

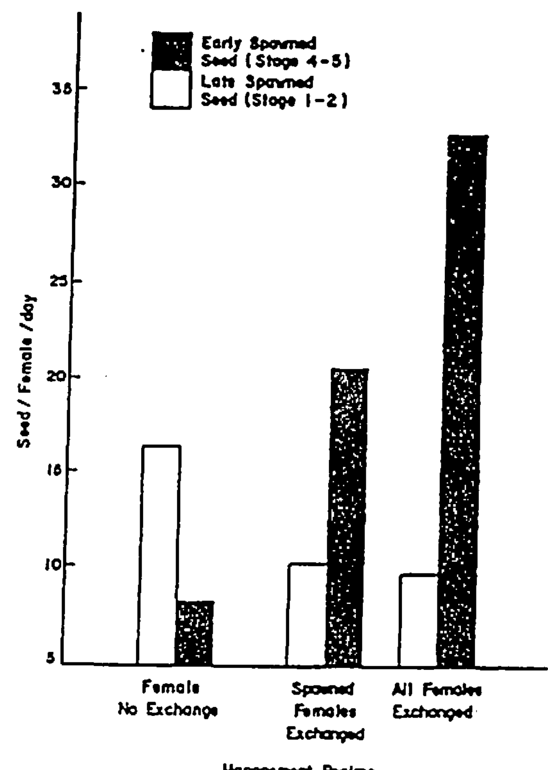

Figure 1. Mean numbers of early spawned (stage 4–5) and late spawned (stage 1–2) seed harvested from individual spawning female *O. niloticus* broodfish per day stocked in spawning tanks under different management regimes over a 125-day experimental period.

harvested from females maintained continuously in the spawning tank were late spawned. The greater numbers of free-swimming fry removed from tanks in which more females were exchanged is also consistent with the conclusion that exchange of all females stimulated earlier, more synchronous spawning (Table 1).

Broodstock management and the resultant difference in timing of spawning had a significant effect on growth characteristics of broodfish; both during incubation and recovery, and over the experiment as a whole. A negative correlation ($P < 0{\cdot}01$) was shown between the duration of mouthbrooding (= seed stage) and weight loss of females in all treatments involving disturbing broodfish for seed harvest (NOEXC, $r_s = -0{\cdot}69$; SPEXC, $r_s = -0{\cdot}66$; ALLEXE, $r_s = -0{\cdot}70$). A positive correlation between duration of mouthbrooding and weight gain during the period after seed removal was only shown for females exchanged as a complete group (ALLEXE, $r_s = 0{\cdot}37$). Undisturbed fish (NODIS) fed more intensively (2·6% body weight/day) than in disturbed treatments (1·3–1·4% body weight/day) and gained weight faster than those in the disturbed treatments (females 1·5 g/day compared with 0·6–1·2 g/day, $P < 0{\cdot}05$). Male fish grew best in NODIS treatment (3·0 g/day) and least in NOEXC (1·9 g/day). Treatments with exchange of females were intermediate (NOEXC and SPEXC, 2·4 g/day).

Discussion

Removal of seed from incubating females and exchange of female. *O. niloticus* after a short spawning period for conditioned (rested) females appears to enhance both individual egg and fry yield and synchrony of spawning. Regular harvest of seed from incubating females increased production by a factor of 3 to 40 compared to harvesting fry from an undisturbed population. Spawning activity appeared low in the NODIS treatment but poor incubation success and/or high levels of cannibalism may also have reduced seed yields (Snow, Berrios-Hernandez & Ye 1983; Gregory 1987). Frequent and complete harvest of seed by draining the spawning tanks prevented the build-up of cannibalistic, larger fry and reduced the duration of incubation by mouthbrooding females. Early removal of eggs has frequently been reported to reduce the interspawning interval of female tilapias (e.g. Peters 1983; Lee 1979; Rana 1986) and this may also partly explain the higher seed yields obtained in treatments where seed was removed from the females.

Inhibition of spawning during conditioning (SPEXC, ALLEXC) appeared to encourage females both to spawn earlier and to produce more seed per day once stocked in the spawning tank. Conditioning for 10 days did not appear to cause the atretic deterioration in egg quality that Peters (1983) observed in fish maintained for a period of a week after maturation. Another study indicated that conditioning periods longer than 10 days reduced productivity (Little *et al.*, in press). Both high fish density and stocking as all female groups were probably critical to 'conditioning'. Other studies have shown that ovulation and oviposition do occur in female-only groups (Silverman 1978a,b), but high densities are known to inhibit spawning (Balarin & Haller 1982; Coche 1982).

The effect of reduced IHI points to the advantages of frequent seed harvest for hatchery productivity. Although the trial was continued for a short time, it is unlikely that the increase in productivity observed was simply a short-term phenomenon. A subsequent experiment in the same tanks indicated that seed yields of over 350 seed/kg female/day could be maintained over 200 days if fish were conditioned for 10 days prior to a spawning period of 5 days (Little 1990).

Differences in spawning synchrony, as indicated by seed stage at harvest, explained changes in female weight during the spawning period. Earlier, more synchronous spawning in exchange treatments (ALLEXC, SPEXC) resulted in a longer period of oral incubation and greater weight loss. Spawned females from the total exchange treatment (ALLEXC), which spawned early and therefore incubated longest, subsequently showed more weight gain during the conditioning period than females that had spawned later and incubated for a shorter period. This trend was not shown by the other treatments, perhaps due to competition with non-spawning fish in the spawning (NOEXC) and conditioning (SPEXC) tanks respectively. Growth rate of males was related to breeding synchrony. Apparently, when breeding synchrony was pronounced (ALLEXC, SPEXC), feeding by males was less restricted by reproductive activity and/or competition with females already mouthbrooding and thus unable to eat. The establishment of hierarchies among fish in stable communities which inhibits reproduction has been described for *Oreochromis* spp. (Fishelson 1983; Turner 1986). This phenomenon may not only be a major cause of the low spawning activity observed in treatment NODIS, but may also explain the differential spawning intensity noted among the disturbed treatments. Turner (1986) suggested that stress during harvest could entirely disrupt the structure of territories and social relationships between fish. The exchange of

females after harvest might accentuate this effect, thereby allowing subordinate fish to breed more frequently and synchronously.

Undeveloped *Oreochromis* eggs harvested from the female's mouth have proved difficult to incubate artifically (Rothbard & Pruginin 1975) and improved breeding synchrony ensures that a high proportion of harvested seed are more developed and easier to incubate artifically. If suitable incubation facilities are available, synchronized spawning allows a reduced IHI and much more efficient use of the spawning area.

Acknowledgments

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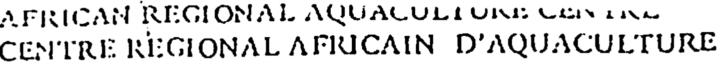

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MASS SYNCHRONIZED SPAWNING OF *TILAPIA GUINEENSIS*

D. Campbell, A. T. Mahatane, And S. O: Aleem

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Mass synchronized spawning of *Tilapia guineensis*

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MASS SYNCHRONIZED SPAWNING OF *TILAPIA GUINEENSIS* AFRICAN REGIONAL AQUACULTURE CENTRE, RAF/82/009

D. CAMPBELL, A.T. MAHATANE* AND S. O. ALEEM

ABSTRACT

A technique for mass synchronized spawning of *Tilapia guineensis* is described. By introducing a single couple into each of a series of 25,0.4m^3 concrete tanks, 102 spawns were obtained from a total of 179 couples with 1355 ± 726 (mean ± SD) fry produced per spawn. Using fish captured from the wild, 64% spawned in 3.3 ± 2.01 days. 48% of the fish that had been previously used and kept in ponds spawned in 3.8 ± 5.75 days. Fish produced during a one week period were of sufficiently uniform size to avoid cannibalism when introduced into ponds.

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Tilapia guineensis (Bleeker, 1862) is a euryhaline species found along the West Coast of Africa from Senegal to Angola (Philippart and Ruwet, 1982). A substratum spawner, the fish will reproduce in ponds. However, the newly hatched fry weigh only about 2mg (Legendre, 1983) and easily fall prey to other species, particularly *Sarotherodon melanotheron* fry (FAO, 1969; Dadzie, 1981). Although not readily apparent in pond systems, cannibalism by both larger fry and adults of *T. guineensis* is also a problem, particularly at the yolk sac stage (Campbell, unpubl.). Survival in spawning ponds is very poor, and production of juveniles has been a problem when attempting to culture the species in the Niger River Delta.

A simple means of producing enough seed fish for stocking is necessary for aquaculture development. This paper presents the results obtained from an attempt to produce *T. guineensis* fry in large numbers and the behavioural differences observed between wild and used broodfish in the degree of synchronization of spawning.

MATERIALS AND METHODS

The work was from September 1985 to April 1986 at the Buguma brackish water fish farm located in the Niger River Delta. 25 separate concrete tanks measuring 65 X 90 X 70cm or $0.4m^3$ were used to spawn the fish. Each tank was filled to 50cm depth giving about 300 l volume of water. The water was filtered and pumped from tidal ponds. Salinity was 11 - 20 ppt and water temperature 29-34°C during the period of the experiments. After filling, agricultural lime ($CaCO_3$) was sprinkled in the tanks to precipitate suspended matter allowing clear observation of the tank bottom.

Once the tanks were filled there was no further water circulation or exchange.

A spawning cycle or test began by draining and cleaning all the tanks and filling them with new brackish water. In each test 40 - 50 sexually mature fish with a standard length ranging from 8 - 16cm (15 - 220g) were visually sexed and a male and female of the same size were introduced into each tank. Fish were chosen and paired with no attempt made to ascertain their readiness to spawn. Each spawning cycle lasted 2 - 3 weeks and the fish were not fed throughout the experiment. The tanks were monitored at least 3 times daily for the presence of fertilized eggs. As soon as the eggs were found, the male fish was removed to avoid cannibalism. The female was left to guard and ventilate the eggs. The fry were collected and transferred to a nursery pond when the yolk sack was absorbed.

Broodfish were initially captured by seining fish from unused tidal ponds and were referred to as "wild" broodfish. After completion of a cycle, all fish used in the test were placed in a 200 or 400m^2 earthen pond with a density of 0.2 - 0.5 fish/m^2. The ponds were initially limed ($CaCO_3$) at a rate of 10kg/are and fertilized with inorganic fertilizer (N:P:K 15:15:15) at an initial rate of 5kg/are. Bi-monthly fertilization at a rate of 2.5kg/are continued as long as the fish were in the ponds, and dried brewers waste was given as a supplementary feed. In further spawning cycles, these fish were referred to as "used" broodfish.

In each cycle, some mortality of the broodfish occurred, usually due to poor handling when stocking, but on some occasions fish would jump from the tanks, be killed in fighting, or remove the drain plug from the tank when attempting to build a nest.

These fish were not replaced during that particular test, nor taken into account for further calculations.

The time from stocking to spawning was recorded. In each cycle, the percentage of couples spawning was determined from the number of couples which spawned or remained at the end of the cycle, and mortality was not considered. Used and wild broodfish were compared for days to spawning and percentage spawning using Students' t test. To study the relationship between the standard length of the female and the number of fry produced, the result of 25 individual spawns were counted and the female measured.

RESULTS

Nine spawning cycles were completed producing over 120,000 fry. A total of 102 spawns were recorded, with a mean number of 1355 $\pm$ 726 fry per spawn. In analysing the results, a difference became apparent in spawning behavior and degree of synchronization between stocks of wild and used broodfish. These differences are presented in Table I.

TABLE I

Results of spawning *T. guineensis*.

	Wild	Used	Totals
Total number of couples	96	83	179
Total spawning	62	40	102
Mean days to spawning	3.3$\pm$2.01	8.8$\pm$5.75	
Number of trials	5	4	9
Percent of couples spawning per trial			
– mean	64 %	48 %	
– minimum	50 %	35 %	
– maximum	88 %	60 %	

The 3.3 mean days to spawning of the wild fish is significantly different ($P<0.05$) from the value of 8.8 days from the used broodfish. Of all the wild broodfish introduced into the tanks, 44% spawned within 3 days, and 62% within the first week. In one instance, the fish spawned only 3 hours after stocking, in another 10 hours.

The spawning rate of the wild fish (64%) was not significantly different ($P<0.05$ from the value of the used fish (48%). Moreover, with the used fish, the spawning was more protracted and erratic (Fig. 1).

The number of fry produced per female varied substantially (Table II) although all broods counted originated from wild females.

TABLE II

Standard length of female number of fry produced.

Standard length cm	Minimum number	Maximum number	Mean number	Standard deviation	Number observations
8 - 9	200	1633	707	575	5
9 - 10	531	2301	1145	526	10
10 - 11	1273	2051	1562	359	4
11 - 12	550	2342	1380	894	4
12 - 13	537	1904	1160	592	4
13 - 14	1451	2714	2232	549	4
14 - 15	927	3116	1919	1108	3

Regression analysis gives $Y = 219.9\ X - 1019.5$ ($r = 0.635$) where X is the standard length and Y the number of fry produced. In terms of the number of the number of fry produced and the lowest mortality, fish of 10 - 12 cm standard length gave the best results although this also have been a function of the tank size as the larger fish were certainly more stressed in the small area.

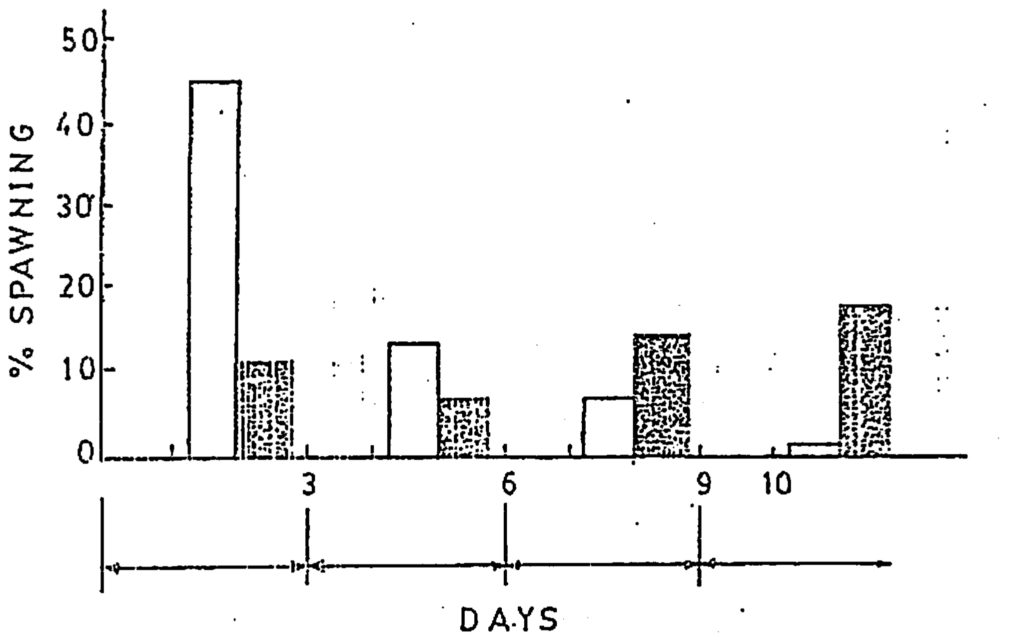

Fig. 1: Percent of broodstock spawning 'wild' and 'used' fish

In each spawning cycle, 10,000 - 35,000 fry were produced. Those spawning in the first week, as was the case of almost all of the spawning fish from the wild, produced fish of a relatively uniform size that could be introduced into a single pond with no fear of cannibalism.

DISCUSSION

The method proved to be a viable means of producing large numbers of *T. guineensis* fry. Using only wild broodfish would be recommended as the time to spawning and the degree of spawning synchronization is significantly better than that of the used broodfish.

The difference may be due to the condition of the fish. Fertilization and supplemental feeding with only dried brewers waste were probably not sufficient to maintain all the fish in good physical condition.

What is more difficult to explain is the degree of synchronization obtained, particularly from the wild fish. In substratum spawners, pair formation, courtship, nest building, and spawning are time consuming and spawning frequency can vary from 24 - 84 days (Fryer and Iles, 1972). Furthermore, the fish were stressed during handling and suffered a complete change of environment. Individuals longer when 15cm standard length would usually die or jump out of the tanks.

With smaller fish, the fact that 44% of the wild fish spawned during the first 3 days, and in some cases in as little as 3 or 10 hours, is remarkable. It could be either that the tank environment stimulates the fish or removes an inhibitory factor present in the wild. The change in substrate, water quality, the absence of predators, or forced association may be the stimulus. Alternatively the stress on the fish during capture and handling might have

stimulated the release of reproductive hormones. It was not possible to determine the stimuli involved.

The concrete tanks used on the farm were previously built for other purposes, and were not necessarily the ideal size or configuration. It is quite possible that better results would have been obtained by using larger tanks. Alternatively very small ponds would perhaps be used. What is important is that this proved to be a reliable technique for producing large numbers of uniform size fry for stocking.

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Predicted Response to Selection for Early Growth in *Tilapia nilotica*

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Abstract

Heritability estimates and genetic and phenotypic correlations were determined for length and weight at 45 and 90 days in the Auburn strain of *Tilapia nilotica*. Heritability estimates were determined by half-sib analysis from an experiment that contained 16 paternal half-sib families or 32 full-sib families, that is, two full-sib families nested in each of 16 paternal half-sib families. Average length and weight were 63 mm and 4.9 g at 45 days, and 99 mm and 18.6 g at 90 days. Sire heritability estimates ranged from 0.04 to 0.10, and dam heritability estimates ranged from −0.02 to 0.54. Dam heritability for length at 45 days was the only heritability estimate that was significantly different from zero ($P = 0.05$). At 45 days, environmental and dominance genetic variance were the major contributors to phenotypic variance, while at 90 days environmental variance accounted for virtually all of the phenotypic variance. Genetic and phenotypic correlations between length and weight at 45 and 90 days ranged from 0.94 to 1.12. Predicted gains from mass selection were small because of low heritabilities and small standard deviations. Low genetic variation may have been caused by drastic reductions in the effective breeding number during transfer of the fish stocks from the Ivory Coast to Brazil and from Brazil to Auburn. Problems caused by reduction of genetic variance as a result of reductions in effective breeding number are discussed.

All breeding work with tilapia has been directed towards the production of monosex populations in order to prevent reproduction and consequent stunting of growth that occurs as a result of their early sexual maturity and high fertility. If selection for early growth is feasible, it may be possible to improve growth so that tilapia reach marketable size before becoming sexually mature and thus eliminate the need for monosex culture.

Before embarking on a selective breeding program it is possible to predict its practicality by estimating the heritability for the trait that will be selected. The heritability of a metric trait (one that can be measured) is one of its most important properties. It expresses the portion of phenotypic variance that is attributable to additive genetic variance. Additive genetic variance is the variance of breeding values and is the most important component of phenotypic variance because it is the chief genetic determinant of the response to selection (Falconer 1960).

In tropical fishponds, *Tilapia nilotica* matures between 4 and 6 months of age (Ruwet et al. 1976). To predict the efficiency of selection for early growth, heritability estimates for length and weight prior to 4 months of age are needed.

This project represents the first quantitative genetic analysis of any *Tilapia* species to determine if growth rate can be improved by selective breeding. The objectives of this study were (1) to estimate heritability for length and weight at 45 and 90 days in *T. nilotica*; (2) to estimate genetic and phenotypic correlations between these traits; and (3) to predict possible gains from mass selection programs.

Methods

Heritability estimates and standard errors were determined from intraclass correlations of paternal half-sib families as described by Becker (1975). In this procedure, phenotypic variance is partitioned into its genetic and environmental variance components by analyzing data gathered from a series of full-sib families (full-

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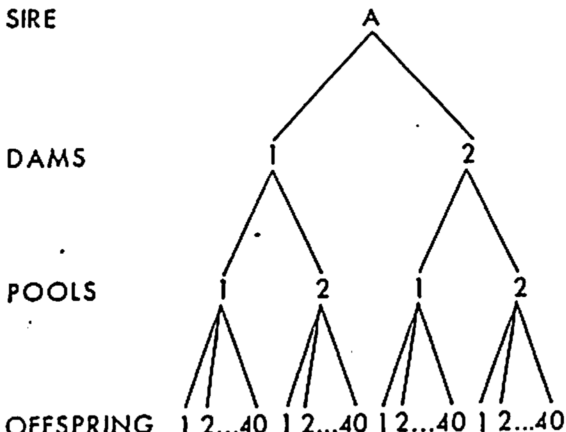

FIGURE 1.—*Schematic diagram of one paternal half-sib family (half-sib family A) or two full-sib families (full-sib families A1 and A2) used to determine heritability estimates and genetic correlations. Sixteen such half-sib families or 32 full-sib families were used in the experiment with* Tilapia nilotica.

sibs share two parents in common) that are nested within a series of half-sib families (half-sibs share one parent in common).

The experimental design used in this experiment was a nested, completely randomized design which contained 100 offspring per pool (40 of which were measured), two pools (replicates) per dam, two dams per sire and 16 sires (Fig. 1).

Estimates of variance components were obtained from the statistical model

$$Y_{ijkl} = \mu + S_i + D_{ij} + E_{ijk} + W_{ijkl},$$

where:

- Y_{ijkl} is the observed phenotype of the lth offspring in the kth pool from the jth dam mated to the ith sire;
- μ is the population mean;
- S_i is the random effect of the ith sire; this effect is assumed to be normally distributed with a mean of 0 and variance σ_s^2 $[N(0, \sigma_s^2)]$;
- D_{ij} is the random effect of the jth dam mated to the ith sire; $[N(0, \sigma_d^2)]$;
- E_{ijk} is the random effect of the kth pool within the ijth family; $[N(0, \sigma_e^2)]$;
- W_{ijkl} is the environmental and genetic deviation of the individual fish from the pool mean; $[N(0, \sigma_w^2)]$.

Variance components used to calculate heritability estimates were determined by analysis of variance (Table 1). Each sire group was a half-sib family, so σ_s^2 was the covariance of half-sibs; each dam contributed a full-sib family, so σ_d^2 was the covariance of full-sibs less the covariance of half-sibs; the environmental variance component and the remaining genetic variance components are found in σ_w^2. The genetic and environmental composition of variance are described in Becker (1975). All components of phenotypic variance could not be estimated within the scope of the experiment so it was assumed that epistatic variance and maternal variance were zero. Once those assumptions were made, then

$$\sigma_s^2 = \tfrac{1}{4} V_A;$$
$$\sigma_d^2 = \tfrac{1}{4} V_A + \tfrac{1}{4} V_D;$$
$$\sigma_w^2 = \tfrac{1}{2} V_A + \tfrac{3}{4} V_D + V_E;$$

where V_A is the additive genetic variance, V_D is the dominance genetic variance and V_E is the environmental variance components of phenotypic variance (V_P); V_A, V_D, and V_E were determined by simultaneous solution of the mean squares.

Sire heritability estimates (h_s^2) and dam heritability estimates (h_d^2) were determined as follows:

$$h_s^2 = \frac{4\sigma_s^2}{\sigma_s^2 + \sigma_d^2 + \sigma_e^2 + \sigma_w^2};$$

$$h_d^2 = \frac{4\sigma_d^2}{\sigma_s^2 + \sigma_d^2 + \sigma_e^2 + \sigma_w^2};$$

where σ_s^2, σ_d^2, σ_e^2, and σ_w^2 are now estimates. Sire estimates of heritability contained only V_A and were accurate estimates ($h_s^2 = V_A/V_P$); h_d^2 contained both V_A and V_D and were an indication of the upper limit of heritability, $h_d^2 = (V_A + V_D)/V_P$.

When two or more traits are measured simultaneously, correlations between the traits can be determined by analysis of covariance as described by Becker (1975). Genetic correlations and phenotypic correlations between length and weight at 45 and at 90 days, and the standard errors for genetic correlations were calculated. Fish were not individually identified, so it was not possible to pair lengths and weights between the 45- and the 90-day samples.

Spawning

To produce the paternal half-sib families, a series of 80 breeding sets, each consisting of

TABLE 1.—*Analysis of variance that was used to determine variance components of heritability estimates for* Tilapia nilotica.

Source	df	Expectation of mean squares
Total (N)	$N - 1$	
Sires (S, s)	$S - 1$	$\sigma_w^2 + 40\sigma_p^2 + 80\sigma_d^2 + 160\sigma_s^2$
Dams (D, d)/Sires	$S(D - 1)$	$\sigma_w^2 + 40\sigma_p^2 + 80\sigma_d^2$
Pools (P, p)	$D(P - 1)$	$\sigma_w^2 + 40\sigma_p^2$
Within (offspring) (W, w)	$P(W - 1)$	σ_w^2

one male and three females that were randomly chosen, were established. Each breeding set was assigned to a circular plastic pool, 3.05 m in diameter, on July 23, 1978. Females in all pools were checked daily for spawning activity. Females incubating eggs or fry could be easily detected because they become territorial and their mouths become melanic. As soon as a female released her fry they were removed by seine and dip net (7 meshes/cm). The territorial nature of the brooding female and the habit of newly released fry of remaining in close proximity to the mother allowed the collection of each family with no mixing. Each family was then transferred to one of 10 nursery pools and placed in separate cages (7 meshes/cm) to maintain its integrity.

One of the major goals of the experiment was to produce the families within a short period because initial size advantages are important and are maintained (Wohlfarth and Moav 1972; Hulata et al. 1976). This was accomplished when 89 families were collected during an 8-day period, July 30–August 6. Sixteen paternal half-sib families or 32 full-sib families were randomly chosen for the experiment.

Stocking

The spawning pools were drained and refilled to a depth of 71 cm; each pool contained 5,200 liters of water. On August 14, each pool was fertilized with 44 kg/hectare of 20-20-5 fertilizer and 55 kg/hectare of Auburn Number 3 fish ration (44% protein) ground to a meal. On August 17, the 32 families were stocked with each family being randomly assigned to two pools. One hundred fry from each family were stocked in each of the two replicates. The average length of the fry at stocking was 14 mm.

Feeding

Tilapia were fed ground Auburn Number 3 ration throughout the experiment. While in the cages, the fry were fed the fine meal three times daily; after stocking, the fish were fed twice daily. A fine meal was used so that food particle size could not become a source of environmentally induced length variance as has been shown in carp (Wohlfarth 1977). Feeding rates were gradually increased from 6.7 kg/hectare-day on August 17 to 47.5 kg/hectare-day on October 6. To prevent possible oxygen depletion in pools, the daily ration was not increased after October 6.

Sampling

Forty randomly chosen fish from each replicate of each family were measured to the nearest millimeter and weighed to the nearest 0.1 g 45 and 90 days after their collection. The fish used in the 45-day sample were treated with acriflavin to help prevent disease problems arising from possible stress and were restocked.

Temperature

On October 4, all pools were covered with 0.15-mm clear polyethylene in order to maintain warm water temperatures despite dropping air temperatures due to seasonal changes. Daily maximum and minimum water temperatures were taken at a depth of 23 cm from August 24 until the experiment was terminated. Average maximum and minimum water temperatures during the experiment were 27 and 22 C, respectively.

TABLE 2.—*Mean, standard deviation, and coefficient of variation (CV = 100·SD/mean) for length at stocking and for length and weight at 45 and 90 days for* Tilapia nilotica.

	Mean	SD	CV
Stocking length (mm)	14	2	14
45-day length (mm)	63	5	8
45-day weight (g)	4.9	1.3	26.5
90-day length (mm)	99	8	8
90-day weight (g)	18.6	5.1	27.4

TABLE 3.—*Analysis of variance and the derived variance components for length and weight at 45 and 90 days in Tilapia nilotica.*

		Variance components			
		45-day		90-day	
Source	df	Length	Weight	Length	Weight
Sire	15	0.880	0.022	1.250	0.218
Dam	16	4.794	0.173	−0.397	0.290
Pool	32	1.794	0.161	4.997	1.853
Within (offspring)	2,490	27.743	1.630	70.591	25.587

Results and Discussion

The fish grew from an average size of 14 mm at stocking to 63 mm and 4.9 g at 45 days, and 99 mm and 18.6 g at 90 days (Table 2). Survival rates were 95% or greater in 81% of the pools, and 91% or greater in 94% of the pools. Only four pools had survival rates less than 91%, the lowest being 72%.

Heritability Estimates

Variance components that were used to calculate heritability are presented in Table 3, and heritability estimates are present in Table 4. The only heritability estimate that was significantly different ($P = 0.05$) from zero was h_d^2 for length at 45 days. The h_d^2 showed a marked, although not significant, decrease between 45 and 90 days. At 45 days, environmental and dominance genetic variance were the greatest contributors to phenotypic variance, while at 90 days the environmental variance component accounted for virtually all of the phenotypic variance (Table 5). A possible explanation for this change is that the fish were growing rapidly and undergoing rapid physiological changes during this period, and different genes were operating at the two ages; thus the changes in V_A, V_D, V_E, and heritability. The negative h_d^2 for length at 90 days is a biological impossibility. It is the result of sampling or statistical error. The negative value suggests that true heritability is low (Gill and Jensen 1968).

TABLE 4.—*Sire* (h_s^2) *and dam* (h_d^2) *heritability estimates ± SE for length and weight of* Tilapia nilotica *at 45 and 90 days.*

	h_s^2	h_d^2
45-day length	0.10 ± 0.19	0.54 ± 0.23
45-day weight	0.04 ± 0.14	0.35 ± 0.19
90-day length	0.06 ± 0.06	−0.02 ± 0.07
90-day weight	0.04 ± 0.06	0.04 ± 0.08

The low heritability estimates discovered for *T. nilotica* in this study are in agreement with those found for length, weight, or growth in carp (*Cyprinus carpio*: Moav and Wohlfarth 1968, 1976; Kirpichnikov 1971), rainbow trout (*Salmo gairdneri*: Aulstad et al. 1972; Müller et al. 1979), and Atlantic salmon (*Salmo salar*: Ryman 1972; Refstie and Steine 1978).

Large heritability estimates for length, weight or growth have been reported for Atlantic salmon (Gunnes and Gjedrem 1978), channel catfish (*Ictalurus punctatus*: Reagan et al. 1976) and rainbow trout (von Limbach 1970; Aulstad et al. 1972). Sire heritabilities for length and weight in Atlantic salmon are large (0.31–0.38) and are significantly different from zero. However, the standard errors associated with the heritability estimates for channel catfish and rainbow trout render them not significantly different from zero. Additionally, the heritability estimates for length and weight for channel catfish are probably artificially inflated because there was assortative mating, and the families with larger fish were fed more than those with smaller fish.

Gall (1975), Chevassus (1976), and Gall and Gross (1978a, 1978b) reported moderately high heritability estimates (0.2–0.3) with small standard errors for body size and weight of rainbow trout. However, the heritability estimates were determined by correlations of full-sibs ($h^2 = V_A + \frac{1}{2}V_D/V_P$), and this is an inflated estimate of true heritability ($h^2 = V_A/V_P$).

Correlations

Genetic and phenotypic correlations were calculated and are shown in Table 6. Large positive correlations were expected, and the find

TABLE 5.—Additive (V_A), dominance (V_D), and environmental (V_E) variances expressed as a percent of the total variance for length and weight at 45 and 90 days in Tilapia nilotica.

	V_A (%)	V_D (%)	V_E (%)
45-day length	11	47	42
45-day weight	5	33	62
90-day length	7	0ᵃ	93
90-day weight	4	1	95

ᵃThis was assumed to be zero for ease in calculation; $\sigma_D^2 < 0$.

TABLE 6.—Sire (r_s) and dam (r_d) estimates of genetic correlations and phenotypic (r_p) correlations between length and weight at 45 and at 90 days, and the standard error for genetic correlations in Tilapia nilotica.

Day	r_s	r_d	r_p
45	1.09 ± 1.31	0.98 ± 0.61	0.94
90	1.12 ± 1.61	ᵃ	1.01

ᵃ $\sigma_d^2 < 0$ so r_d could not be calculated.

ings are consistent with those found for length and weight in channel catfish (Reagan et al. 1976) and Atlantic salmon (Gunnes and Gjedrem 1978; Refstie and Steine 1978). These results indicate that (1) selection for one trait will also improve the other; (2) weight can be accurately estimated by measuring total length.

Responses to Selection

Response to individual selection (mass selection) can be predicted using the following formula (Falconer 1960):

$$R = i\sigma_p h^2;$$

where R is the response per generation of selection, i is the selection intensity, σ_p is the standard deviation of the trait under selection, and h^2 is the heritability of the trait under selection.

Although heritability estimates for *T. nilotica* were smaller at 90 days, the predicted responses to selection were larger (Table 7). This is because the standard deviations were larger at 90 days. In either case, the predicted responses to selection were small. The small heritabilities coupled with small phenotypic variances indicate that there is little genotypic variance in the Auburn strain of *T. nilotica*, thus making progress through mass selection difficult.

Indirect selection is used when direct selection is difficult because the desired trait has low heritability or is difficult to measure. Weight is more important than length in marketable fish, but direct selection for weight is difficult because it is hard to obtain accurate weights. The large positive genetic correlations between length and weight indicate that indirect selection for weight by direct selection on length would be possible, but low heritabilities and small variances make it doubtful that indirect selection for weight would be of any practical use for this strain of *T. nilotica*.

An explanation for the lack of genetic variation in the Auburn strain of *T. nilotica* is the history of the fish. The *T. nilotica* at Auburn came from The Centro de Pesquisas Ictiológicas, Pentecoste, Ceará, Brazil, in 1974. Auburn's foundation stock was between 100 and 200 small juveniles that were seined from a single pond that contained an estimated 5 to 10 pairs of brooders. The foundation stock for the fish station at Pentecoste was 50 to 100 fish which had come from the Station de Recherches Piscicoles, Bouake, Ivory Coast, in 1971 (L. L. Lovshin, Auburn University, personal communication). The Auburn strain of *T. nilotica* has thus undergone at least two reductions in effective breeding number, and it is very likely that these inadequate sample sizes have reduced the genetic variance in the population. The variance data show that even though Auburn maintains around 150 to 200 randomly mating pairs, there is little genetic variance in the Auburn strain of *T. nilotica*.

Should it be necessary, the genetic variation of the Auburn strain of *T. nilotica* could be reestablished by outbreeding the fish to wild African strains or fish from other fish stations. To maximize genetic variation, these fish should be allowed to mate at random for several generations to break up any linkage groups that may exist.

The problem of a narrow genetic base is one that should be given consideration in aquaculture because the unintentional restriction of a gene pool can lead to serious problems. Kincaid (1976) has shown that certain levels of inbreeding can adversely affect survival and weight of rainbow trout. To date, fish culturists have given most of their attention to the development of improved diets, proper stocking densities, et

TABLE 7.—*Predicted responses to three levels of mass selection for one generation for length (mm) and weight (g) in* Tilapia nilotica.

Level of selection	Predicted response for one generation			
	Length at 45 days	Weight at 45 days	Length at 90 days	Weight at 90 days
1%[a]	1.4	0.2	1.5	0.5
2½%[b]	1.2	0.1	1.3	0.4
10%[c]	0.9	0.1	1.0	0.3

[a] Corresponds to selecting 1 out of 100 fish.
[b] Corresponds to selecting 5 out of 200 fish.
[c] Corresponds to selecting 10 out of 100 fish.

cetera, but yields can not be optimized if genetically inferior fish are used. An additional problem is that the environmental improvements and manipulations that are developed for a population with a narrow genetic base may only be effective for such a gene pool and may not be applicable to other populations. It is imperative that proper care be taken to avoid the unintentional restriction of gene pools and unintentional inbreeding in fish stocks. If this is not done, much of the effort directed towards environmental improvements in aquaculture may be wasted.

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Abstract:

The seed of *Oreochromis niloticus* can be produced using a variety of methods which are variably intensive with respect to the use of broodfish, concentrate feeds, land,and water. Criteria are defined for various measures of efficiency allowing comparison between systems under different economic conditions. In addition to the normal productivity measures (e.g. seed/kg female/month and seed/m^2/day) seed output/unit concentrate feed and seed output/unit water used are compared. System net yields are contrasted in which the output of marketable seed, recruits and growth of broodfish is compared. Earthen ponds produced first feeding fry in numbers 2-100 times more than clearwater tanks and 1-5 times more than hapas-in-ponds in terms of amount of concentrate feed given. Harvested first feeding fry suitable for hormonal sex reversal represented only between 1.7 and 11.6% of total system net yield the remainder of production consisting of fry too large for sex reversal, recruits and net weight gain of broodfish (depending on system).

Key-words: Efficiency indicator, Tank, Hapas, Earthen Pond, Concentrate Feed.

Introduction:

Oreochromis are unusual among the important cultured fish species for their ease of seed production and yet, paradoxically, for the difficulties that hatcheries face in the synchronous production of large quantities of seed. This is particularly important when first feeding fry are required for hormonal sex reversal, since large numbers are required to make the process economically viable. Techniques such as frequent egg harvest from incubating females are known to improve individual female productivity (Lee, 1979; Verdegem and McGinty, 1987) and exchange of broodfish to improve synchrony of breeding (Little, Macintosh and Edwards, 1993). Commercial hatcheries have begun to adopt these techniques in Thailand. Open pond spawning and collection of first feeding fry as described by Liao and Chen (1983) and Rothbard et al (1983) are still commonly used to mass produce fry suitable for hormonal sex reversal. The adoption of more intensive systems will have consequences for the use of land, labour, water ,broodfish and feeds. The relationship of inputs to outputs of three types of seed production system including removal of first-feeding fry from the perimeter of open earthen ponds, and harvest of seed from mouthbrooding females spawned in hapa-in-ponds and tanks supplied with filtered, recirculated water respectively are compared in the current paper with a view to establishing suitable efficiency parameters.

Methods

Description of systems:

Main features (see Figure 1);

The experimental facilities were based on the campus of the Asian Institute of Technology. All broodfish were obtained from the same stock (*Oreochromis niloticus*, Chitralada strain), previously raised in fertilised earthen ponds and used in experimental units at a sex ratio of 1:1. The quanitiy of harvested seed of all types was estimated by counting and weighing sub-samples of 100 seed and then bulk weighing the total batch to two decimal places.

The earthen ponds (area 1740 m^2) used were made by excavation of a clay soil of the Rangsit series; these soils are characterised by their low permeability. Ponds were filled and maintained to a depth of 1 metre with irrigation water and fertilised using septage at 150 kg COD/ha/day. First feeding fry were removed from the shallow perimeter of ponds by dip-net 3-6 times daily for durations of between 105-116 days.

All seed were removed from incubating females held in hapas and tanks as individual clutches every 5 days and combined on the basis of stage of development (except for one experimental treatment in which free-swimming fry were removed from the perimeter of the tank). Seed was staged at harvest

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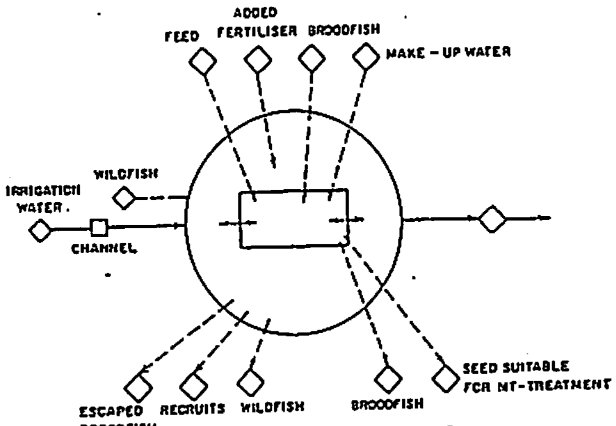
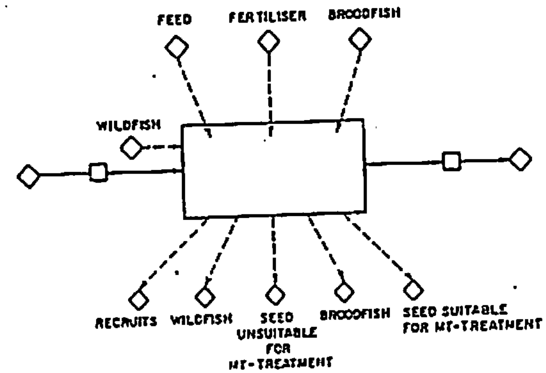
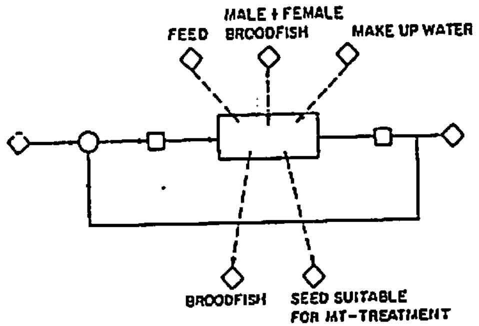
HAPA - IN - POND



EARTHEN POND



TANK RECIRCULATED WATER SYSTEM



LEGEND : after EIFAC , 1986

▭ A rearing unit

◇ Input or output from the system

○ A unit of process that changes water quality so as to make it significantly more or less suitable for rearing organisms within the system

—→ Movement of water or other liquid

- - → Other types of transfers

□ A process necessary to the system but has no significant direct effect on water quality

Fig. 1

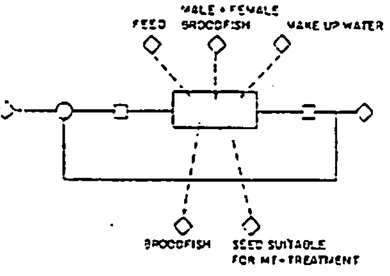


Fig. 1

using an abritary scale of development that could be determined by eye (Little et al, 1993). Mixed batches of seed dropped by the females and recovered form the tank or hapa bottom were estimated separately. All seed was incubated in down-welling incubators receiving water recirculated through a slow-sand filter system to the yolk-sac absorbed stage. Several brrodfish management strategies in tanks and hapas were compared including brrodfish exchnage, variable stocking density and sex ratio/Nylon hapas (area, spawning=40m², conditioning 5.4 m²) were suspended to a depth of 60 cm in similar earthen ponds as above. Circular, concrete tanks (area; spawning= 12.6 m², conditioning 1.8m² depth 1m) were located within a recirculation system giving total water exchange at least three times per day. Seed was removed over experimental periods ranging from to days 106-201 days.

Water consumption in tanks was estimated for ponds and hapas on the basis of mean evaporative losses. Broodfish and other fish in the systems were counted and batch weighed on stocking and final harvest when the systems were drained.

All three systems used a floating catfish pellet (30% crude protein; Charoen Pokaphand) fed three times daily *ad libitum* for breeding and conditioning fish.

Results:

Intensified production of *Oreochromis* fry with respect to breeding area is possible in more controlled environments such as hapas, and particularly, tanks with water exchange. Productivity is still several times higher than in earthen ponds, even if the area required for conditioning fish and the unutilised area between hapas (i.e. the total area) is considered (Table 1). An output of 1 million fry/month requires a minimum of 160 m² of tanks supplied with clean water, 200m² of spawning hapa area or 6000m² of open pond. Efficiency of water use is best in intensive tank breeding of *Oreochromis*, in excess of 10,000 fry/m³ water, compared to less than 500 fry/m³ in open ponds. Incubation of

Table 1 Range of efficiency of water use in the production of *Oreochromis* fry in concrete tank (12.6 m²), hapas-in-pond (40 m²) and earthen pond (1740 m²) systems

	Water Area		Water Volume
	Fry/m² spawning/ day	Fry/m² total/day	Fry/m³
Tanks	14-225	14-196	826-13380
Hapas	30-180	6-45	1300-1888
Ponds	1-6	1-6	110-473

seed harvested from hapas or tanks approximately doubles water requirements however. Irrigation quality water was used in open ponds whereas deep groundwater was the original source of water in the recirculated systems (tank breeding and incubation).

Differences in broodfish productivity were related to the intensity of management. Broodfish condition can be maintained and breeding synchrony enhanced in tank and hapa systems when fish are handled, and seed harvested, frequently (Little et al, 1993). The complete harvest of seed in such systems is also important in the reduction of cannibalism related losses. Productivity in tanks in which free-swimming fry were removed daily (rather than by harvest of eggs and yolk-sac fry direct from the mouth) was very low (360/kg female/month, Table 2).

Highly skewed sex ratios improved the output of fry per kg male. A sex ratio of 4:1 (female:male) gave more than double the fry output compared to a sex ratio of 1:1 (4556 compared to 10765 fry/kg male/month). Frequent harvest of fry (6x daily) and the stocking of small broodfish optimised output of fry/kg female in earthen ponds.

Table 2 Range of *Oreochromis* broodfish productivity in concrete tank (12.6 m²), hapas-in-pond' (40 m²) and earthen pond (1740 m²) systems

	Broodfish	
	Fry/kg female/month	Fry/kg male/month
Tanks	361-4523	
Hapas	2729-8463	2118-10765
Ponds	2022-5077	

Fertilised earthen ponds were 2-40 times more efficient than tanks in the use of pelleted feed (Table 3). Efficiency of feed use in hapas was intermediate. Typical system FCR's were under 3 and the proportion of net production as first feeding fry suitable for sex reversal was under 15% for all systems. Broodfish growth made up most of the net production in tanks and hapas (Table 4), but the growth of recruits was more important in earthen ponds in which harvest of fry was incomplete.

Discussion:

Oreochromis spp. are characterised by their high degree of parental care of small batches of seed. These features make an analysis of their seed production efficiency more comparable to livestock than other commercial fish species in which induced spawning of a few fecund individuals can produce many seed at the same time. The relevance of improved seed production efficiency is clearer for *Orechromis* than, for instance, the carps in which fecundities of 100,000 eggs/female are the norm.

The batch spawning habit of *Oreochromis* also necessitates that the basis for comparison between systems is clear and relevant to commercial systems since data obtained from short term studies (less than 3 months) do not reflect commercial conditions. Early peaks in seed production followed by rapid declines are a feature of *Oreochromis* fry production systems (e.g. Guerrero and Guererro, 1985) and can lead to elevated productivity predictions.

Conventional production of tilapia seed in which spawning and nursing occur in the same pond has been described (Broussard et al, 1983), as have adaptions to this method to produce seed suitable for sex reversal, both continuously (Liao and Chen,

Table 3 Range of feed utilisation efficiency in *Oreochromis* fry production in concrete tank (12.6 m²), hapas-in-pond (40 m²) and earthen pond (1740 m²) systems.

	Feed	
	Fry/g feed	System FCR
Tanks	< 1-5	1.9-2.7
Hapas	2-10	1.5-2.5
Ponds	9-40	0.2

Table 4 Range of yields of yolk-sac absorped fry and source of byproduct yield by type from concrete tank, hapa-in-pond and earthen pond systems.

	Byproducts			
	Yolk-sac absorped fry as a % of net yield	Type of Byproduct		
		Broodfish	Recruit	Large fry
Tanks	5.7-11.6	✓	-	-
Hapas	2.8-12.8	✓	-	-
Ponds	1.7-7.8	✓	✓	✓

1983) and by batch (Rothbard et al, 1983). Practically, the poor productivity and sustainability of seed output from such systems can be counteracted by an increase in production area but this option may not be possible if land costs are high. In Northeast Thailand the high opportunity cost of hatchery land was identified as being a major constraint to extensive, open pond tilapia fry production (Little et al, 1987). Consideration of seed output in terms of *production* area or *total* area, particularly in the case of hapa systems, will depend on whether hapas are suspended in earthen ponds (high opportunity cost) or in communal waters (Beveridge, 1984). When hapas are suspended in a lake or resevoir the seed output/area of spawning hapa is of more relevance to a hatchery operator than output per unit area of lake or reservoir.

Quality of water is a major factor in the sustainability of yields and the higher output of fry from tanks in this study reflects the higher initial quality of (ground) water used compared to the surface, irrigation water in ponds. Hapa-in-pond systems are also more subject to variability in water quality than tanks supplied with water recirculated through a suitable biofilter (Little, 1990). *Oreochromis* seed yields in hapas and tanks using water from Laguna lake were affected by seasonal declines in quality (Batao, 1988). Other studies in which seed production was monitored in hapas in tanks (e.g. Hughes and Behrends, 1983) and/or for only short culture periods may not be affected by such water quality deterioration. In contrast to the low productivity per unit area, earthen ponds can be more efficient in terms of seed/kg broodfish than hapas or tanks, despite the incomplete harvest of fry inherent in this method. The importance of hierarchy in the control of *Oreochromis* breeding in arenas has been indicated (Turner, 1986 Little et al, 1993) and suggests that this can be a major constraint in individual fish productivity within intensive systems.

Differences in the value of male or female broodfish will affect the importance of output/kg broodfish. The YY males used to produce all male *Oreochromis* without direct use of hormones, for example, are expensive and fry output/kg male will be a more important efficiency indicator than seed/kg female or seed/kg of all broodfish.

Stocking broodfish at low density in fertilised earthen ponds improved the efficiency of concentrate feed use (fry/kg feed) compared to more intensive systems. The concept of 'sparing' concentrates with natural feeds for phytophagous fish raised in semi-intensive systems (Hepher, 1988) can be cost effectively applied to *Oreochromis* broodfish management. This efficency parameter could also indicate overfeeding and in the determining optimal strategies where cost of feed or logistics restrict their use.

The system feed conversion ratio reflects the growth of broodfish rather than seed production and although earthen ponds appear efficient in terms iof feed use, fry yields are low when expressed as a percentage of total net production. MT-treatable fry represent only a small proportion of total net yield, even in intensive systems. The nature of byproducts reflect the management of broodfish and seed harvest. Broodfish growth is the main source of production in tanks and hapas where seed harvest is regular and complete but recruits, derived from fry that evaded harvest earlier in the production cycle, make up most of the final yield in earthen ponds. Larger fry of indeterminate age were also removed in considerable numbers from earthen ponds at the same time as treatable fry but were not considered sex reversible. Liao and Chen (1983) do not mention these fry in their description of practices in Taiwan and the twenty day complete cycle described by Rothbard et al (1983) ensures near synchronous breeding and similar aged/sized fry suitable for sex reversal. Popma (1987) describes the successful sex reversal of larger, uneven-sized fry after harvest by edge seining.

There are many other factors that could be considered when evaluating different fry production systems. Yields in terms of labour, for example, (found to be similar for ponds, hapas or tanks; Little, 1990) are very important. The choice of system for *Oreochromis* seed production should also consider the ease of drainage of tank and pond systems, availability of suitable hapa material and sites for their use, and the risks associated with contamination of broodfish with wild breeding populations.

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PRACTICAL GUIDE
ON
MODULE 2b: Marking of Tilapia

Introduction:

Generally, fish marking is divided into two. (a) fin clipping which involves removing some of fin or spines and (b) tagging which involves insertion, attachment, or injection of a foreign object or substance. Marking is an efficient method of distinguishing an individual or group of individual fish in a breeding program. The GIFT project is dependent on an efficient marking system to identify the groups, strains, families and individual tilapia. It is also important for tracing pedigrees because each generation of fish is identified based on the unique tags or markings. There are two major type of marking used in the GIFT Project, first is the tagging which uses fingerling floy tag (for fingerlings) and PIT tags (for breeders) to identify individual fish while the second type of marking uses pelvic or pectoral fin-clipping, spine uprooting and plastic discs to identify groups of fish.

Behavioral Objective

Given all the materials necessary for marking the fish and background information on the subject, the trainee will fix and prepare floy tags and plastic disc tags and perform and should be able to:

(a) tag at least 60 fingerlings
(b) PIT tag 10 fish breeders
(c) fin-clip (pectoral and pelvic) 20 fingerlings
(d) uproot dorsal spine of 10 breeders in two hours with minimal stress and without mortality on the fish.

Intermediate Objectives

Discuss the importance of marking in a breeding program
Specify the types of marking suitable for the fish
Fix and prepare floy tags and plastic disc tags
Assemble all the materials needed for marking

Mark the fish by floy, plastic disc, PIT, pectoral, pelvic and dorsal spine uprooting

Materials

A. Fixing and preparation of floy and plastic disc tags

1. Fingerling floy tags
2. Vinyl threads
3. Needles
4. Plastic disc
5. Card board/cartolina paper
6. Paste
7. Pentel pen
8. Ballpen
9. Scissors
10. Scotch tape
11. Bond papers

B. Floy and plastic disc tagging

1. Fingerling and breeders
2. Anesthesia
3. Fixed and prepared floy tags and Pit tags
4. Plastic disc
5. Card board/cartolina paper
6. Paste
7. Pentel pen
8. Ballpen
9. Scissors
10. Scotch tape
11. Bond papers

C. PIT tagging

1. Breeders
2. Anesthesia
3. Towels
4. Tubs
5. Strainers/scoop nets
6. Aerators
7. Clean fresh water
8. Identity tags

9. Implanter (modified syringe)
10. Identity tag reader

D. Fin-clipping and dorsal spine uprooting

1. Towels
2. Anesthesia
3. Basins
4. Strainers
5. Aerators
6. Scoop nets
7. Surgical scissors
8. Tubs
9. Clean aerated fresh water
10. Disinfectant (merthiolate)
11. Cotton ball or cotton buds
12. Hand gloves
13. Tissue paper
14. Masking tapes
15. Record sheets
16. Net covers
17. Tally counters
18. Twissors

Procedures

A. Fixing and Preparation of Floy tags

1. Prepare materials
2. Cut several 6 inch vinyl threads
3. Make single or double knot on one end of the thread
4. Put the tag and make another knot to lock the tag
5. Insert the thread with floy tag into the needle
6. Put plastic disc
7. Arrange in sequence in card board/cartolina paper with the corresponding numbers

B. Floy tagging and Plastic disc tagging

1. Prepare materials (see above)
2. Anesthetize fingerlings in 0.3 g MS222/li of water
3. Position the fingerlings in a swimming position facing your left hand

4. Locate the insertion site between sixth and seventh dorsal spine above the lateral line
5. Insert the needle underneath one scale and insert through the tissue
6. Attach plastic disc for further anchorage to securely lodged the tag
7. Make three loopholes untieable knot
8. Cut the thread with 1-2 inch length for further growth of the flesh
9. Put tagged fish in a recovery basin with clean aerated freshwater

C. PIT Tagging

1. Prepare the materials (see above)
2. Anesthetize the fish 0.3 g or more depending on the size of the fish
3. Surgeon/implanter's position with elbows braced against body
4. Load identity tag in the needle line up the tip of the tag with the slot in the needle
5. Position the fish lay on its right side with the head of the fish facing your left hand
6. Locate insertion site on the left ventral side of the fish adjacent to the anus, soft area
7. Insert needle underneath one scale and insert through the tissue
8. Post operative recovery spacious basin with clean, aerated freshwater

D. Fin-clipping

Pelvic fin-clipping

1. Prepare the materials (see above)
2. Anesthetize the fish in 0.3 g or more depending on the size of the fish
3. Hold the fish at the anterior head portion, tail end towards your body
4. Turn the fish ventrally and cut the pelvic fin at the base
5. Apply disinfectant on the wound with cotton balls or buds
6. Put in recovery tubs, with clean aerated fresh water

Pectoral fin-clipping

1. Prepare the materials (see above)
2. Anesthetize the fish in 0.3 g or more depending on the size of the fish
3. Hold the fish at the anterior head portion, tail end towards your body
4. Turn the fish on its side and cut the pectoral fin at the base
5. Apply disinfectant on the wound with cotton balls or buds
6. Put in recovery tubs, with clean aerated fresh water

Dorsal spine uprooting

1. Prepare the materials (see above)
2. Anesthetize the fish in 0.3 g or more depending on the size of the fish
3. Hold the fish at the anterior head portion, tail end towards your body
4. Position the fish in swimming position, covering the eyes with white towel
5. Spread the web of dorsal spines, count from the first anterior and locate the spine for uprooting. The first dorsal spine should not be cut.
6. Rip or tear membrane on both sides of the spines to be uprooted
7. Hold the spine and bend carefully until the bones break
8. Loosen the broken spine by twisting and moving sideways then uproot with twissor
9. Apply disinfectant on the wound with cotton balls or buds
10. Place in a recovery tubs, with clean aerated freshwater

MASTERY TEST/END OF MODULE TEST

Given the materials for marking the fish, task is to mark (tagging and fin-clipping) the fish with less stress and no mortality. Performance will be observed based on the condition of the fish after marking and the number of fish tagged within one hour period.

PRACTICAL GUIDE/EXERCISE

ON

Module 3a : ASSESSMENT OF SEXUAL MATURATION

INTRODUCTION

A precise method for recording the sexual maturation stages of live fish in an applied breeding program and hatchery operations is necessary. Examination on the external characteristics of the genital papilla conditions as RS (ready to spawn), S (swollen), HS (had spawned) and NR (not ready to spawn) in female breeders could be used. Better understanding on the different stages of gonadal development will be useful to further assess the spawning condition of female tilapia. Recording of females readiness to spawn will facilitate the time of fry collection.

Behavioral Objectives

Given all the necessary background information about the subject matter and materials needed, trainee will be able to assess correctly the sexual maturation of eight female tilapia breeders based on papilla condition and gonadal development.

Materials

Breeders (female only)
Tubs
Basins
Strainers
Aerators
Scoop nets
Masking tapes
Pens/pentel pens
Record sheets

Hand gloves
Freshwater
Towels
Tissue paper
Anesthesia
Petri dish
Siphon

PROCEDURES:

A. Assessment of Sexual Maturation based on papilla condition

1. Prepare all the materials listed above except petri dish and siphon.
2. Properly label the tubs/basins with RS, S, HS and NR.
3. Scoop five to ten breeders and place in a tub.
4. Anesthetize in a previously prepared solution (refer to manual on Module 7 for details)
5. Take but the sedated fish from the anesthetic solution. Take one fish at a time.
6. Examine the papilla condition whether RS, S, NR or HS (refer to Table 1 of Module 10a for detailed description).

B. Assessment of Sexual Maturation based on stages of Gonadal Maturity

1. Prepare all the materials listed above.
2. Anesthetize the fish as in previously prepared solution
3. Take out the sedated fish from anesthetic solution
4. Lay the fish on its right side facing your left hand on a wet towel
5. Apply gentle pressure on the abdomen of the fish to collect eggs for gonad examination. Refer to Table 2 of Module 10a for detailed description of gonadal development.

MASTERY TEST

Given the materials for assessing/scoring the sexual maturity of fish, task is to score the fish whether as RS, S, HS or NR based on papilla condition and gonadal maturity. Performance will be checked whether the fish are scored properly.

PRACTICAL GUIDE
ON
MODULE 4a : Breeding Strategies

Introduction

Fish genetics research and breeding programs are an effective means of contributing to increases in the productivity and production efficiency of aquaculture. The possibilities for improving productivity through selective breeding are especially promising because fish have high fertility, short generation intervals and high gnetic variability compared with other farmed animals. Purebreeding programs hinge around three genetic parameters: heritability, genetic variations and genetic correlations between traits. Depending on heritability for a given trait, different types of purebreeding strategies can be adopted: mass selection, within-family selection, family selection, progeny testing and a combination of one or more types.

Behavioral Objective

Given all the materials necessary for the estimation of some genetic parameters and background information on the subject, the trainee will compute for heritability and response to selection using calculator and SAS procedures. The following will be done prior to estimation:

(a) body weights correction for the sex effects

(b) produce same outputs (calculator and SAS procedures)

Intermediate Objectives

Discuss the importance of estimating genetic parameters in a breeding program
Assemble all the materials needed in the estimation of genetic parameters
Short introduction about SAS software (e.i. how to get into SAS and perform simple SAS procedures)

Materials

Scientific Calculator
Records of harvest data
Yellow paper
Pencil/ballpen
Computer
Diskettes

Procedures

Note: All calculations or analysis (heritability and response to selection) will be performed first, using handheld calculator then followed by using SAS procedures for comparison. All the data are already entered into the computer in the directory: d:\gift\training

1. Collect all the necessary materials to compute for heritability and response to selection. Follow the steps given in the text or from the previous lecture.

2. Correct the body weights for the effect of sex prior to estimating heritability and response to selection.

3. Perform SAS procedures to compare the outputs.

4. Run SAS procedures to produce computer outputs

Heritability:

1.) To work into SAS declare your working directory by typing the ff:

```
LIBNAME IN 'D:\GIFT\TRAINING';
RUN;
        /*then press F8 to submit*/
```

2.) To correct for the sex effects type the following SAS procedures:

```
PROC MEANS DATA=IN.________;
VAR BW;

PROC SORT;
BY SEX;

PROC MEANS;
VAR BW;
BY SEX;
RUN;
        /*press F7 then type file 'prn' to print the output then go
          back to the program editor and continue typing the following:
```

```
DATA IN._______;
SET IN._______;
IF SEX=1 THEN CBW=BW*(GM/MWM);
IF SEX=2 THEN CBW=BW*(GM/MWF);
RUN
          /* GM= grand mean
             MWM= mean weight of male
             MWF= mean weight of female*/

PROC SORT;
BY SEX;


PROC MEANS;
VAR CBW;
BY SEX;
RUN;
     /* Note:  it should be that the mean weights (CBW)
        of male and female are the same after correction
        otherwise error must have been made*/
```

3.) To produce ANOVA or GLM output type the following:

```
PROC ANOVA DATA=IN.________;
CLASS SIRE DAM;
MODEL CBW=SIRE DAM(SIRE);
RUN;

PROC GLM DATA=IN.___________;
CLASS SIRE DAM;
MODEL CBW=SIRE DAM(SIRE)/SS1 SS3;
RANDOM SIRE DAM(SIRE);
RUN;
```

4.) Retrieve the **heritab.prg** (see attached) for computing the heritability by typing the following in the program editor in the command line:

include 'd:\gift\training\heritab.prg'

5.) Then enter all the values from the **ANOVA or GLM output** into the heritab.prg (SS_S, SS_D, d.f. (sire), d.f. (dam), d.f. (error) and the MS_W or MSerror

6.) After inputting all the values then submit the program for processing by pressing **F8**

7.) Go to the output window by pressing **F7** then print the output by typing **file 'prn'**

8.) Compare the two outputs (calculator and SAS outputs) . Calculator and SAS outputs must jive otherwise, repeat.

Response to selection

To correct for the sex effects type the following SAS procedures:

```
PROC MEANS DATA=IN.________;
VAR BW;

PROC SORT;
BY GROUP SEX;

PROC MEANS;
VAR BW;
BY GROUP SEX;
RUN;
        /*press F7 then type file 'prn' to print the output then go
          back to the program editor and continue typing the following:

DATA IN.________;
SET IN.________;
IF SEX=1 AND GROUP='SELECTED' THEN CBW=BW*(GM/MWMS);
IF SEX=2 AND GROUP='SELECTED' THEN CBW=BW*(GM/MWFS);
IF SEX=1 AND GROUP='AVERAGE' THEN CBW=BW*(GM/MWM);
IF SEX=2 AND GROUP='AVERAGE' THEN CBW=BW*(GM/MWFA);
IF SEX=1 AND GROUP='ISRAEL' THEN CBW=BW*(GM/MWM);
IF SEX=2 AND GROUP='ISRAEL' THEN CBW=BW*(GM/MWFI);
RUN
        /* GM= grand mean (across groups)
           MWMS= mean weight of male (Selected group)
           MWFS= mean weight of female (Selected group)
           MWMA= mean weight of male (Average group)
           MWFA= mean weight of female (Average group)
           MWMI= mean weight of male (Israel group)
           MWFI= mean weight of female (Israel group)*/
```

```
PROC SORT;
BY GROUP SEX;

PROC MEANS;
VAR CBW;
BY GROUP SEX;
RUN;
            /* Note:  it should be that the mean weights (CBW)
               of male and female in all the groups are the same
               after correction otherwise error must have
               been made*/
```

PRACTICAL GUIDE / EXERCISE
ON
MODULE 5a - Characterization of Tilapia Using Truss Morphometry

I. Title: Photodocumentation:

Introduction:

Image processing consists of methods to enhance images such as contrast and edge detection, so that the desired details of the images are more evident.

An image is treated as a two-dimensional pattern of brightness that is produced by an optical system such as a camera.

Advantages of images such as photographs are: provision of accuracy and clarity; permanent record; ease of sampling and labor requirement.

Data acquisition is by photographic approach because the program to measure the linear distance is based on photographic image.

Objective:

Given the necessary materials and background information on the subject, the trainee will produce clear fish images / photographs for morphometric analysis.

Materials:

SLR camera
Kodalith film
styropore board
marker (white sheet)
pentel pen
pins

ruler
copy stand/tripod
anaesthesia
recovery basin
anaesthetizing solution

Procedure:

1. Prepare copy stand or tripod at a height that can capture the whole body outline of the fish
2. Set the ASA to 25. Load the film
3. Mount the camera
4. Anaesthetize 6 pieces of fish at a time (breeder size)
5. Fasten the left uppermost side of fish into the styropore board. Make sure that the fish is immobile to ensure clear shots.
6. Place the ruler as a calibration device prior to measurements
7. Position the marker at the lower left side, This will indicate the name, strains, sex and date of documentation.
8. Focus the camera to the image, check for the light by slowly and lightly pressing the shutter (opening 5.6 or light indicator is yellow orange) and then press the shutter .
9. Repeat procedures 1 to 9.

Mastery test: The trainee shall produce clear picture/photographs as an output suited for digitization. Evaluation can be done a week after the photodocumentation.

II. Title : Data Gathering by Caliper method/Digitizer

Introduction:

The image typically seen on photographic film (prints, negatives, slides, x-rays) are continuos tone images composed of graded series of gray tones or colors that at least in principle blend smoothly into one another to reconstruct the original.

The process of converting a continuos tone image to digital format is termed "digitizing" . A digitizing pad (graphic tablet) is a device for getting positional information into a computer with cursor (resembles a mouse with cross hair or "bulls eye"). This pointing device is preferred than a stylus (ball point pen) because the point being digitized is more visible and easier to keep steady.

Distinct advantages offered by digital formats are: speed which digital images can be acquired and reproduced; their portability, and facilitate quantifications of morphology by increasing the accuracy.

Objective:

To collect morphometric data by digitization.

Materials:

Digitizing tablet
slide projector
transformer
processed slides
projector positioner slides
computer, printer & printer papers, diskettes
CAMA program
slide tray

Procedure:

1. Assume the slides had been processed. Load slides into tray.
2. Prepare the slide projector at a level height of the digitizer.
3. Plug the digitizer onto the transformer.
4. Open the computer and initialize, load the CAMA program.
5. Check for the perpendicular projection. If this cannot be attained, the program cannot be opened further. Many attempts should be tried.
6. Assume the program has been opened, select the sequence measurement to progress (this time, slide sequence measurements, a sequence measurements to complete the 21 distances).
7. Project the first slide into the vertically hanged digitizing pad, calibrate the image (state in the menu driven program) based on the standard length of the image (i.e. to calibrate the image, point to the tip of the mouth and to the tip of the caudal peduncle to get the actual length of the image).
   The linear measurement is automatically saved both into a file or printer.
8. Repeat procedures 6 to 7 until all photographs were digitized.

NOTE: When errors are encountered, during measurements, there are options in the program to cancel and repeat the process, either by selecting single measurement option.

Mastery Test: This will be based on the efficiency of performing procedures. The output should not bear different directions of lines due to uncontrolled cursor movements. Scores may vary on the body outline of fish that can be produced.

III. Title: Data Management

Introduction:

In data analysis, a well managed data is crucial to ascertain the correct information contained in each cell. The saved worksheets file of the collected morphometric data by digitization will be parsed to structure the data. Database is the basic key prior to analysis. An uncleaned datasets will always project outliers in the resulting analysis.

Objective: To arrange a CAMA stored data into a well structured Lotus worksheet file and be able to translate the file into logarithmic transformation.

Materials:

diskettes
Computer with Quatro
CAMA files with stored data sheets

Procedures:

1. Encode data in quatro.
2. Format data in Foxpro.

Mastery Test: The trainee shall produce a well structured datasets ready for SAS runs. A print out of a short and prepared datasets will be evaluated. (print-out).

IV. Title: Size Correction

Introduction:

Size factor plays a crucial role in morphometry. Any information must relate only to shape that contains more information on taxonomic groupings minus the size effects. Sexual dimorphism is evident in tilapias, with males normally bigger than females.

Objective:

To create a standard measure of body size given a set of fish measurements.

Materials:

Edited & translated .SSD file
Computer with SAS program

Procedure:

1. For each sex, male and female, type a SAS program that will create a variable column to contain the standard measurement of size, call it Xp variable . $Xp = x1 + x2 + x3 + x21/21$.

2. To derive the corrected size for that particular sample of fish:

   correct size = observed truss divided by predicted length; the predicted length or size is derived from the regression procedure.

   Repeat correcting from x1 until x21 variables.

Mastery Test: The task is for the trainees to establish the standard measure of body size and use this standard size to correct the observed size and establish a new set of variable for a corrected size.

FIELD GUIDE

Module 5b: Characterization of Tilapia Population Using Isozyme Electrophoresis

INTRODUCTION

The importance of genetic variation within populations, especially hatchery populations, enables populations to adapt to changing environments. High levels of variation is associated with greater survival and better growth performance of some fish populations. Inbred populations of Tilapia, on the other hand, are known to display morphological abnormalities, lower survival rates and poor growth performance. Thus, it is always desirable to maintain as much variation as possible by avoiding excessive inbreeding.

Variation is also the basis for breeding programs. In order for a selection program to succeed, the broodstock must have a wide range of genetic traits from which desirable qualities are selected. Affinities between stocks are established by assessing the genetic variation within a group and comparing these with others. Information on the relationships between stocks is crucial in arriving at broodstock and hatchery management decisions.

Isozyme electrophoresis is one technique by which variation may be evaluated. With this technique, a sample of genetic characteristics are visualized on starch gels. These patterns are evaluated to quantify variation within the populations and establish the degree of affinity between them. Isozymes were chosen over other molecular markers because of the simplicity of the technique and the moderate need for equipment and supplies to perform the procedures.

BEHAVIORAL OBJECTIVES

After the training, the participant appreciates the need to evaluate genetic variation, its applications to their current research as well as the simplicity of using isozyme markers. The span of the training is not enough to make the trainee able to independently gather and evaluate the data but should increase interest to develop expertise work in collaboration with others in this area.

INTERMEDIATE OBJECTIVES

Dissect/ prepare samples for electrophoresis
Cook starch gels and load samples on to the gels
Carry-out the electrophoresis, gel slicing and staining procedure
Record bands/ assign allele designations
Perform simple standard statistical analyses on the data
Interpret information from the data

MATERIALS NEEDED

Equipment:
Electrophoresis power supplies
Buffer tanks
Cold cabinet/ refrigerator

Others:
30 Fish samples
Gel molds
Cutting saw
Plastic sheets
Staining sheet
Potato starch hydrolyzed for electrophoresis
Various enzyme substrates, co-factors and activators
2% Agar preparation
Graphing paper

PROCEDURES

1. Prepare materials listed above. Choose stains that develop fast and would have stable patterns.
2. Show participants how to dissect tissues from whole fish and prepare these for loading into the starch gels.
3. Let participants dissect tissues and prepare samples themselves.
4. Demonstrate how to cook and set starch into the molds.
5. Allow participants to carry out this procedure in pairs.
6. Show participants how to load samples.
7. Let participants load their own samples into the gels.
8. Run gels under standard conditions for at least five hours.
9. Prepare stains for the gels as well as the stains which the participants will use according to standard recipes.
10. Show participants how to slice, pick up and stain gels.
11. Let participant do these steps with their own gels.
12. Have the participants read the patterns on their gels and record the data.
13. Demonstrate how to enter and analyzed data using a microcomputer.Data will be analyzed to determine simple genetic parameters only.

MASTERY TEST

The test for this module would be for each pair of participants to perform electrophoresis on samples and interpret the information gathered from the gels.

PRACTICAL GUIDE / EXERCISE
ON
FIELD GUIDE:
MODULE 6a: Maintenance of Live Tilapia Germplasm

I. Title: Inventory of Stocks

INTRODUCTION

Inventory of stocks is important in maintaining the live tilapia germplasm because this checks the health condition and survival of the fish. This can be done early in the morning or late in the afternoon monthly and quarterly in tanks and ponds, respectively. During inspection, the insertion site of floy tag, pit tag or any fish mark should be noted if there is any infection.

BEHAVIORAL OBJECTIVES

Given all the background information about the subject matter and materials needed for inventory of stocks, the trainees will be able to conduct proper/effective inventory and checking of stocks (record at least five fish per trainee) without mortality and with minimal stress on the fish.

MATERIALS:

Pit tag reader
Batteries (for pit tag reader)
Tubs
Scoop nets (big mesh size)
Towels
Record Sheet
Pen / pentel pen
Masking tapes
Holding facilities (sorting/checking of stocks)
Tables
Chairs
Freshwater
Cages
Aerators

Tissue paper

Note: three to four person are needed

PROCEDURES:

1. Prepare all the materials listed above.
2. Prepare tanks prior to reading of individual pit tag
3. Properly labeled the tubs with freshwater (e.g. G1 for Male and Female)
4. Scoop the fish in cages and place in tubs. Take one fish at a time.
5. Lay the fish on top of the table and cover with soft wet towel
6. Using the PIT tag reader, get the PIT tag number of the fish.
7. Record the pit tag number and other fish mark of the fish.
8. Check the health condition of the fish.
   (if not in good condition isolate the fish in a separate tank)
9. Check the recorded tag number against the masterlist
   (Separate the fish if the tag number is not found in particular fish populations
10. Place the inventoried fish in labeled tubs and stock in clean tanks.
11. Repeat the same procedure from 4 to 12 until you finish all the stocks
12. Be sure to count the fish and make a location map after each stocking.

MASTERY TEST

Given the materials for inventory of stocks, task is to record 5 to 10 individual fish mark (pit tag, floy tag, fin clip and etc.) check against the masterlist and examine the health condition of the fish with less stress and mortality. Performance will be observed based on the condition of the fish after conducting the inventory within 5 hours.

PRACTICAL GUIDE / EXERCISE
ON
MODULE 6b - Cryopreservation of Tilapia

I. Title: Collection and Motility Scoring of Milt

Introduction:

The first step involved in cryopreservation is the collection and scoring of spermatozoa. This involves a process whereby milt collected will be checked in the laboratory to see its viability for long term preservation. This step is crucial because milt quality can be affected by sample contamination brought about by improper milt collection.

Objective:

Given the step by step procedure on collection and motility scoring of spermatozoa, the trainees will be able to collect uncontaminated milt and score its motility according to the standard procedures developed by the university of Stirling in Scotland.

Materials:

Capillary tubes
Absorbent paper, tissue paper or towel
Inverted microscope
Marker pen
Appendorf tubes/microcap tubes
Styrofor box with cracked ice
Small amount of tap water at 25-28°C for checking sperm motility
Finpipettes (100-200 μl) set at 200 μl
Finpipette tips

Procedure:

A) Collection of Milt (Field)

1. Select fish and ensure careful handling.
2. Apply abdominal pressure to clear bladder of urine.
3. Dry genital papilla and remove all moisture and mucus with absorbent towel.
4. Hold selected broodfish with both hands.
5. Place 100 µl capillary tube at the tip of the genital papilla.
6. Gently press the abdomen, and draw the collected sample into the tube by capillary attraction.
7. Put collected milt into labeled appendorf tube/micro cap and store in a Styrofoam box with cracked ice.

B) Checking Sperm Sample (laboratory)

1. Check sample by placing a small amount on a glass slide. view under the microscope.
2. Sperm sample should be immotile at this stage.
3. Only immotile samples should be used.
4. Activate sperm sample by the addition of small amount of water.
5. Check sperm motility.
6. Give motility score of 5 if 100% of sample is moving upon activation and 0 if none is moving.
7. Now store sperm sample in the fridge and use as soon as possible.

Mastery test:

At the end of the exercise, the task are for the trainees to collect uncontaminated sample and to score sperm motility.

Reference: Manual on Cryopreservation of Tilapia by Dr. Krishen J. Rana & Anne Gilmour from the University of Stirling, Scotland.

II. Title : Estimation of Sperm Density

Introduction: Although this step is optional in an ordinary cryopreservation activity, this procedure is done to know sperm concentration in a given volume sample. This is important especially in conducting researches, wherein materials used should be of known concentration.

Objective: Given the step by step procedure on estimating sperm density using a Neubaeur Haemacytometer counter, the trainees will be able to estimate sperm density of collected spermatozoa procedures developed by the University of Stirling in Scotland.

Materials:

Spermatozoa sample put in Styrofor box with cracked ice
Neubaeur Haemacytometer counter
Inverted microscope
Marker pen & paper
Appendorf tubes/microcap tubes
Finpipette (5-40 µl) & (200-1000 µl)
Finpipette tips for 5-40 µl & 200-1000 µl
Tally counter
Deactivator solution containing 0.7 g/100 ml NaCl and 0.6 g/100 ml KCl in distilled water

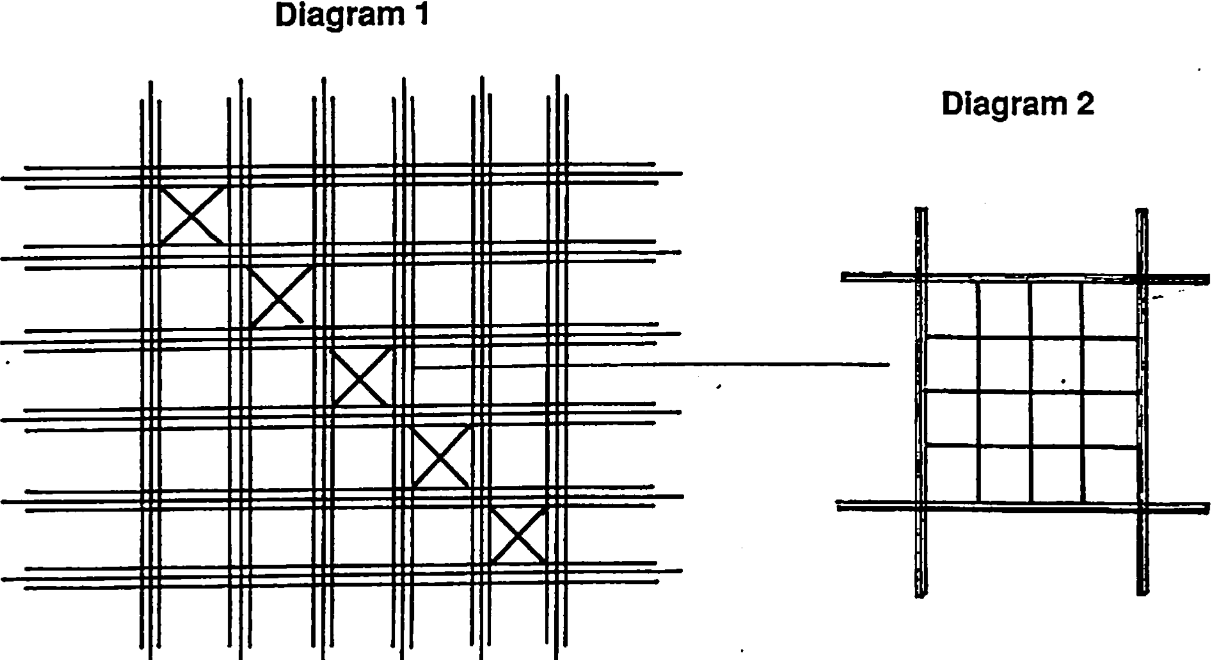
Procedure:

1. Make a dilution with deactivator which is practical for counting a range between 1 in 500 and 1 in 1000 is suggested.
2. Carefully place a drop of this solution on the Neubauer slide counter and cover with the designated cover slip.
3. Leave the slide for approximately 10 min. to allow the sperm to settle into one plane.

4. Count 5 large square. This should be done 3 times and the average no. of cells calculated.

5. To estimate conc./ml, use average count x dilution x 50000

Example: if average no = 200 and dilution = 500
cell conc. (no. per ml) = 200 x 500 x 50000 =
5.0×10^{9}



Procedure

1. Count the no. of sperm in 5 of the squares marked with an I on diagram (1)
2. Each of the squares is divided into 16 diagrams (2)
3. This procedure should be repeated 3 times, with different groups of 5 squares
4. Calculate average = Total number (numbers from the 3 different counts) divided by 3
5. Use this figure in the above formula to give cell conc./ml

Fig. 2. Steps for estimating sperm density (cell conc./ml) using Haemacytometer.

1. Count the number of sperm in 5 of the squares marked with an x on diagram 1
2. Each of the squares is divided into 16 on diagram 2
3. This procedure should be repeated 3 times with different groups of 5 squares.
4 Calculate average = Total no. (numbers from the 3 different counts) divided by 3.
5. Use this figure in the above to give cell conc./ml.

Mastery Test: At the end of the exercise the task is for the trainees to estimate sperm density of collected spermatozoa at cell conc. per ml.

Reference: Manual on Cryopreservation of Tilapia by Dr. Krishen j. Rana & Anne Gilmour from the University of Stirling, Scotland.

III. Title: Fill Dewar and check Programme in KRYO 10

Introduction:

Prior to continuing this step, it is important that trainees have already read the Manual of Operation of KRYO 10. This is important so that trainees will know the machine, its parts and function and way of operation.

Checking the program should be initiated prior to freezing the sample. This should be done especially there are other programs loaded in the machines. This is to make sure also that the program activated is the right program used in freezing tilapia spermatozoa.

Objective:

Given the necessary materials and Manual of Operation of KRYO 10, the trainees will be able to fill up dewar with liquid nitrogen and check program set in KRYO 10 according to the standard operating procedures explained in KRYO 10 Manual of Operation.

Materials:

Liquid Nitrogen
KRYO 10
Manual of Operations of KRYO 10

Procedures:

A) Filling of the dewar and connection to the KRYO 10

1. Use face mask and protective gloves, fill the dewar 1/2 full with liquid nitrogen.
2. Attach the pump to the dewar with the valve in an open position.
3. Switch on the heater, close the valve lever and wait until the pressure rises to 5 on the gauge.
4. Check the program installed in KRYO 10.

B) Checking Programme in KRYO 10

1. Using the instructions provided turn on the machine

2. Select program menu
3. Give access code
4. Select view
5. Check the following parameters:
   Room temperature i.e. 20 °C
   Cooling rate is -5°C per minute
   Target temperature set at -60 °C
   Hold step here for 2 minutes
6. Select exit menu
7. Turn off the machine.

Mastery Test: At the end of the exercise the task is for the trainees to fill up dewar with liquid nitrogen and check program installed in KRYO 10.

Reference: Manual of Operation of KRYO 10.

IV. Title: Preparation of Sample for Cryopreservation

Introduction: The procedure involved here includes labeling of straws and goblets, computation of the needed milt, cryoprotectant and modified fish ringer in a required number of straws. Later when this had been settled the three ingredients are mixed in the following order; modified fish ringer, 10% methanol then the milt sample. The milt sample is added when all the materials had already been prepared. Also, it is at this point that the 30 minutes equilibration time starts.

Objective: Given the necessary materials and step by step procedure in cryopreservation, the trainees will be able to preserve tilapia spermatozoa following the standard procedures developed by the University of Stirling in Scotland.

Materials:

- Modified Fish Ringers
- 10% Methanol
- Small plastic or glass containers for dilutions
- Milts sample
- Marker pen & paper
- Finpipettes 200-1000 μl & 2-10 ml
- Finpipette tips for 200-1000 μl & 2-10 ml
- Beads
- Straws
- Cryo pen
- Plunger
- Tissue

Procedure:

A) Labeling of Straws and Goblets

1. Choose straw, goblet and bead to be used ensuring the colors are matched with the list of straw/bead combination assigned for each strain of fish.

2. Label straw and goblets using cryo pen and written strain code, PIT tag number of Fish ID and date of freezing in each individual straw.

B) Preparation of Sperm Sample

1. Decide on required dilution
2. Calculate amount of straws desired
3. Mix modified Fish Ringers + 10% methanol and sperm sample.
4. A maximum of 30 minutes should be allowed from mixing the milt with extender and methanol to placing in the KRYO 10. The amount of straws which can be reasonably filled within this span should be calculated.

C) Straw Filling and Sealing

0.5 ml straw

1. Place unplugged end of straw into prepared milt sample, insert plunger into plugged end of straw.
2. Depress the plunger drawing the sample through until the plug has gelled.
3. Seal at the opposite end with a bead applying enough pressure to ensure that the bead is firmly inserted to the straw.
4. Dry the straws with absorbent paper to avoid ice forming during the freezing process and the straws adhering to one another.
5. The time spent for this procedure should be kept within 30 minutes equilibration time.
6. Arrange the straws in the KRYO 10 prior to freezing.

0.25 ml straw

1. Follow step 1-2 for 0.5 ml straw
2. Dip the unplugged end of the straw into a small amount of water and then immediately into a small container of tightly packed gel powder.
3. Follow step 4-6 for 0.5 ml straw.

Mastery Test: At the end of the exercise the task is for the trainees to label straws and goblets, prepare sperm samples, fill up straws and freeze sperm samples using KRYO 10.

Reference: Manual on Cryopreservation of Tilapia by Dr. Krishen J. Rana & Anne Gilmour from the University of Stirling, Scotland.

V. Title: Thawing of Straw after freezing

Introduction: Thawing of straws is one of the most critical variables affecting the success of cryopreservation. This step is done to rehydrate cells prior to checking post freezing motility score of spermatozoa. It is suggested that rapid warming should be done to prevent ice crystal formation. Frozen sample from liquid nitrogen with -196 °C temperature are thawed in water bath with 40-41°C temperature to prevent recrystalization which are thought to be the main causes of cryoinjury.

Objective: Given the necessary materials and step by step procedure in thawing cryopreserved milt, the trainees will be able to check after freezing motility score of tilapia spermatozoa following the standard procedures developed by the University of Stirling in Scotland.

Materials:

Water bath 40 °C . This can simply be a bucket
Thermometer
Timer
Scissors
Slide and microscope
Tap water at approximately 27 °C
Face visor and protective gloves and tweezers

Procedure:

1. Use visor and gloves
2. Tweezers can be useful here as gloves may be too clumsy to handle the straws
3. Identify straw and immediately plunge into the water bath and gently agitate for 8 seconds.
4. Wipe the straw and cut the unmarked end.

5. Place onto a microscope slide and activate with the addition of 100 µl tap water by carefully mixing.
6. Give motility score from 1-5 when 5 is equivalent to 100% motility

Mastery Test: At the end of the exercise the trainees will be able to thaw cryopreserved samples and give after freezing. sperm motility score..

Reference: Manual on Cryopreservation of Tilapia by Dr. Krishen J. Rana & Anne Gilmour from the University of Stirling, Scotland.

VI. Title: Check Viability of Cryopreserved Milt

Introduction:

This step is done to check the success of cryopreservation. Eggs collected from ovulated female tilapia are fertilized with thawed cryopreserved spermatozoa. Two hours after fertilization, number of eggs that reached morula stage of egg development are counted to compute for the fertility rate. This last step is the true measure of success of cryopreservation.

Objective:

Given the necessary materials and step by step procedure in artificial fertilization, the trainees will be able to check viability of cryopreserved milt by using it to fertilize tilapia eggs. following the standard procedures developed by the University of Stirling in Scotland.

Materials:

Ovulating tilapia
Thawed cryopreserved milt
Scissors
Petri disc
Tap water
Tissue paper or absorbent paper
Incubator
Fine brush or feather

Procedure:

1. Select ovulating female tilapia and ensure careful handling
2. Strip ovulated female by applying abdominal pressure
3. Collected eggs are divided into aliquots of approximately 400 eggs.
4. Empty cryopreserved sperm suspension over eggs.

5. Activate spermatozoa by the addition of small amount of tap water.
6. Gently agitate eggs and sperm mixture and let stand for 5 minutes.
7. Rinse egg with tap water
8. Transfer fertilized eggs to incubating jars
9. Control egg movement by adjusting water flow
10. After 2 hours remove eggs from incubating jars and count eggs that reached morula stage of development

Mastery Test: At the end of the exercise the trainees will be able to check viability of cryopreserved milt by using it to fertilize tilapia eggs

PRACTICAL GUIDE/EXERCISE
ON
Module 7a: ORGANIZATION AND COORDINATION WITH TEST STATIONS

Introduction

In preparation for on-station and on-farm research work, there is a need to select test stations, farms and culture systems (rice fish, waste food and cages) suitable for genetic trials. For instance, for rigorous evaluation of strains or strain crosses under different culture systems and various geographic locations, the GIFT project selected experimental sites in rice paddies, earthen ponds, waste fed and cage systems. Government and non-government stations/institutions played key role in the execution of these experiments by providing required facilities, and/or helping establish contact with local farmers for on-farm trials.

In this exercise, the trainees will follow the protocol for organizing experiments in test stations/farms based on the experience of the GIFT Project.

Objective

To perform under simulated conditions the step-by-step procedures in organizing experiments and coordinating with test stations or farms.

Procedure

Assuming a project has been assigned to conduct on-farm/station experiment (Evaluation of genetically improved breed of tilapia in ponds). For on-station trial a potential site will be the BFAR in Muñoz and the farms in the nearby areas. A request letter was already forwarded to the BFAR-NFFRTC, Mu ñoz for site visit. This letter of request was granted and a scheduled visit to the station and farms was arranged. The scheduled visit will be on July 30, 1997.

The task is for the trainees to carry out the following:

Trainees will be grouped into two:

GROUP 1 - ON-STATION TRIAL
GROUP 2 - ON-FARM TRIAL

STEP I. Coordinate with station head.
State the purpose of visit and discuss.
Inspect/evaluate the facilities.

STEP II. Note the importanr items such as:
- water source
- pond area
- outlet/inlet
- security of fish
- others

STEP III. If the site is suitable for trial brief the station head about the experiment. Request permission to use the facilities and assistance.

STEP IV. For on-farm triai. ask the assistance of the station to assist the group for a farm visit and evaluation.

STEP V. During farm visit follow the items listed in STEP II.

STEP VI. Coordinate with tha station head/farmers about the activities .

STEP VIII. Record the data during the visit and make a group presentation.

PRACTICAL GUIDE / EXERCISE
ON
MODULE 8a- Transport of Fish Germplasm

Introduction:

Techniques in packing and fish transport play an important role in the conduct of the experiment. Since early 1990's the GIFT Project has been involved in transporting tilapia from Africa in to the Philippines, fingerling distribution to farmers in different regions of the country and also to INGA member countries in Asia. Through this experience. the GIFT Project has developed techniques in packing and transporting tilapia at minimal mortality.

Objective:

To specify and apply under simulated conditions the basic steps in transporting livefish germplasm with consideration with the ICES/EIFAC Codes of practice and manual of procedures for transfer of fish.

Materials:

Laboratory Materials

Thermometer
Weighing balance
Tally hand counter
Holding tanks with Aeration
Ruler

Transport Materials

Plastic bags
Pandan Bags
Rubber band
Filled Oxygen tank with rubber hose
Scoop nets
Basins/tubs
Aerators
Styrofoam boxes

Old news paper
Packaging tape
Scissors
Pentel pen
Ice cubes
Plastic pail

Procedure:

Trainees will be divided into two groups. Each group will be packing fish for transport locally and abroad.
To perform the task, the following assumptions are given:

For fish transport to other country (Case 1)

GIFT fingerlings with size range of 1.5 - 2 g will be transported. The Bureau of Fisheries and Aquatic resources has issued the Fish Health Certificate and permit to transport. Based on the laboratory results taken from 30 pieces of fish samples. the fish for transport are healthy and free from parasites and diseases. Special permit to load was also issued by the Bureau of Customs and the Airway bill was pre-arranged at airport's cargo section.

The time and date of flight is on 30 July 1997. 1300 hrs. (1:000 p.m.). Loading time at the station is at 0500 hrs (5:00 a.m.).

For fish transport to test station (Case 2)

A total of 600 pieces GIFT fingerlings with size range of 1.5 - 2 g will be transported to the test station. The fish were conditioned in the tank and are now ready for transport. A letter of request to the Station Head was given and the stocking date was coordinated.

The scheduled arrival of fish to the station is on 30 July 1997, 1500 hrs (3:00 p.m.). Loading time at the station will be at 0500 hrs (5:00 a.m.).

Steps:

A. Plastic bag preparation:

1. Check the bags for any damage or puncture.
2. Insert old newspapers between two plastic bags for plastic bags protection.
3. Fill plastic bags with unchlorinated tapwater (6-8 liter per bag)

note:

For air transport. bags with newspaper will be oxygenated , packed and placed inside the styrofoam boxes. The fingerlings will be transferred to these bags prior to the scheduled flight.

4. Place plastic bags with water inside pandan bags

B. Fish Loading

1. Collect fish from holding tanks. Transfer the fish in plastic basins/tubs.
2. Using a scoop net. transfer the fish in plastic bags. Count the number of fish per bag. Ccunting can be done by volumetric method actual/manual counting.
3. Introduce oxygen in plastic bags at about 2/3 of the bag's volume.
4. Fasten upper end of plastic bags with rubber band.
5. Place ice cubes on top of the bags.

C. Transport of Fish

1. Arrange and file the bags inside the vehicle. Keep the bags away from pointed materials/object.
2. Check the bags every hour to make sure that there is no oxygen leaks or bag damage.
3. Bring extra plastic bags and rubber band and portable oxygen tanks.

D. Water Replenishment and Styrofoam Box Packing (For air transport only)

1. Upon arrival at the airport Cargo Section, transfer the fish from the bags to styrofoam boxes.
2. Drain the water and transfer the fish slowly into the bags with newspaper linings.
3. Introduce oxygen in plastic bags at about 2/3 of the bag's volume.
4. Present the cargo for inspection
5. After inspection, close the styrofoam boxes and seal it with packing tape.
6. Cargo will be carried to crate.

E. Unloading and Stocking

1. Upon arrival at the destination, monitor the temperature of the water in the tank or pond where fish will be stocked.
2. Be sure that the recipient of fish is informed about the protocols on fish handling and quarantine.

NOTE: The trainees will be graded according to the efficiency of work and percentage of survival or success of transport.

PRACTICAL GUIDE / EXERCISE
ON
MODULE 8b - Quarantine and Postmortem of Tilapia

I. Title: Prophylactic Treatment for Out-going Tilapia Germplasm

Introduction:

Prior to fish transport/shipment, stocks usually undergo at least 2 weeks of quarantine. This is to ensure that fish being transported are free of parasites and diseases. Samples are also given to BFAR Fish Health Laboratory for Health examination. Disinfection of fish using 20 ppm bath of $KMnO_4$ is a routine procedure during quarantine. This is usually done two days after harvest and two days before transport/shipment.

Objective:

Given the materials and step-by-step procedures in prophylactic treatment, the trainees will be able to give a bath of 20 ppm $KMnO_4$ to tilapia fingerlings following the procedure demonstrated.

Materials:

Basin
Fine mesh net
Aerators
Pre-measured $KMnO_4$
Stop watch
Freshwater/tap water

Procedure:

1. Disinfect tank where fish will be held after treatment.
2. Fill with water.
3. Collect fish to be treated.
4. Thoroughly mix pre-measured $KMnO_4$ with water.
5. Put fine mesh hapa in the basin with $KMnO_4$.

6. Aerate the mixture.
7. Take 5 fish and dip in the basin with KMnO4 mixture for 45 sec.
8. Rinse fish in basin with tap water.
9. Put treated fish in disinfected container (Tank or aquaria).
10. Observe fish.
11. Repeat step 7-10 for the rest of the fish, taking 15-20 fish at a time depending on size.

Mastery test:

At the end of the exercise, the task is for the trainees to give prophylactic treatment of 20 ppm to tilapia fingerlings.

II. Title: Fish Postmortem Examination

Introduction:

Postmortem examination is necessary when the cause of disease or infection is not known. Live fish which shows evidence of disease should be taken out and killed immediately. Postmortem examination should be performed hygienically. Thus, after examination.. any fish remains should be properly disposed of and the examiner should adequately disinfect himself and his utensils.

Objectives:

Given the materials and step-by-step procedure in fish postmortem examination, the trainees will be able to conduct postmortem examination following the standard procedure developed by the BFAR Fish Health Unit.

Materials:

Microscope
Microscope slide
Cover slip
Scalpel
Mounted needle
Fine scissors
Hand knife
Hand lens
Bottles with 10% formal saline solution

Procedures:

1. Kill the fish by a sharp blow on a skull or by cutting the spinal cord with a sharp scalpel.

2. Keep the fish wet throughout the examination.

Skin

1. Take scrapings for examination.
2. Scrape fish with sharp scalpel in an anterior to posterior direction and place some mucus and ephitelial tissues on a slide with a drop of water.
3. Spread scrapings thinly, add a drop filtered water and cover with a cover slip.

4. Examine under the microscope using high powered objective (HPO) of the stereo microscope.
5. Examine the entire fish under the low power objective (LPO) of the stereo microscope. Be sure to examine under the fins and other areas of the body for presence of large parasites.

Gills

1. Remove operculum and examine inside.
2. Remove a whole gill and place on a slide or in a petri disc (add water if necessary) and examine under low power objective.
3. Separate the primary lamellae with needles to observe large monogenea and crustacea. Examine any lesions in detail under high power.
4. Cut off lamellae and remove gill arch. Place lamellae on a slide and spread thinly-crop if necessary with coverslip. Examine under high power.

Other Organs

1. Make an incision along the ventrum, from anus to head.
2. Remove the abdominal wall to expose the viscera.
3. Examine any abnormalities under HPO
4. Remove alimentary canal and associated organs by cutting across the esophagus and around the anus.
5. Examine the surface of the alimentary canal and organs by scrapping the contents onto a slide and observe under HPO.

Internal Organs

1. Make a squash preparations from the heart, liver, gall bladder, spleen, kidney, gonads, urinary bladder and swim bladder and observe under HPO.

Eyes

1. Dissect eyes and open nares. Examine under LPO and HPO for helmints.
   Dissect carefully out the separate tissues of the eyes to determine the location as the site is helpful for identification.
2. Remove the skin and slice some muscle tissues for helminth larvae and protozoan cyts.
3. Squash some muscle tissues between slides or glass plates and examine over a bright light source.
4. Open the cranial cavity.
5. Examine a slide and make smear of the brain tissue.

Mastery test:

At the end of the exercise, the task is for the trainees to conduct postmortem examination following the standard procedure set by the BFAR Fish Health Unit.

MODULE PLANS

MODULE PLAN	Page: *1 of 2*
	Module No.: *1b*

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins.	Introduction	Introduce the module and explain the objectives of the module
10 mins.	Transparency 1b.3 - 1b.9	Describe research and testing facilities used in conducting GIFT breeding experiments. Explain the need to be familiarized with these facilities. Enumerate the various components of research and testing facilities used in GIFT Project. Show Transparency 1b.3 - 1b.4. Explain that facilities under each component have been specially designed to suit the requirements of breeding/selection activity Give specific examples of these facilities. Show Transparency 1b.5 - 1b.9.
15 mins.	Slide Presentation:	Tell trainees they will now see slides that will show/describe the research and testing facilities used in GIFT Project as an example. Ask them to note on a piece of paper key important points about the facility. (i) Show slide describing Quarantine facilities. Explain its purpose. Emphasize the importance of isolating the quarantine facility from others. Describe how drainage system was put up(Slide 1b.1 and 1b.2). (ii) Present slide describing the facilities used for truss morphometric characterization of tilapia. Explain briefly its use (Slide 1b.3). (iii) Show slides illustrating facilities for maintenance of live fish germplasm and cryopreserved sperm. Explain the use of reference collection tanks, (Slide 1b.4) holding pond facilities, (Slide 1b.5) cryopreservation laboratory and the various equipment within the cryopreservation laboratory (Slide 1b.6 - 1b.7). (iv) Show slides describing facilities for breeding and family testing experiments: Hapa net cages (Slide 1b.8) Artificial incubating system (Slide 1.9) B-net cages (Slide 1b.10) Earthen ponds (Slide 1b.11) Rice paddies (Slide 1b.12) Floating/fixed net cages (Slide 1b.13, 1b.14) Explain briefly the use of these facilities

Please turn over

MODULE PLAN		Page: *2 of 2* Module No.: *1b*
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
15 mins.	**Discussion/Question and Answer**	Encourage trainees to ask questions for clarifications.
78 mins.	**Practical Application - Minimum facility requirement to run a given activity (Discussion)**	Instructor to explain the practical application of the Module. Cite specific examples. Discuss factors/problems to consider in running a minimum facility.
10 mins.	**Mastery Test** **MTQ-1b**	Distribute MTQ - 1b and collect completed questionnaires
10 mins.	**Test Answers** **MTA - 1b**	Distribute MTA - 1b and discuss suggested answers with the trainees
10 mins.	**Mastery Test (Suggested Answers)**	Trainor to discuss to trainees the Suggested Answers of the Mastery Test.
75 mins	**Tour of Research Facilities/Field Demonstrations**	Bring trainees to the project site and identify facilities. Demonstrate, if necessary, the use of research facility
20 mins.	**Opinion Questionnaire**	Distribute opinion questionnaire. Get trainees to complete and hand in to instructor. Make improvements to the course as necessary.
5 mins.	Feedback	Instructor to give feedback to trainees.
TOTAL	*Time of Module 1b:*	*4 hours and 21 mins.*

MODULE PLAN		Page: 1 of 2 Module No.: 2a
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
5 mins.	**Introduction (Transparency 2a.1 - 2a.2)**	Show title of module (Transparency 2a.1). The procedure of gathering and recording important biological data or phenotypic traits will be taught in detail. Present module objectives.
10 mins.	**Plan specific activities prior to sampling and recording of data (Transparency 2a.4)**	The following should be ascertained prior to data gathering (i) What is the objective of breeding experiment? (ii) What are the pertinent data which needs to be collected? (iii) How often one should gather these data? (iv) How these data will be collected? (v) Who should collect the data?
5 mins.	**Description of GIFT data forms (Transparencies 2a.5 to 2a.8)**	Show Transparencies 2a.5 to 2a.8 describing the data sheet forms for recording of information during specific activities such as: a) breeding and collection of fry b) fish transfer to B-net cages c) Communal stocking and regular sampling Enumerate the variables in each data form and explain in detail how data or information are recorded.
20 mins.	**Slide\Transparency**	Show a complete package of films slide describing how different data are to be gathered in the field and recorded: Before Spawning: hapa number, sex of breeder, tag number, individual body weight, standard length, body depth, caudal fin bars, maturity condition After Spawning: hapa number, sex of breeder, tag number, body weight, standard length, body depth, caudal fin bars During Fry Collection: hapa number, batch, date of fry collection, date of fry collected (dead & alive), bulk weight of fry collected, mean weight, dimension of hapa

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MODULE PLAN		Page: 2 of 2 Module No.: 2a
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
		<u>During transfer of post-fry from nursing hapa to B-net cage</u>: Hapa number, dimension of hapa, previous number (nursing hapa), survival of fry from each hapa, number of post fry counted from all hapa and stocked in hapa 2 size of B-net cage, body weight per family. <u>Before tagging</u>: weight measurement and survival. <u>After tagging: and before stocking</u> ;Sample number, tag number, tag color, initial body weight, initial standard length, initial body depth. <u>Final sampling during grow-out</u>: Sample number, tag number, tag color, sex of the fish, final body weight, final standard length, final body depth, maturity condition
10 mins.	**Question & Answer Discussion**	Instructor to encourage trainees to ask questions for clarification on matter presented Instructor to stress to trainees that batch no. of fish is important for recording in data analysis.
10 mins.	**Summary (Transparency 2a.9)**	Instructor to summarize important points about the module. Instructor to remind trainees about the activities for their practicals.
25 mins.	**Progress test**	Distribute PTQ-2a. Collect completed questionnaires. Distribute PTA-2a.
440 mins.	**Demonstration/ Practicals**	Instructor to bring trainees to the experimental site. Distribute Sampling forms and Practical Guide on this module. Trainees will anesthetize/weight fish individually. Each of them will determine the weight, length, body depth, sex, tag no. & condition remarks of fish. Each trainees will closely observe how to collect and record data. Instructor will distribute materials and will ask each trainees to perform the task that has been demonstrated.
15 mins.	**Feedback**	Review the performance of trainees in general. Provide corrective feedback. Answer all queries.
10 mins.	**Opinion questionnaire**	Distribute opinion questionnaire and collect completed sheets.
Total	***Time of Module 2a:***	***9 hours and 10 mins.***

MODULE PLAN	Page: 1 of 2 Module No.: 2a (Attachment)

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins.	**Introduction Transparencies 2aSa.1 and 2aSa.2**	Present title of module (Transparency 2a.1) and explain how the present module fits with all others. Introduce module objectives (Transparency 2a.2) and explain importance of anesthetization to reduce stress during handling. Stress the importance of minimizing handling stress to fish and give pointers to lessen physical injury to fish during tagging/handling.
10 mins.	**Terminology (Transparency 2aSa.3)**	Instructor to define anesthetic and explain its effect on fish. Instructor to define fish marking and it's importance. Instructor to give examples of anesthetics. • Chlorobutanol • MS - 222 • Quinaldine
5 mins.	**Examples of Anesthetic (Transparency 2aSa.4 & 2aSa.5)**	List examples of anesthetics used on tilapias by GIFT Project and describe its effect on fish. Point out precautions and state which anesthetic was found suitable for tilapia.
5 mins.	**(Transparency 2aSa.5 - 2aSa.10; Slides 2aSa.1 - 2aSa.2)**	Show and explain Transparencies and slides describing the step-by-step procedure on anesthetization. Instructor to emphasize on the process and determining the dosage required for the fish to be anesthetized.
5 mins.	**Discussion**	Encourage trainees to ask question for clarification on matters presented. Slide showing for each type of tags.
5 mins.	**Summary (Transparency 2aSa.11)**	Instructor to summarize important points about anesthetization of Tilapia. Tell trainees the disadvantages of prolonged exposure to the anesthetic solution.
5 mins.	**PROGRESS TEST**	Distribute PTQ - 2aA. Collect Progress Test Questionnaires. Distribute PTA - 2aA and discuss suggested answers with trainees.
170 mins	**Demonstration and Practicum**	Given all the materials required including sample live tilapia specimen, the instructor will demonstrate how to properly anesthetize the fish, emphasizing on the precautions to be observed during the process.
		Instructor will distribute Group trainees into 3 and let each group perform anesthetization on a given sample of live tilapia.

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MODULE PLAN	Page: 2 of 2 Module No.: 2a (Attachment)

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins.	Opinion questionnaire	Distribute opinion questionnaire and collect completed sheets. Make improvements to the course necessary.
15 mins.	Feedback	Instructor to comment on performance of trainees and provide corrective feedback.
TOTAL	*Time of Module 7 =*	*3 hours and 57 minutes*

MODULE PLAN	Page: 1 of 2 Module No.: 2b

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins.	**Introduction/ Discussion (Transparency 2b.1 - 2b.5)**	Instructor to present module. Show trainees the objectives of module. Discuss to trainees about fish marking and it's importance. Enumerate to trainees the different types of fish marking.
5 mins.	**Discussion**	Discuss to trainees (to be illustrated/drawn in white board) the different shapes used in plastic disc tagging.
45 mins.	**Slide Presentation Procedure (Transparency 2b1. - 2b.6)**	Show slide films 2b.1 - 2b.6 illustrating the various types of marking tilapia. Instructor to describe each type of tag and its suitability for use in tilapia based on GIFT experiments. Emphasize the specific purposes for each distinct feature of tag (e.g. letters, numbers, colors codes). Show slide films describing the step-by-step procedures in preparation of floy tags. Instructor to explain clearly the ff.: a) importance of proper tag fixing b) procedure of keeping a master list of tags for each environment Show slide films describing the step-by-step procedure in fish marking (floy tagging, PIT tagging and fin clipping) (Note: Each slide film illustrates a specific step). Instructor should encourage trainees to ask questions or clarify some points as each step is shown on slide.
5 mins.	**Summary**	Instructor to summarize important points about fish marking (show transparencies). Stress important points based on the objectives of the module presented.
10 mins.	**Progress test (PTQ - 2b)**	Distribute PTQ-2b. Collect Program Test Answers. Distribute PTA-2b and discuss suggested answers with trainees.
66 mins.	**Demonstration/ Exercise**	Given all the materials required in fish marking, the instructor will group the trainees into 2 batches. Each batch of trainees will closely observe how fish marking is actually performed on an individual fish. Instructor will entertain queries for better understanding of participants. After the observation, Instructor will distribute tagging materials and live fingerlings (20 pcs. each) for each trainee for floy & plastic disc tagging. Another 5 pcs. breeders for PIT Tagging and 15 fish for fin clipping will also be distributed. The task will be performed following the instructions stated in Practical Guide on Fish Marking.

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MODULE PLAN		Page: 2 of 2 Module No.: 2b
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
294 mins.	**Practicals**	After demonstration, each trainee, provided with all the required materials, will have real practice on all steps involved in each type of marking tilapia. Practical instructor to closely observe each trainee and make sure that he assigned the necessary skill.
15 mins.	**Feedback**	Review the performance of trainees in general. Provide corrective feedback. Answer all queries.
10 mins.	**Opinion questionnaire**	Distribute opinion questionnaire and collect completed sheets.
TOTAL	*Time of Module 2a:*	*7 hours and 58 minutes*

Module Plan	Page: *1 of 2*
	Module No.: *2c*

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
10 mins.	**Introduction Transparencies 2c.1 and 2c.2**	Explain how the present module relate with all other modules. Present title and objective of module. Inform trainees there will be an end of module practical exam/exercise.
15 mins.	**Transparency 2c.3 to 2c.4.5**	Define database in general and other related terms. Go over/Refer to definition of terms when necessary. Instructor to demonstrate on how to modify data structures. Explain the importance of efficient database management in breeding/genetic improvement program. Describe datasets generated from GIFT Project experiments.
10 mins.	**Components of GIFT database Environment/System Transparency 2c.5**	Show Transparency illustrating the components of GIFT database environment. Explain importance of defining/knowing the environment system. Enumerate the six major components: (1) Database (2) Data dictionary (3) User/system interface (4) Database Management system (5) Database Administration (6) User-groups Explain each major component and describe how these are linked with each other.
5 mins.	**Transparency 2c.6**	Give layman example. Show Transparency illustrating the components and their relationships.
15 mins. *50 mins.*	**Exercise**	Question and Answer session Distribute sample forms (2-3) and background information to facilitate discussion. Modification of data structures. Dissect form to come up with a data structure.
30 mins.	**Transparency 2c.7**	Show again Transparency 11.5 to relate to next subject. Show flow chart of GIFT Project's Database. Tell trainees the chart describes the routine procedures of management of data generated from the GIFT breeding experiments.

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MODULE PLAN		Page: 2 of 2 Module No.: 2c
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
		Explain in detail the procedures for storage, retrieval and analysis of the data.
10 mins.	**Discussions**	Instructor to encourage trainees to ask questions. Instructor to review lessons before the exam.
60 mins.	**Open session**	Inform and introduce to the software packages to be used in Module. Determine the trainees literacy on the topic. Conduct guided practice.
205 mins.	**Demonstrations / Hands on Computer session using two sample datasets**	Using Projection Panel and computer, instructor to demonstrate to trainees the step-by-step routine procedures on how to store, retrieve and analyze datasets using appropriate software.
20 mins.	**Progress test**	Distribute PTQ - 2c. Distribute PTA - 2c. Discuss answers with trainees.
125 mins.	**Mastery test**	Instructor to group trainees into three (with three trainees per group). Do cross referencing with the GIFT database flow chart and GIFT database environment transparencies. Provide necessary references: 1. GIFT Data Dictionary 2. GIFT Stat Manual 3. SAS Procedure Manual (optional)
10 mins.	**Summary/Recap**	Let each group present their solutions. Provide them materials. Provide feedback; obtain feedback from rest of class. Answer queries from trainees and clarify important points.
5 mins.	**Opinion Questionnaire**	Distribute opinion questionnaire. Get trainees to complete questionnaires and hand in to instructor. Make improvements to course as necessary.
TOTAL	***Time of Module 2c:***	***9 hours and 30 minutes***

MODULE PLAN		Page: 1 Module No.: 3a
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
10 mins.	**Introduction Transparencies 3a.1 and 3a.2**	Show Transparency 3a.1(Title of module) and explain how the module relates with all others. Present module objectives (Transparency 3a.2). Tell trainees there will be one Progress test and one Practical Mastery test.
5 mins.	**Protocol for holding & conditioning of breeders**	Instructor to explain the importance and the procedure of holding and conditioning of breeders.
5 mins.	**Assessment of sexual maturity (Transparencies 3a.3) (Slide 3a.1 - 3a.4)**	Instructor to describe protocol on how to assess the maturity based on secondary sexual characteristics of female Nile tilapia. Present Transparency 3a.3 Show pictures or slides (Slides 3a.1 - 3a.4) and describe how to give codes/scores based on the genital papilla of female fish.
5 mins.	**Timing of fry collectiob**	Explain the timing of fry collection. (Show Transparency 3a.3)
15 mins.	**Summary (Transparency 3a.4)**	Instructor to summarize key important points about the module. Show transparency 3a.4.
10 mins.	**Progress test**	Distribute PTQ - 3a . Collect completed questionnaires. Distribute PTA - 3a and discuss answers with trainees.
60 mins.	**Demonstration**	Bring Trainees to the field. Demonstrate/show to trainees tilapia stocks with varying sexual maturity condition of genital papilla. Explain in detail how to distinguish one fish to the other.
100 mins.	**Practicals/ Exercise**	Distribute Practical Guide on Module 3a (Assessment of Sexual Maturation). Trainees will be graded based on the success of spawning using genital papilla of female as indicator. (trainees to inspect their work after 10 days).
10 mins.	**Comments**	Comments on performance of each trainee to provide feedback
10 mins.	***Opinion questionnaire***	Distribute and then collect completed questionnaires.
Total	***Time of Module 3a:***	***3 hours and 50 minutes***

MODULE PLAN		***Page:*** *1 of 1* ***Module No.:*** ***4a***
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
5 mins.	**Introduction Transparencies 4a.1 and 4a.2**	Show title of Module (Transparency 4a.1). Introduce Module objectives (Transparency 4a.2) and tell trainees there will be one progress test and one mastery test.
420 mins.	**Lecture**	Instructor to give background on GIFT breeding strategy. Instructor to give a general review on quantitative genetics and fish breeding; Explain selection and their importance in improving aquaculture productivity; Describe fish breeding protocol including mating, the types of mating and the procedures in developing mating plan.
94 mins.	**GIFT Breeding Protocol (Transparencies 4a.3 to 4a.8)**	Show schematic diagrams (Transparencies 4a.3 to 4a.8) describing the breeding protocols used by the GIFT Project in conducting seven generation experiments. Explain fully each diagram.
15 mins.		Questions and Answers
10 mins.		Instructor to show graph (scale of merit) for Phenotypic traits. Instructor will explain graph & entertain questions/queries.
5 mins.	**Summary**	Instructor to review/remind to trainees the key factors of the module.
20 mins.	**Progress test (PTQ - 4b)**	Distribute PTQ-4b and collect completed questionnaires. Distribute model answers and discuss feedbacks.
230 mins.	**Demonstrations**	In this field, instructor will give a simulated demonstration with emphasis on the steps that should be carried out in sequence, while trainees observe the performance.
720 mins.	**Practicals and Mastery test**	Trainees will refer to Trainee Material 4a (Manual on Breeding of Tilapia) as reference material. Trainees will be grouped comprising 3 members per group. Let each group practice all the steps in breeding under close supervision. Comment on performance of each group to provide feedback
5 mins.	**Opinion questionnaire**	Distribute and then collect completed questionnaires
Total	***Time of Module 4b:***	***25 hours and 24 minutes***

MODULE PLAN		Page: *1 of 2* Module No.: *5a*
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
10 mins.	**Introduction Transparencies 5a.1 and 5a.2**	Present title of module and explain how this relates with previously taken up modules (Transparency 5a.1) Introduce module objectives (Transparency 5a.2)
15 mins.	**Lecture (Transparencies 5a.3 and 5a.6)**	- Present details of subject matter - Define and explain morphometry • Truss • Conventional - State advantages of truss morphometry
10 mins.	**Transparency 5a.7**	Describe body morphology of fish. • external • indexed Explain how to arrive at 21 indexes Discuss coding of fish slide & advantage of recording on film (Kodalith)
5 mins.	**Procedures of Data collection (Transparency 5a.8 - 5a.10)**	Introduce photodocumentation. Explain the requirements, mounting of camera, techniques of data collection, digitization and measurements (Transparency 5a.8 -5a.10).
5 mins.	**CAMA Analysis**	Introduce CAMA (Computer Aided Monoscopic Analysis). Give emphasis on definition. Refer to CAMA Manual for details.
5 mins.	**Data Management (Transparencies 5a.11)**	Explain how to prepare worksheets and translate files, structuring of data FOXPRO (Transparency 5a.11)
60 mins.	**STATISTICAL ANALYSIS (TRANSPARENCIES 5a.12 TO 5a.16)**	Discuss procedure on how to analyze and interpret data. Explain multivariate analysis, PCA and interpretation. Define terms for clarifications.
10 mins.	**Recap**	Instructor to summarize all important points taken up in the module. Answer queries and clarify.
10 mins	**Progress test (PTQ - 5a)**	Give PTQ - 5a to trainees.
200 mins	**Practicum**	Photographic collection of samples in groups; Mastery test is based on clarity of picture outputs (evaluation can be done after a week due to developing process)
240 mins.	**Practicum**	Digitization (ready made pictures are available for purposes of getting data. Mastery test is based on digitizing process; if lines are in different directions, (unable to control the cursor) scores may vary on the performance of digitizing.

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MODULE PLAN		Page: 2 of 2 Module No.: 5a
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
300 mins.	**Practicum**	Data management , translation (ready made data and programs) are available for translation and analysis for SAS, size correction using PCA. Mastery test is based on the output from SAS Trainee should be able to plot the results.
10 mins.	**Mastery Test**	Computing for Means.
20 mins.	**Mastery Test**	Computing for Predicted size.
60 mins.	**Mastery Test**	Individual Assessment of trainees.
5 mins.	**Comments**	Comment on performance of class to provide feedback.
5 mins.	**Opinion Questionnaire**	Distribute and then collect completed questionnaires.
TOTAL Time of Module 5a:		***16 hours and 16 minutes***

MODULE PLAN	Page: 1 of 3 Module No.: 5b

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
10 mins.	**Lecture 1** **Introduction** **(Transparency 4b.1 and 5b.2)**	Present title and objective of module. Tell trainees how this relates with other modules.
40 mins.	**Definition/ Explanation** **Transparency 5b.3 and 5b.4** **Additional Transparencies:** * **Nature of Variation** * **Quantifying Variations at morphological meristics, proteins, DNA** **Slide Presentation**	Define electrophoresis. Explain its use and importance in fish population genetics -Define isozyme markers; Explain how to interpret gel banding patterns. Cite examples of these in some electrophoretic studies in teleosts, in freshwater species in particular & their application -Describe the progress made on tilapia particularly the studies made in GIFT Project. Show slide 5b.2. Describe how to prepare fish samples, process tissue samples. Emphasize that dissection and extraction of tissues involves a more delicate procedure
5 mins.	**Summary of Protocol (Slide 5b.1)**	Present Slide 5b.1 illustrating the flow of steps for starch gel electrophoresis. Explain in detail the diagram.
3 mins.	**Starch gel Preparation (Slide 5b.3)**	-Show slide 5b.3. Enumerate the chemicals/materials needed. Describe how to prepare correctly the starch gel mold.
20 mins.	**Sample application (Slides 5b.4 to 5b.11)**	Show slides 5b.4 to 5b.11. Explain the following: -degassing a starch solution by suction -pouring the starch solution into the mold -releasing the starch gel from the mold by means of a scalpel blade -cutting a longitudinal section of gel mold for sample application -separating the anodal from the cathodal portions for sample application -marking the edges and mid-portion of the gel with tracking dye -sample application from the left and to the right of the gel -aligning the anodal and cathodal pieces in preparation for electrophoresis

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MODULE PLAN		Page: 2 of 3 Module No.: 5b
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
3 mins.	**Setting up a Gel for Electrophoresis (Slides 5b.12 and 5b.13)**	Show Slides 5b.12 and 5b.13. Explain the detailed procedure of setting up the gel ready for electrophoresis.
2 mins.	**Slicing the Starch Gel (Slides 5b.14 to 5b.19)**	Present Slide 5b.14 to slide 5b.19. Explain how to slice gel horizontally into thin slabs. Emphasize the need for slicer's dexterity in accomplishing the task.
5 mins.	**-Staining recipes and Allele Nomenclature** **-Interpretation of Banding Patterns**	Tell trainees about staining recipes used in Project's electrophoretic studies on tilapia. Explain standardized nomenclature and how to read electrophoretic mobility of enzyme in a zymogram.
30 mins.	**Lecture 2**	
20 mins.	**Discussions**	Encourage trainees to ask questions. Instructor to clarify important points.
10 mins.	**Exercise**	Instructor gave sample exercise for trainees to work on (reading the bands).
10 mins.	**Progress test (PTQ - 5b)**	
10 mins.	**Feedback**	Distribute suggested answers and discuss with trainees
240 mins.	**Practical demonstrations 1**	Instructor to bring trainees to the Genetics Laboratory. Instructor to demonstrate the step by step procedure of making starch gels & preparing samples. Prepare gels / samples to run on the next day. Trainees are grouped in pairs.
30 mins.	**End of day 1**	1. Review of activities for the day 2. Summary of lab exercise from the previous day
90 mins.	**Practical demonstrations 2**	Group trainees in 5 (assuming there are 10 trainees). Each group comprising 2 trainees per group will perform the procedure. Instructor to closely observe the performance per group. Demonstrate how to load samples & prepare electrophoretic gels. Set gels to run - 5 hours.
	Lecture 3/ Practicals	Data Analysis (Introductory) What to do with bands * Computing allele frequencies * Determining Hardy-Weinberg equilibrum * Polymorphism * UPGMA * Genetic distance or identity

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MODULE PLAN		Page: *3 of 3* Module No.: *5b*
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
120 mins.	**Mastery test (MTQ - 5b)**	Analysis of data from gels
120 mins.	**Practicals/ Demonstrations 3**	Slicing Staining and Scoring gels
15 mins.	**Feedback**	Review the performance of trainees in general. Provide corrective feedback.
10 mins.	**Opinion questionnaire**	Distribute opinion questionnaires and collect completed sheets.
Total	***Time of Module 5b:***	***13 hours and 21 minutes***

MODULE PLAN	Page: 1 of 2 Module No.: 6a

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins	**Introduction (Transparency 6a.1)**	Show Transparency 6a.1 (Title of Module) and explain importance of maintenance of tilapia germplasm.
5 mins.	**Objectives (Transparency 6a.2)**	Present module objectives (Transparency 6a.2). Tell trainees there will be one practical exercise and one mastery test during the course.
10 mins.	**Importance (Transparency 6a.3)**	Explain in detail the importance of maintaining live tilapia stocks. Define *ex-situ* conservation and their use in aquaculture and for breeding.
5 mins.	**(Transparency 6a.4)**	Tell trainees the live tilapia stocks being maintained by the GIFT Project. Enumerate the various strains and why these are being kept.
5 mins.	**(Transparency 6a.5)**	Show Transparency 6a.5 and briefly explain how to maintain stocks in tanks and ponds.
15 mins.	**Marking of stocks (Transparency 6a.6 & 6a.8)**	Describe the procedure on how to identify the stocks using fish marking (floy tagging, PIT tagging, fin clipping). Show Transparency in Preparation of Masterlist. Show sample table of Masterlist and explain in detail how it is prepared. Emphasize the need for keeping a masterlist of fish stocks.
5 mins.	**Detailed Procedure (Transparency 6a.7)**	Tell trainees the importance and the procedure of maintaining replacement or back-up stocks
5 mins.	**Inventory of stocks (Transparency 6a.9)**	Specify importance of conducting inventory of stocks. Present Transparency 6a.9 and explain procedure on how inventory is conducted.
15 mins.	**Routine maintenance (Transparency 6a.10 - 6a.15)**	Describe the step-by-step procedures in routine maintenance of stocks. Present Transparency 6a.10 to 6a.15 and discuss the following: a) Feeding b) Fertilization c) Maintaining good water quality (ponds, tanks) d) Daily monitoring/inspection of stocks

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MODULE PLAN	Page: *2 of 2* Module No.: *6a*

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins.	**Summary (Transparency 6a.16)**	Instructor to summarize key points in module : a) importance of maintaining live germplasm b) routine maintenance c) inventory of stocks
10 mins.	**Discussion**	Ask trainees the important points they have learned. Encourage trainees to ask questions and clarify important points.
10 mins.	**Progress test (PTQ - 6a)**	Distribute PTQ - 6a. Collect completed questionnaires. Distribute PTA - 6a. And discuss answers with trainees.
10 mins.	**Field Demonstration**	Practical instructor bring trainees to the GIFT facility for maintenance of tilapia germplasm. Emphasize on the importance of conducting the actual inventory of stocks aside from performing routine maintenance activities. In the field , instructor will demonstrate how the actual inventory of stocks is done, while trainees observe the performance.
85 mins.	**Practical exercise/Mastery test**	Using Practical Guide on Module 6a, Trainees will perform inventory of stocks.
10 mins.	**Opinion questionnaire**	Distribute and then collect completed questionnaires.
10 mins.	**Comments**	Comment on the performance of trainees to provide feedback.
Total	***Time of Module 6a:***	***3 hours and 41 mins.***

MODULE PLAN		Page: 1 of 4 Module No.: 6b
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
5 mins.	**Introduction Transparencies 6b.1 and 6b.2.**	Present title of module and discuss how the present module fits with other modules Introduce module objectives and discuss the important points they have to remember from the lecture. Tell trainees that there will be one mastery test to be given at the end of the discussion.
10 mins.	**Trainee assessment (Transparency 6b.3 & 6b.4)**	Instructor to ask trainee to define: *ex situ* conservation, gene bank & Cryopreservation. Instructor to define the terms mentioned and explain its importance in aquaculture and genetic improvement.
5 mins.	**Safety precaution**	Present some helpful tips and safety measures that must be observed when working in the Cryopreservation laboratory Refer to attachment 4 of Module 6b.
5 mins.	**Flowchart of Protocol for Cryopreservation of tilapia spermatozoa Transparency 6b.5**	Show Transparency 6b.5 and briefly mention the steps involved in the Cryopreservation of tilapia spermatozoa.
5 mins.	**Materials**	Instructor presented materials to be used in cryopreservation.
10 mins.	**Preparation of extender Transparency 6b.6**	Instructor to define extender (Transparency 6b.6a) and explain its use in Cryopreservation. Enumerate different types of extender for tilapia. Describe the procedure for preparing the extender for tilapia. Show the chemical composition of Modified Fish Ringers (Transparency 6b.6b).
5 mins.	**Selection of Milt donor**	Explain criteria for selecting fish to be used as milt donor and the preparations needed to prepare fish prior to milt collection.
5 mins.	**Collection of Milt from tilapia broodfish (Transparency 6b.7 - 6b.8)**	Explain procedure for collecting tilapia milt from male broodfish. Show Transparency 6b.7 - 6b.8. Stress the importance of collecting uncontaminated milt and proper handling of collected milt upon collection.
15 mins.	**Exercise for sperm counting**	Instructor presented formula for sperm counting. Exercise for calculating amount of Milt, Methanol & Extender (on white board). Instructor asked for queries regarding the exercise.

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MODULE PLAN	Page: 2 of 4
	Module No.: 6b

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins.	**Labeling of Straws (Transparency 6b.11)**	Enumerate information that should be written in individual straws namely: Fish Code, PIT tag number date of freezing & trainee number. Inform trainees about coding of straws and beads used to facilitate cataloguing of samples (Transparency 6b.11).
5 mins	**Transfer of KRYO 10 and Run Program (Transparency 6b.14)**	Emphasize that prior knowledge on how to operate the machine and run the program are important in performing the activity. Trainee must be familiar on all the display features of the KRYO 10 machine. Show Transparency 6b.14a. Tell trainees that samples should be loaded immediately as soon as the machine displays the alarm (Transparency 6b.14b). Discussed record/catalogue form for cryopreservation.
5 mins.	**Recap**	Instructor write the steps of cryopreservation and review the steps to trainees.
10 mins.	**Progress test**	Distribute Progress test (Part 1) for Module 6b.
10 mins.	**Progress test**	Distribute Progress test (Part 2) for Module 6b.
120 mins.	**Practicals (CRYO Lab)**	Trainees proceed to cryo lab for practicals1 (Collection and motility scoring of milt) Trainees are divided into three groups. Instructor demonstrates collection of milt using capillary tube. (note: First group proceed to practicals while the next group are asked to prepare the solution to be used and the last group to solve the problem [individually] written on the white board)
20 mins.	**Reference Tanks**	Trainees proceed to GIFT Reference tanks. Instructor gave demo. on collection of milt.
5 mins.	**Check Sperm Motility (Transparency 6b.9)**	Proceed to Cryo lab. Define sperm motility, Explain procedures for checking and scoring sperm motility. Show Transparency 6b.9

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MODULE PLAN	Page: 3 of 4 Module No.: 6b

Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
5 mins.	**Estimation of Sperm Density (Transparency 6b.10)**	Explain to trainees that although this step is optional, estimating sperm density is important especially in conducting researches. Show the trainees the materials/equipment needed and explain procedure on how to estimate the sperm density using haemacytometer counter per ml as a unit. Show Transparency 6.10a and 6.10b. Show/do some computations in the board.
85 mins.	**Practicals**	Practicals 2 (Estimation of Sperm density). Instructor to demonstrate checking of motility under a microscope.
15 mins.	**Practicals**	Practiclas 3 (Check program in Kryo 10).
5 mins.	**Filling of straws with Milt and Sealing (Transparency 6b.13)**	Enumerate materials needed in filling and sealing straws. Trainees must be familiar with the use of micropipette & syringe (Transparency 6b.13). Tell trainees to seal the other end of the straws depending on the size of straws. Emphasize that only 30 minutes is allowed to do this step.
5 mins.	**Preparation of Tilapia Milt for Cryopreservation (Transparency 6b.12)**	Enumerate materials/chemicals needed to prepare tilapia milt for Cryopreservation. Define cryoprotectant and explain its use. Describe steps on how to prepare milt ready for Cryopreservation. Tell trainees that tilapia milt should be the last to be added in the mixture (Transparency 6b.12).
75 mins.	**Practicals**	Practicals 4 (Preparation of Sample for Cryopreservation). Instructor to demonstrate to trainees procedures in filling of straws.
5 mins.	**Transfer straws and Store sample in LN2**	Trainee must recognize when to remove the samples and transfer this to dewar containing liquid nitrogen (Transparency 6b.15). Emphasize the importance of wearing proper clothing and accessories when handling hazardous chemicals like liquid nitrogen.
5 mins.	**Thaw Cryopreserved Milt samples (Transparency 6b.16)**	Enumerate the equipment required for thawing the cryopreserved milt samples within the straws and describe its procedure. Show Transparency 6b.16.

Please turn over

MODULE PLAN		Page: *4 of 4* Module No.: *6b*
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
5 mins.	**Check Motility of Cryopreserved Milt samples**	Define procedures in checking and scoring sperm motility.
15 mins.	**Check viability of Cryopreserved Milt samples (Transparency 6b.17)**	Define viability of cryopreserved milt sample. Describe how to collect unfertilized eggs from female tilapia broodfish. Show Transparency 6b.17a - 6b.17d. Describe the stages of egg development for ease in identification. Explain to the trainees what is meant by fertility rates and its importance in checking viability of cryopreserved milt.
5 mins.	**Demonstration**	Instructor to show trainees on how to strip eggs . Instructor to demonstrate to trainees procedures on Thawing activity. • cutting of straws • examining cryopreserved milt under a microscope.
55 mins.	**Practicals**	Practicals no. 5 (Thawing of straws after freezing). Instructor to distribute Cryo preservation record for each trainees to fill out.
20 mins.	**Cryopreservation activity**	Instructor to stressed the importance of the viability of tilapia milt. Instructor to enumerate the materials needed & to brief trainees of the procedures. Instructor to check the temperature of water (should be 40°). Demonstration by instructor on thawing of straw, cutting end of straw and scoring of eggs.
85 mins.	**Practicals**	Practicals no. 6 (Fertilization of eggs).
5 mins.	**Opinion questionnaire**	Distribute questionnaire and collect completed sheets.
TOTAL	***Time of Module 6 =***	***10 hours and 58 minutes***

MODULE PLAN		Page: 1 of 2 Module No.: 7a
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
15 mins.	**Introduction (Transparency 7a.1 & 7a.2)**	Show title of (Transparency 7a.1). Explain module objective and how this relates with previous modules. Show Transparency 7a.2. Briefly introduce the topic by going over the important points stressed in the module. Tell trainees there is one case study to check if trainees have achieved module objectives.
60 mins.	**Lecture (Transparency 7a.3 -and 7a.5)**	-Explain rationale for conducting trials/experiments in selected farms/stations(Transparency 7a.3 & 7a.4). -Emphasize importance of efficiency in organization/coordination with the selected test stations/farms. -Present list of test stations which participated as research partners of GIFT Project (Transparency 7a.4). -Describe how GIFT Project coordinated with these test stations/farms in terms of conducting experiments and note the lessons learned from the experience. -Instructor to emphasize the roles of test stations/farms in implementation of the experiment.
80 mins.	**Protocol (Transparency 7a.6 to 7a.7)**	-Explain in detail the flow-chart of activities for on-station and on-farm coordination. Show Transparency 7a.6 & 7a.7. -Describe how the following activities are carried out, following GIFT Project experience: • Planning/Scheduling of on-farm/on-station activities • Identification of test environments • Coordination with test stations/farms • Briefing of station/farm managers on the entire study and specification of requirements for implementation of tasks. • Monitoring on-farm/station activities • Fish Stocking/Monitoring • Fish Harvest • Providing feedback to station/farm manager on study
60 mins.	**Case Study**	-Distribute instructions on case study - Coordination with Test Station -Trainees to visit accredited GIFT test stations for actual interview.

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Module Plan		Page: 2 of 2 Module No.: 7a
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
20 mins.	**Discussions**	-Encourage trainees to ask questions; Instructor to clarify important points. -Instructor to comment on trainees performance in convincing on-station managers/farmers.
360 minutes	**Case Study**	-Distribute instructions on case study - on-farm coordination. -Trainees to visit accredited GIFT stations for actual interview.
10 mins.	**Summary (Transparency 7a.8)**	Instructor to summarize key important points about the module. Show Transparency 7a.8.
15 mins.	**Discussion on Case Study**	-Discuss model answer to case study.
5 mins.	**Opinion Questionnaire**	-Get trainees to complete and hand in to Instructor. -Make improvements to the course as necessary.
Total	***Time of Module 7a:***	***10 hours and 25 minutes***

MODULE PLAN		Page: 1 of 2 Module No.: 8a
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
5 mins.	**Introduction (Show Transparency 8a.1)**	Present title of module and explain how the present module relates with all others. Emphasize that transport of live fish germplasm is only necessary when there is a valid reason to transfer fish germplasm from one country to another and that the activity must satisfy the conditions set by the international Codes of Practice.
5 mins	**Transparency 8a.2**	Present module objectives.
5 mins.	**Policies of fish transfer (Transparency 8a.3)**	Explain the policies/regulations for transfer of fish germplasm. Show Transparency 8a.3
5 mins.	**Procedures of Importation/Exportation (Transparency 8a.4 - 8a.5)** **(Transparency 8a.6 - 8a.8)** **(Transparency 8a.9 - 8a.11)**	Show trainees the schematic diagram of procedures followed in the GIFT Project during germplasm collection from Africa (Transparency 8a.4) and flow of activities during transport of live tilapia germplasm from Africa to the Philippines (Transparency 8a.5). Briefly introduce INGA and its member countries. Show Transparency 8a.6. Discuss recommended protocol for exchange of fish germplasm among INGA member countries. Refer to attachment 1 of Module 8a. Show Transparency 8a.7 - 8a.8. Cite examples of regulations followed by Philippines for importation. Show Transparency 8a.9. Describe protocol prior to and during transport of live tilapia germplasm from Philippines to INGA member countries. Present flow of activities during laboratory fish health examination (Transparency 8a.10)and steps in packing of tilapia germplasm for transport (Transparency 8a.11).
15 mins.	**Slide presentation**	Show slides 8a.1 to 8a.5 describing GIFT experiences on transport/shipment of fish.
10 mins.	**Discussions**	Ask trainees the important points they have learnt and encourage trainees to ask questions and clarify important points.
10 mins.	**Summary (Transparency 8a.11)**	Instructor to summarize key important points in module . (Show Transparency 8a.11)
15 mins.	**Progress Test**	Distribute PTQ. 8a to each trainee and collect completed questionnaires

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MODULE PLAN		Page: 2 of 2 Module No.: 8a
Time	Main Units/Steps/Aids	Contents: - Summary of Activities, points to be emphasized, etc.
10 mins.	**Comments/Feedback**	Discuss PTA. 8a and comment on the performance to provide feedback to trainees
110 mins.	**Practical Demonstrations**	Instructor to distribute Practical Guide/Exercise on Packing and Transport of Live Fish Germplasm. Instructor will demonstrate each step in packing and loading of live germplasm, while trainees observe the performance. Emphasize that the steps should be carried out in sequence.
375 mins	**Group activity**	Instructor to explain mechanics/procedures of the exercise/mastery test. Trainees will perform actual transport of fish to designate place. Instructor will observe closely the performance of trainees.
60 mins.	**Report (writing/reporting)**	Trainees to give their report on Mastery test (transport).
15 mins.	**Comments Discussions/Feed back**	Comment on performance of the class to provide feedback
5 mins.	**Opinion Questionnaire**	Distribute and then collect completed questionnaires. Make improvements to the course as is necessary.
TOTAL	***Time of Module 8a:***	***4 hours and 30 minutes***

MODULE PLAN		Page: 1 of 2 Module No.: 8b
Time	**Main Units/Steps/Aids**	**Contents: - Summary of Activities, points to be emphasized, etc.**
10 mins.	**Introduction** **Objectives** **Transparency 8b.2**	Show Transparency 8b.1 (Title of Module). Tell trainees that in previous module, the procedures for transport of fish germplasm were explained. In this module, the methods on how to quarantine and diagnose disease/parasite for newly transported germplasm will be discussed. Show Transparency 8b.2. Present the objectives. Emphasize to the trainees important points they have to know from the lecture based on the objectives presented. Tell trainees that a mastery test will be given at the end of the discussion.
5 mins.	**Quarantine Definition/Importance Transparencies 8b.3 & 8b.4**	Present Transparency 8b.3 and 8b.4 Explain what is meant by quarantine. Emphasize that quarantine is a 'must' to newly transported fish germplasm.
5 mins.	**Transparency 8b.5** **Routine Quarantine Procedures for In-coming Tilapia Germplasm Transparency 8b.6**	Present Transparency 8b.5. Specify the procedure to be followed prior to the arrival of new fish germplasm. Present Transparency 8b.6. Enumerate and explain the important things to do upon arrival at quarantine facility.
10 mins.	**Routine Quarantine Procedures for Out-going Tilapia Germplasm Transparency 8b.7**	Present Transparency 8b.7 and explain schematic diagram of quarantine procedures followed by GIFT team for outgoing tilapia germplasm.
10 mins.	**Transparency 8b.8**	Present Transparency 8b.8 Stress to the trainees the practical ways of avoiding contamination of fish stocks while conducting quarantine.
10 mins.	**Transparency 8b.9**	Present Transparency 8b.9 Introduce to the trainees Table 1. which summarizes the disease and problems encountered by the GIFT team. Show some slides describing how the different parasites look like and slides describing symptoms of infections/diseases encountered. Present the life cycle of ICH as an example.
5 mins.	**Transparency 8b.10**	Present transparency 8b.10 and enumerate steps involved in treating infected fish.
5 mins.	**Transparency 8b.11**	Present transparency 8b.11 and show trainees how to calculate amount of food required by infected fish at a given period of treatment.
5 mins.	**Transparency 8b.12**	Present transparency 8b.12 and show trainees how to calculate Ask trainees to refer to Treatment Conversion Chart provided in Attachment 4.

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TRAINING OBJECTIVE FORMS FOR MODULES

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

Page: *1 of 1*

Objective Derived From Task/Sub-task/ (Short Title): Research and Testing Facilities

Ref. Nos.:

Job Aids — **Yes** ☐ **No** ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given the necessary materials and background information on research and testing facilities used in GIFT Project.

Behavior : Specify the minimum facility requirements for conducting breeding/selection work; explain the importance of major facilities required in fish breeding program.

Standard : According to specification standards set by GIFT Project

B. MASTERY TEST (Brief outline)

Real performance test in a simulated condition. The whole mastery test can be given in a training room by asking the trainee to specify all the necessary facility requirements for conducting fish breeding/selection work and explain briefly their corresponding use or importance.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
1.0	01	Specify the minimum basic facility requirements for conducting quarantine of germplasm.	Classifying
	02	Determine basic quarantine facility to avoid contamination of stocks.	Rule Using
	03	Specify facilities for maintenance of live fish germplasm.	Classifying
	04	Specify facilities for maintenance of cryopreserved germplasm.	Classifying
	05	Specify facilities for breeding of tilapia.	Classifying
	06	Categorize facilities used during different phases of selective breeding experiment.	Classifying
	07	Determine importance of the facilities used for each phase of selective breeding.	Rule Using

Completed by: **Date:**

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

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Objective Derived From Task/Sub-task/ (Short Title): Sampling and recording of phenotypic traits

Ref. Nos.:

Job Aids Yes ☒ No ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Give the necessary materials and sample data forms

Behavior : Collect and record biological data

Standard : According to standard sampling and recording procedures developed by GIFT Project and with 100% accuracy

B. MASTERY TEST (Brief outline)

Real performance test in a simulated condition, the trainee, given the necessary materials and sample data forms will collect and record biological data according to standard sampling and recording procedures developed by GIFT Project and with 100% accuracy.

Ref. No. Sub Task	Ref. No. *Int. Obj.*	Intermediate Objectives	Type of S/K/A
2a	01	Determine the objective of the breeding experiment	Rule Using
	02	Identify pertinent data which needs to be collected	Classifying
	03	Assign who should gather and record data	Classifying
	04	Devise data sheet form	Problem Solving
	05	Coordinate with other members of the group who will participate in sampling and data gathering	Problem Solving
	06	Schedule sampling of breeders prior to stocking in breeding hapa	Rule Using
	07	Assemble all the materials needed for sampling breeders	Classifying
	08	Collect breeders to be sampled	Classifying

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TRAINING OBJECTIVE

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Ref. No. Sub Task	*Int. Obj.*	**Intermediate Objectives**	**Type of S/K/A**
	09	Apply anesthetization procedure to breeders before sampling	Physical Skill, Rule Using
	10	Specify and record breeding hapa number where a selected breeder is to be stocked	Classifying, Physical Skill,
	11	Differentiate the sex of male breeder from female breeder	Discriminating
	12	Recognize the sex of fish breeder	Discriminating
	13	Identify breeder through its Pit tag or Floy Tag	Classifying
	14	Read and record the tag number	Rule Using, Physical Skill
	15	Weigh each breeder and record initial weight	Rule Using
	16	Measure and record standard length	Rule Using
	17	Measure and record body depth	Rule Using Physical Skill
	18	Specify caudal fin bars	Classifying
	19	Specify the schedule dates for collection of fry	Classifying
	20	Define sexual maturity	Classifying
	21	Describe indices of sexual maturity	Rule Using
	22	Examine condition of papillae/belly and buccal cavity of breeder	Rule Using
	23	Determine scoring system to indicate status of sexual maturity	Rule Using
	24	Construct reproductive index in each brood fish	Problem Solving
	25	Schedule sampling of breeders 10-14 days after stocking	Rule Using
	26	Assemble all the materials needed for sampling of breeder	Classifying
	27	Collect breeders to be sampled	Classifying

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TRAINING OBJECTIVE

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Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
	28	Collect and record data such as hapa number, sex, tag number, body weight, standard length	Classifying Physical Skill
	29	Specify and record the number of hapa where fry are collected	Classifying Physical Skill
	30	Define batch variable	Classifying
	31	Specify and record the batch number spawned families	Classifying Physical Skill
	32	Specify and record the complete date when tilapia fry are collected	Classifying Physical Skill
	33	Specify and record the complete date when fry are stocked in nursing hapas	Classifying Physical Skill
	34	Sort the fry collected	Classifying
	35	Separate the dead fry from alive fry	Classifying
	36	Count and record the number of dead and alive fish	Classifying
	37	Define bulk weight of fry	Classifying
	38	Weigh in bulk the fry collected and record	Discriminating Physical Skill
	39	Compute for mean weight of individual fry based in bulk weight	Problem solving
	40	Record mean weight	Physical Skill
	41	Specify and record size of hapa where fry are stocked	Classifying
	42	Schedule sampling of post fry prior to transfer in B-net cage	Rule Using
	43	Assemble all the materials needed	Classifying
	44	Collect the post fry to be sampled	Classifying

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TRAINING OBJECTIVE

Project: GIFT Manual of Procedures			Page: 4 of 4
Ref. No.		**Intermediate Objectives**	**Type of S/K/A**
Sub Task	***Int. Obj.***		
	45	Specify the dimension and number of hapa where post fry are harvested	Classifying
	46	Count and record the number of fry harvested from each replicated hapa 1	Rule Using Physical Skill
	47	Count and record the number of fry stocked in hapa 2	Rule Using Physical Skill
	48	Specify and record the dimension of B-net cage where post fry are transferred	Classifying Physical Skill
	49	Define body weight per family	Classifying
	50	Schedule sampling of fingerlings prior to tagging	Rule Using
	51	Assemble all the materials needed to sample fingerlings	Classifying
	52	Collect at random 30% of the population per B-net cage	Classifying
	53	Apply anesthetization procedure to fingerlings before sampling	Rule Using
	54	Collect and record the sample number, tag number, tag color, initial body weight, standard length, initial body depth, maturity condition	Classifying Physical Skill
	55	Schedule final sampling of fish	Rule Using
	56	Assemble all the materials needed in final sampling	Classifying
		Collect and record the sample number, tag number, tag color, sex of fish, final body weight, final standard length, final body depth, maturity condition	Classifying Physical Skill
Completed by:			**Date:**

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures		Page: 1 of 1
Objective Derived From Task/Sub-task/ (Short Title): Anesthetization of tilapia	Ref. Nos.:	Job Aids Yes ☒ No ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given the necessary materials and background information on the subject

Behavior : Specify and apply anesthetization procedures on a given sample of fish

Standard : With minimal stress and 100% restoration of mobility of fish after a designated period of exposure in recovery water

B. MASTERY TEST (Brief outline)

Real performance test in a wet laboratory, the trainee with all the necessary materials will anesthetize tilapia. The anesthetized fish after exposure in recovery water should have minimal stress and should regain 100% mobility after a designated period.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	*Int. Obj.*		
4.0	01	Define what is anesthetic	Classifying
	02	Specify materials necessary for anesthetization	Classifying
	03	Determine effect of anesthetic on fish	Rule Using
	04	Recognize the materials necessary for anesthetization	Discriminating
	05	Weigh a small amount of anesthetic powder needed to prepare the solution	Discriminating
	06	Estimate the time required for fish to be immersed in anesthetic solution	Discriminating
	07	Realize negative side effects brought to fish during prolonged exposure to anesthetic solution	Problem Solving
	08	Place anesthetized fish in recovery basin to regain full mobility	Physical Skill
Completed by:			Date:

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures		Page: *1 of 2*
Objective Derived From Task/Sub-task/ (Short Title): Marking of tilapia	Ref. Nos.:	Job Aids Yes ☐ No ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given necessary materials for tagging the fish and background information on the subject

Behavior : Perform different marking procedures (floy-tagging, PIT tagging, fin clipping and dorsal spine uprooting) in tilapia

Standard : With minimal stress on the fish and should be able to tag at least 60 pieces of fish per hour, PIT tag 10 fish breeders, pin clip 20 fingerlings and uproot dorsal spine of 10 breeders in 2 hrs..

B. MASTERY TEST (Brief outline)

Real performance test in a wet laboratory, the trainee will be given all the necessary materials and will be asked to perform the different fish marking procedures on tilapia. Fish marking should be done with minimal stress on the fish and trainee should be able to tag at least 60 pieces of fish per hour, PIT tag 10 fish breeders, pin clip 20 fingerlings and uproot dorsal spine of 10 breeders in 2 hrs.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
5.0	01	Define fish marking	Classifying
	02	Specify importance of fish marking in a breeding program	Classifying
	03	Determine effects of tagging/marking on fish	Rule Using
	04	Determine advantages and disadvantages of different types of tags/marking procedure	Rule Using
	05	Specify the kind of tag suitable for the fish	Discriminating
	06	Assemble all the materials needed for fixing the tags	Classifying
	07	Assign tags according to groups of fish and the environments where these fish will be stocked	Classifying
	08	Arrange all the materials that will be used for tagging	Classifying

TRAINING OBJECTIVE

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Ref. No.			
Sub Task	***Int. Obj.***	**Intermediate Objectives**	**Type of S/K/A**
	09	Match the fingerlings with the assigned tag numbers	Discriminating
	10	Specify the correct side of the fish body for marking	Classifying
	11	Determine the specific site/location in fish body for marking	Rule Using
	12	Employ the marking procedure	Physical Skill, Rule Using
	13	Transfer marked/tagged fish in recovery water to regain mobility	Physical Skill, Rule Using
Completed by:			Date:

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures		Page: *1 of 2*
Objective Derived From Task/Sub-task/ (Short Title): Management of Genetic Database	Ref. Nos.:	Job Aids Yes ☒ No ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given sample datasets from breeding experiments, computer and necessary background information on the subject

Behavior : Specify and apply procedures for storage, retrieval and analysis of datasets

Standard : With accuracy and according to standard database management protocols

B. MASTERY TEST (Brief outline)

Real performance test in a classroom, the trainee given sample datasets generated from breeding experiment and background information on the subject, will store, retrieve and analyze data with accuracy and according to standard database management protocols

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
12.0	01	Define database management	Classifying
	02	Specify objectives of genetic database management	Classifying
	03	Check softwares/programs used in storing, retrieval and analysis of data	Rule Using
	04	Review application and operation of these programs/softwares	Classifying
	05	Review procedures on management of genetic database	Discriminating
	06	Collect all accomplished forms with dataset entries	Classifying
	07	Check if correct entries have been inputted into their respective boxes or columns in data form	Rule Using

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TRAINING OBJECTIVE

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Ref. No.		**Intermediate Objectives**	**Type of S/K/A**
Sub Task	***Int. Obj.***		
	08	Double check, clear and look for dubious entries of data	Classifying
	09	Arrange accomplished forms chronologically and put controls in each form	Classifying
	10	Check if footers and headers of data forms are properly filled out	Rule Using
	11	Define data structures	Classifying
	12	Specify functions of data structures	Classifying
	13	Specify procedures for defining data structure	Classifying
	14	Specify user-interface that will handle data entry activities for GIFT Project	Classifying
	15	Specify appropriate procedure of editing data	Classifying
	16	Define updating of file	Classifying
	17	Run for an updated file to check again if error still exists	Rule Using/ Physical Skill
	18	Perform a regular clean-up and back-up of the master file	Rule Using/ Physical Skill
	19	Choose appropriate software in analysis of genetic data	Discriminating
	20	Apply statistical analysis procedure using the Program/Software	Physical Skill/ Rule Using/ Problem Solving
Completed by:			**Date:**

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

Objective Derived From Task/Sub-task/ (Short Title):

Breeding of tilapia

Ref. Nos.:

Job Aids — **Yes** ☒ **No** ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : In the classroom and in field laboratory under simulated conditions

Behavior : Specify the step-by-step field procedures for conducting the breeding experiments which led to genetic improvement of farmed tilapia and estimate the important genetic parameters.

Standard : In accordance with the protocol adopted by the GIFT Project

B. MASTERY TEST (Brief outline)

Given all the needed background information, trainees will specify (by written examination) the step-by-step field procedures for conducting breeding experiments; trainees, in groups, will be asked to estimate the important genetic parameters (heritability and response to selection).

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
4a	01	Review fish breeding protocol/quantitative genetics	Discriminating
	02	Specify objective, strategy of the breeding experiment and expected output	Classifying
	03	Specify required facilities, materials, manpower requirements	Classifying
	04	Schedule activities of the breeding experiment	Rule Using
	05	Choose stocks that will be used as breeders	Discriminating
	06	Determine the number of breeding hapas	Rule Using
	07	Determine the number of breeders required per hapa net cage	Rule Using

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TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

Ref. No. Sub Task	Ref. No. Int. Obj.	Intermediate Objectives	Type of S/K/A
4a	08	Determine importance/protocols in conditioning of tilapia breeders prior to spawning	Discriminating; Rule Using
	09	Specify principle of mating plan	Classifying Rule Using
	10	Develop a mating plan prior to stocking of breeders	Problem Solving
	11	Determine all pertinent data to be gathered prior to stocking of breeders in breeding hapa	Rule Using
	12	Determine appropriate procedures in fry collection	Rule Using
	13	Specify data to be gathered during fry collection	Classifying Discriminating
	14	Determine procedures in rearing tilapia fry/fingerlings	Classifying; Rule Using
	15	Determine the total number of fingerlings to be tagged and stocked in test environments	Rule Using
	16	Know pertinent data to be recorded during grow-out of fingerlings	Discriminating
	17	Determine procedures for gathering/recording data on growth, survival, reproduction and other phenotypic traits	Discriminating; Rule Using
	18	Collect pertinent data during harvesting	Classifying
	19	Assemble all the materials needed in estimating genetic parameters	Physical Skill Rule Using
	20	Correct the body weights for the effect of sex prior to estimating heritability and response to selection; Run SAS procedures to produce computer outputs	Discriminating; Rule Using

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TRAINING OBJECTIVE

Project: GIFT Manual of Procedures		
Objective Derived From Task/Sub-task/ (Short Title): Characterize fish population through morphometry	Ref. Nos.:	Job Aids Yes ☒ No ☐

A. TERMINAL OBJECTIVE ☐ POST TRAINING OBJECTIVE ☐

Conditions : Given the necessary materials and background information on the subject

Behavior : Characterize fish population by using truss morphometry

Standard : According to standard procedures described in truss morphometry

B. MASTERY TEST (Brief outline)

Real performance test in a laboratory, the trainee with all the necessary skill will perform with accuracy the various truss morphometric procedures (photo documentation, data gathering by caliper method/digitizing, data analysis and interpretation). Scoring key will be used in measuring the accuracy.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	*Int. Obj.*		
6a.0	01	Define truss morphometry	Classifying
	02	Review technique of photodocumentation	Discriminating
	03	Assemble the materials, equipment necessary to carry out the task	Classifying
	04	Choose the fish samples to be measured	Discriminating
	05	Review external body morphology of fish	Discriminating
	06	Specify landmark points in fish body	Classifying
	07	Position the camera to take lateral view photos of fish samples for linear measurements	Rule Using
	08	Determine the operationalization of CAMA	Rule Using
	09	Specify the CAMA system requirements	Classifying

TRAINING OBJECTIVE

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Ref. No.			
Sub Task	***Int. Obj.***	**Intermediate Objectives**	**Type of S/K/A**
	10	Collect morphometric measurements by digitizing the landmark points	Classifying
	11	Specify the statistical analysis to be used	Classifying
	12	Conduct the Analysis	Rule Using, Problem Solving
	13	Interpret the results	Rule Using
	14	Construct the graphs or graphical illustration to describe the genetic variability of fish population	Problem Solving, Rule Using
Completed by:			**Date:**

TRAINING OBJECTIVE

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Objective Derived From Task/Sub-task/ (Short Title): Characterize tilapia by biochemical means using starch gel electrophoresis	**Ref. Nos.:**	Job Aids **Yes** ☒ **No** ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given the necessary materials for tagging the fish and background information on the subject

Behavior : Characterize a given fish population by biochemical means using starch gel electrophoresis

Standard : According to standard procedures described in starch gel electrophoresis (See Fish. Tech. Manual No. 11, December 1991)

B. MASTERY TEST (Brief outline)

Real performance test in a laboratory, the trainee with all the necessary materials will perform starch gel electrophoresis in a given fish population.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
6b.0	01	Define electrophoresis	Classifying
	02	Specify importance of starch-gel electrophoresis in population genetics	Classifying
	03	Collect fish samples suitable for biochemical characterization	Classifying
	04	Photodocument fish samples for morphometric analysis and then freeze	Physical Skill
	05	Specify the parts of fish body where tissues will be obtained	Classifying
	06	Dissect and extract the tissue	Physical Skill
	07	Determine procedure on how to properly process the tissue extracted	Rule Using

Please turn over

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures	Page: *2 of 2*

Ref. No. Sub Task	Ref. No. *Int. Obj.*	Intermediate Objectives	Type of S/K/A
	08	Assemble the materials that will be used in preparing the starch gel	Classifying
	09	Cook starch gels and load samples onto the gels	Physical skill, Rule Using
	10	Carry out the electrophoresis, gel slicing and staining procedures	Physical skill, Rule Using, Discrimina-ting
	11	Record band/assign allele designations	Discriminating
	12	Perform simple standard statistical analyses on the data	Discrimina-ting; Rule Using
	13	Interpret information from the data	Discrimina-ting; Rule Using
Completed by:			Date:

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures		Page: 1 of 2
Objective Derived From Task/Sub-task/ (Short Title): Maintain Live Tilapia Germplasm	Ref. Nos.:	Job Aids Yes ☒ No ☐

A. TERMINAL OBJECTIVE ☐ POST TRAINING OBJECTIVE ☐

Conditions : Given the necessary background information on the subject, under simulated condition

Behavior : Identify and apply routine procedures for maintaining important live fish germplasm

Standard : According to standard procedures followed in GIFT Project

B. **MASTERY TEST** (Brief outline)

Real performance in a simulated laboratory conditions, the trainee, with all the background information on the subject and clear instructions, will describe how to maintain important live fish germplasm and apply its routine procedures.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	*Int. Obj.*		
6a	01	Define ex-situ conservation of fish	Classifying
	02	Specify purpose/objective of maintaining live collections of fish germplasm	Classifying
	03	Develop cost effective procedure or means of maintaining live tilapia collections	Classifying
	04	Identify strains or fish populations that will be kept as live collections	Classifying
	05	Pit tag tilapia stocks to differentiate individuals	Classifying, Physical Skill
	06	Condition stocks in B-net cages for 14 days to prepare for breeding	Physical Skill

Please turn over

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

Page: *2 of 2*

Ref. No. Sub Task	Int. Obj.	Intermediate Objectives	Type of S/K/A
	07	Apply procedures in breeding stocks in hapa net cages	Physical Skill, Rule Using
	08	Separate 50 fry from each family and stock in 1mx1mx1m hapa net	Classifying
	09	Separate males and females from each family/strain, floy tagged and stock in B-net cages	Classifying, Physical Skill
	10	Apply procedures for rearing until they are ready for pit tagging	Physical Skill
	11	Transfer pit tagged breeders to ponds separated by strain	Classifying, Physical Skill
	12	Develop a masterlist of stocks to be maintained in all holding tanks and ponds	Problem Solving
	13	Organize routine maintenance activities	Rule Using
	14	Monitor physico-chemical characteristics of water in ponds and tanks	Rule Using
	15	Check feeding requirement of stocks in tanks and ponds	Rule Using
	16	Specify amount of fertilizers required in ponds	Classifying
	17	Check the number of fish that remain in tanks and ponds	Classifying
	18	Monitor health conditions of stocks	Rule Using
	19	Check for my unusual behavior or fish mortality	Rule Using
	20	Specify pertinent parameters or data to be recorded in form	Classifying
Completed by:			Date:

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures		**Page:** *1 of 3*
Objective Derived From Task/Sub-task/ (Short Title): Cryopreservation of tilapia milt	**Ref. Nos.:**	Job Aids **Yes** ☒ **No** ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given the tilapia milt sample and all the necessary equipment and materials

Behavior : Cryopreserve the milt and test the viability of the sample

Standard : With accuracy and according to the protocols developed by University of Stirling in Scotland

B. MASTERY TEST (Brief outline)

Real performance test in a cryopreservation laboratory, the trainee with all the necessary materials will cryopreserve the milt of a given fish sample. The activity should be performed with accuracy and according to the protocols developed by the Institute of Aquaculture, University of Stirling, Scotland.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
10.0	01	Review ex situ conservation in general and cryopreservation of the species	Discriminating
	02	Specify equipment and materials needed	Classifying
	03	Specify the population or stocks to become donor of milt samples	Classifying
	04	Determine appropriate extender to be used in cryopreserving the species	Rule-Using
	05	Determine dosage of extender solution to be prepared	Rule Using
	06	Determine the main parts and function of the KRYO 10	Rule Using
	07	Specify procedures for operating the equipment	Classifying
	08	Choose breeders for milt collection	Discriminating

Please turn over

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
	09	Assemble all the materials to be used	Classifying
	10	Specify procedures for collecting tilapia milt	Classifying
	11	Apply procedures for milt collection	Physical Skill
	12	Identify stages for estimating sperm density	Classifying
	13	Identify procedures in counting all concentration using haemacytometer	Classifying
	14	Use haemacytometer and compound microscope	Rule Using
	15	Get average cell count	Rule Using
	16	Convert average cell count into sperm cell concentration	Rule Using
	17	Specify procedure for preparing milt with extender	Classifying
	18	Calculate the amount of methanol to be added to the sperm sample	Rule Using
	19	Calculate the number of straws which can be reasonably filled within the time span given	Rule Using
	20	Assemble the material for filling straws with milt	Classifying
	21	Assign colors of straw and bead to a particular milt sample of a given strain	Classifying
	22	Gauge proper timing to fill the straw with milt and seal	Rule Using
	23	Specify general safety procedures to be observed	Classifying
	24	Identify protective clothing required for cryopreservation activity	Classifying
	25	Specify procedures for operating the equipment	Classifying
	26	Define all signals in the machine	Classifying
	27	Transfer straws containing milt sample and run the program	Physical Skill

Please turn over

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures			Page: *3 of 3*
Ref. No.		**Intermediate Objectives**	**Type of S/K/A**
Sub Task	***Int. Obj.***		
	28	Gauge proper timing to remove samples from KRYO 10 and transfer in liquid nitrogen	Rule Using
	29	Specify general safety procedures when handling liquid nitrogen	Classifying
	30	Wear proper clothing when opening dewar with cryopreserved milt samples	Physical Skill
	31	Observe safety measures when opening dewar	Attitude
	32	Specify materials/equipment for thawing cryopreserved milt samples	Classifying
	33	Immerse cryopreserved milt samples into waterbath	Physical Skill
	34	Define motility of milt	Classifying
	35	Specify procedure for scoring motility of tilapia milt	Classifying
	36	Estimate motility of tilapia milt	Rule Using
	37	Define viability of milt	Classifying
	38	Compare different stages of tilapia milt	Classifying
	39	Develop cryopreservation record data form	Problem solving
	40	Identify cryopreserved milt samples through color of straws, beads and dividers	Classifying
	41	Record pertinent cryopreservation data	Rule Using
Completed by:			**Date:**

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

Objective Derived From Task/Sub-task/ (Short Title):
Organization and Coordination with Test Stations

Ref. Nos.:

Job Aids — Yes ☐ No ☒

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given the background information on the subject

Behavior : Specify the basic steps in organizing/coordinating with selected test stations/farms

Standard : In accordance with the procedures adopted by the GIFT Project.

B. MASTERY TEST (Brief outline)

In classroom, trainees will be grouped into two and will conduct field survey to selected farm/station. The trainees will record all data during the visit and will make a report for group presentation.

Ref. No. Sub Task	*Int. Obj.*	Intermediate Objectives	Type of S/K/A
11.0	01	Recognize importance of conducting genetic evaluation experiments in selected test stations/farms	Discriminating
	02	Determine test environments or culture sites needed	Rule Using
	03	Assign overall coordinator for on-station/on-farm	Classifying
	04	Specify schedule of activities	Classifying
	05	Specify protocol for conducting on-farm experiment	Classifying
	06	Survey potential stations/farms	Classifying
	07	Inform station/farm managers about the planned experiment and possibility of considering it as test station/farm	Physical Skill/ Rule Using
	08	Visit the potential study site for survey	Physical Skill/ Rule Using

Please turn over

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures			Page: 2 of 2
Ref. No.			
Sub Task	***Int. Obj.***	**Intermediate Objectives**	**Type of S/K/A**
	09	Inform manager about the study, its objectives, activities	Physical Skill/ Rule Using
	10	Evaluate existing research facilities	Discriminating
	11	Determine source of water	Rule Using
	12	Specify outlet/inlet of ponds/tanks	Classifying
	13	Evaluate security of fish in test station/farm	Discriminating
	14	Define roles of station/farm collaborators/cooperators	Classifying
	15	Define roles of station/farm as collaborators/cooperators	Classifying
	16	Record all pertinent data during farm/station survey	Rule Using
Completed by:			Date:

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures

Objective Derived From Task/Sub-task/ (Short Title): Transport of Live Fish Germplasm

Ref. Nos.:

Job Aids Yes ☐ No ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given the background information on transport procedures adopted by GIFT Project.

Behavior : Specify and apply the basic steps in transporting live fish germplasm.

Standard : With consideration of the ICES/EIFAC Codes of Practice and Manual of Procedures for transfer of fish.

B. MASTERY TEST (Brief outline)

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	***Int. Obj.***		
2.0	01	Check policies/regulations for transfer of fish germplasm.	Rule Using
	02	Specify conditions set by international codes of practice.	Classifying
	03	Check protocols set by INGA for importing and exporting live carp and tilapia germplasm.	Rule Using
	04	Review import and export procedures.	Discriminating
	05	Determine source or place from where fish germplasm will be obtained.	Rule Using
	06	Determine steps in securing clearances and permits from importing/exporting country.	Rule Using
	07	Specify all facilities/materials needed for transport.	Classifying
	08	Classify the required facilities/materials according to phase of transport activity.	Classifying
	09	Discriminate between desired and undesired stocks for transport.	Discriminating

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures			Page: *2 of 2*
Ref. No.			
Sub Task	***Int. Obj.***	**Intermediate Objectives**	**Type of S/K/A**
	10	Perform conditioning of stocks.	-
	11	Determine number of fish to be transported.	Rule Using
	12	Pack live fish germplasm in plastic bags.	-
	13	Examine water quality parameters in transport bags.	Rule Using
	14	Load transport bags containing fish stocks to be transported.	-
Completed by:			**Date:**

TRAINING OBJECTIVE

Project: GIFT Manual of Procedures		Page: 1 of 1
Objective Derived From Task/Sub-task/ (Short Title): Quarantine and post-mortem examination of tilapia germplasm	**Ref. Nos.:**	Job Aids **Yes** ☐ **No** ☐

A. TERMINAL OBJECTIVE ☐ **POST TRAINING OBJECTIVE** ☐

Conditions : Given the fish samples and all the necessary facilities

Behavior : Recognize importance of quarantine and apply its procedures under simulated conditions

Standard : With consideration of INGA protocols on fish transfer and with minimal fish mortality after the quarantine period

B. MASTERY TEST (Brief outline)

Real performance under simulated wet laboratory, the trainees will be grouped and will be asked to apply the quarantine procedures in a given sample of fish stocks. Trainees will be observed and will be scored based on performance.

Ref. No.		Intermediate Objectives	Type of S/K/A
Sub Task	*Int. Obj.*		
3.0	01	Organize 'things to do' prior to arrival or exportation of fish germplasm	Rule Using
	02	Schedule disinfection of all holding facilities and quarantine accessories	Rule Using
	03	Replace water in plastic bags containing fish with clean water from holding tanks	Physical Skill
	04	Check fish stocks during acclimation period of 1-3 days	Rule Using
	05	Monitor stocks closely during the conduct of routine quarantine procedures	Rule Using
	06	Recognize difference between healthy and unhealthy stocks	Discriminating
	07	Examine fish stocks for external sign of diseases and parasites	Rule Using
	08	Diagnose cause of disease or parasitic infection	Problem Solving
	09	Prescribe treatment to infection	Rule Using

Completed by: **Date:**

TASK DESCRIPTION FORMS FOR MODULES

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist		
Duty: Fish breeding	Completed by:	Date:
Task: 2a. Sampling and Recording of Phenotypic Traits	4. References/Standards for the task (If any): 100% accuracy in reading and recording the genetics data and according to the standard procedures developed by the GIFT Project	
1. **Where performed:** Farm/Station		
2. **Triggering Event:** Indication of need to gather pertinent genetics data		
3. **Terminating Event:** Genetics data accurately recorded		

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2a.1	Plan specific activities prior to sampling and recording of data	Inefficient gathering of data	Determine the objectives of the breeding experiment
			Identify pertinent data which needs to be collected
			Assign who should gather and record data
			Devise data sheet forms
			Coordinate with other members of the group who will participate in sampling and data gathering
2a.2	Sample breeders prior to stocking of breeders in breeding hapa	Inadequate data gathered prior to stocking of breeders	Schedule sampling of breeders prior to stocking in breeding hapa
			Assemble all the materials needed for sampling of breeders
			Collect breeders to be sampled
		Stress during sampling	Apply anesthetization procedure to breeders before sampling

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2a.2.1	Determine breeding hapa number		Specify and record breeding hapa number where a selected breeder is to be stocked
2a.2.2	Indicate the sex of breeder		Differentiate the sex of male breeder from female breeder
2a.2.3	Indicate the tag number of breeder		Record sex of fish breeder Identify breeder through its Pit tag or Floy tag
2a.2.4	Determine initial weight of each breeder		Read and record the tag number Weigh each breeder and initial individual weight
2a.2.5	Determine standard length prior to stocking		Measure and record standard length
2a.2.6	Determine body depth prior to stocking		Measure and record body depth
2a.2.7	Indicate caudal fin bar		Specify caudal fin bars
2a.2.8	Determine sexual maturity condition		Define sexual maturity Describe indices of sexual maturity Examine condition of papillae/belly and buccal cavity of breeder Determine scoring system to indicate status of sexual maturity Construct reproductive index in each broodfish
2a.3	Sample breeders after spawning		Schedule sampling of breeders 10-14 days after stocking

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
			Assemble all the materials needed for sampling of breeders
			Collect breeders to be sampled
			Collect and record data such as hapa number, sex, tag number, body weight, standard length, body depth, caudal fin bars
2a.4	Gather data during fry collection		Apply procedures in pond preparation
2a.4.1	Indicate the hapa number		Specify and record the number of hapa where fry are collected
2a.4.2	Determine batch variable		Define batch variable
			Specify and record the batch number of spawned families
2a.4.3	Record data of fry collection		Specify and record the complete date when tilapia fry are collected
2a.4.4	Record date of fry transfer		Specify and record the complete dates when fry are stocked in nursing hapas
2a.4.5	Record number of fry collected		Sort the fry collected
			Separate the dead fry from the alive fry
			Count and record each group (dead and alive fry)
2a.4.6	Determine bulk weight		Define bulk weight of fry
			Weight in bulk the fry collected and record
2a.4.7	Determine mean weight of individual fry		Compute for mean weight of individual fry based on bulk weight

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2a.4.8	Indicate hapa size		Record mean weight Specify and record size of hapa where fry are stocked
2a.5	Gather data during transfer of post-fry from nursery hapa to b-net cage		Schedule sample of post fry prior to transfer in B-net cage Assemble all the materials needed Collect the post-fry to be sampled
2a.5.1	Indicate hapa number and dimension		Specify the dimension and number of hapa where the post fry are harvested
2a.5.2	Record survival from each replicated hapa		Count and record the number of fry harvested from each replicated hapa 1
2a.5.3	Record number of post fry stocked in Hapa 2		Count and record the number of fry stocked in Hapa 2
2a.5.4	Record size of B-net cage		Specify and record the dimension of B-net cage where post fry are transferred
2a.5.5	Record body weight per family		Define body weight per family
2a.6	Sample fingerlings before tagging		Schedule sampling of fingerlings prior to tagging Assemble all the materials needed to sample fingerlings Collect at random 30% of the population per B-net cage Apply anesthetization procedure to fingerlings before sampling Specify and record weight and length measurements of the fingerlings

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2a.7	Sample fingerlings after floy tagging		Assemble all the materials needed to sample fingerlings after floy tagging Apply anesthetization procedure Collect and record the sample number, tag number, tag color, initial body weight, initial standard length, initial body depth, maturity condition
2a.8	Sample fish at harvest		Schedule final sampling of fish Assemble all the materials needed in final sampling Collect and record the sample number, tag number, tag color, sex of fish, final body weight, final standard length, final body depth, maturity condition
2a.9	Tag fingerlings		Determine the total number of fingerlings to be tagged Sort the fingerlings to be tagged according to families and environment Apply appropriate procedures on tagging of tilapia
2a. 10	Stock tagged fingerlings in test environment		Check test environments (pons, cages, etc.) are reading for stocking Specify the number of tagged fingerlings to be stocked per environment Sort the tagged fingerlings to be stocked Record pertinent data on fingerlings prior to stocking
2a. 11	Rear tagged fingerlings in test environment		Check feed requirements of stocks Monitor water quality in test environments Monitor stocks in test environments

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2a. 12	Harvest fish	Stress during harvesting	Sample fish to gather data on growth, survival, reproduction and other phenotypic traits Determine pertinent data to be gathered during grow-out Specify schedule of harvesting stocks Apply appropriate harvesting procedures Collect pertinent data during harvesting Observe the condition of the fish before sampling Put the harvested fish in tanks separately by sex for conditioning prior to transport to Project Site.

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist

Duty: Fish breeding	**Completed by:**	**Date:**

Task: 2b. Marking of Tilapia	4. **References/Standards for the task (If any):** To be performed with minimal stress on fish and that trainee after the course should be able to tag at least 60 fish after one hour.
1. **Where performed:** Field/Laboratory	
2. **Triggering Event:** Indication of need to mark tilapia stocks for identification (for example during communal rearing)	
3. **Terminating Event:** Fish stocks tagged efficiently	

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2b.1	Introduce tagging	Lack of understanding on importance of tagging	Define tagging. Specify importance of tagging in a breeding program.
2b.2	Identify tag suitable for external marking of a given species and stage of fish	Use of the wrong type of tag on fish	Determine advantages and disadvantages of different types of tags. Determine effects of tagging fish
2b.3	Prepare the materials for tagging	Possible use of poor quality tag	Recognize the suitable tag taking into consideration the age and size of the fish. Determine the supplier of appropriate tag.
2b.4	**Floy Tagging** Fixing the Floy tags	Incomplete set of materials Error in assigning tags to group of fish	Assemble all the materials needed for fixing the Floy tags. Assign Floy tags according to groups of fish and the environment where these fish will be stocked.
2b.5	Prepare materials for tag insertion	Unfamiliarity with the tagging materials	Arrange all the materials that will be used for Floy tag insertion.
2b.6	Prepare the fish		Match the fingerlings with the assigned tag numbers.

Please turn over

TASK DESCRIPTION FORM			
5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2b.7	Position the fish	Stress on fish due to incorrect insertion site	Specify the correct side of the body for tag insertion.
2b.8	Locate the insertion site		Determine the insertion site in the fish body.
2b.9	Insert the needle	Lack of dexterity during tag insertion	Push the needle on the insertion site, attach plastic disc, make three loophole knots and cut thread to desired length.
2b. 10	Place tagged fish in recovery basic	Increased stress of fish due to prolonged handling	Transfer Floy tagged fish in recovery basin to regain mobility
	Pit Tagging		
2b. 11	Prepare materials for tag insertion	Lack of familiarity with Pit tag materials	Arrange all the materials for pit tagging
2b. 12	Load identity tags in the needle		Insert the tag into the hole of the syringe needle
2b. 13	Pit tag the fish	Difficulty in locating Pit tag insertion site	Determine insertion site
		Lack of dexterity in pit tag insertion	Push the plunger of the syringe to securely lodge the tag in visceral cavity
2b. 14	Placed tagged fish in recovery basin	Increase stress of fish due to prolonged handling	Transfer pit tagged fish in recovery basin to regain mobility.

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist	
Duty: Fish breeding	**Completed by:** / **Date:**
Task: 2c. Management of Genetic Database	4. **References/Standards for the task (If any):** With accuracy and according to standard database management protocol; Basic Operational Manual SAS Operational Manuals GIFT Stat Manual
1. **Where performed:** Computer Room/Classroom	
2. **Triggering Event:** Indication of need to manage a huge amount of data generated from breeding experiments	
3. **Terminating Event:** Data compiled into one integrated database following standard procedures for processing, management and access.	

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2c.1	Review Database Management		Define Database Specify objectives of genetic database management Check softwares/programs used in storing/retrieval and analysis of data Review application and operation of these programs/softwares
2c.2	Validate the data collected	Erroneous encoding	Check if correct entries have been inputted into their respective boxes or columns in data form Double check, clear and look for dubious entries of data Arrange accomplished forms chronologically and put controls in each form Check if footers and headers of data forms are properly filled out

Please turn over

TASK DESCRIPTION FORM			
5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
2c.3	Prepare data structures	Lack of understanding on data structures	Define data structures Identify functions of data structures Specify procedures for defining data structure
2c.4	Encode/Edit data		Specify user interface that will handle data entry activities for GIFT Project Identify appropriate procedure of editing data
2c.5	Updating the file		Define updating of file Run for an updated file to check again if error still exists Perform a regular clean-up and back-up of the master file
2c.6	Analyze datasets	Inadequate knowledge in the use of application programs for data analysis	

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist

Duty: Fish breeding

Completed by: **Date:**

Task: 4a. Breeding Strategies for Tilapia

1. **Where performed:** Project/Farm Site

2. **Triggering Event:** Indication of need for selective breeding due to poor performance of stocks

3. **Terminating Event:** Breeding experiment conducted and important genetic parameters (heritability, response to selection) estimated.

4. **References/Standards for the task (If any):**

In accordance with the GIFT Project Protocols.

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
4a.1	Determine objective/strategy of the breeding experiment and expected output	Inadequate knowledge on breeding protocols/ quantitative genetics of fish	Review fish breeding protocols/ quantitative genetics Specify objective, strategy of the breeding experiment and expected output
4a.2	Plan resource requirements/ activities prior to start of breeding experiment	Lack of planning, organization of the experiment	Specify required facilities, materials, manpower requirements; Schedule activities of the breeding experiment
4a.3	Condition tilapia breeders that will be used in breeding experiment		Select stocks that will be used as breeders; Determine the number of breeding hapa net cages Determine the number of breeders required per hapa net cage Clip premaxilla of male breeders before stocking in hapa net cages Record the weights of breeders before stocking Stock the breeders in hapa net cages

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
			Determine the amount of artificial feed required Feed breeders in hapa net cage during conditioning
4a.4	Prepare breeding ponds		Apply procedures in pond preparation
4a.5	Install breeding hapas	Possible presence of holes in hapas which may result in loss of fry	Check breeding hapas before installation in earthen ponds
4a.6	Stock breeders	Inavailability of mating plan	Specify principle of mating plan Develop a mating plan prior to stocking of breeders Determine all pertinent data to be gathered prior to stocking of breeders in breeding hapas Record the important data of breeders prior to stocking
4a.7	Collect Fry 10-14 days after stocking the breeders	Asynchronous spawning High mortality due to stress in fry collection	Specify the schedule dates for collection of fry Apply appropriate procedures in fry collection
4a.8	Gather pertinent data during fry collection	Inadequate data gathered during fry collection	Specify data to be gathered during fry collection Apply appropriate procedures in weighing/counting the fry collected
4a.9	Rear families in nursery hapas	Tendency of fry to be overstocked or under stocked Mortality due to stress in stocking	Determine the suitable number of fry to be stocked in each nursery hapa Stock the known number of fry/post fry in nursery hapas/B-net cages with less stress

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
4a. 10	Tag fingerlings		Determine the total number of fingerlings to be tagged
			Sort the fingerlings to be tagged according to families and environment
			Apply appropriate procedures on tagging of tilapia
4a. 11	Stock tagged fingerlings in test environment		Check test environments (pons, cages, etc.) are reading for stocking
			Specify the number of tagged fingerlings to be stocked per environment
			Sort the tagged fingerlings to be stocked
			Record pertinent data on fingerlings prior to stocking
4a. 12	Rear tagged fingerlings in test environment		Check feed requirements of stocks
			Monitor water quality in test environments
			Monitor stocks in test environments
			Sample fish to gather data on growth, survival, reproduction and other phenotypic traits
			Determine pertinent data to be gathered during grow-out
4a. 13	Harvest fish	Stress during harvesting	Specify schedule of harvesting stocks
			Apply appropriate harvesting procedures
			Collect pertinent data during harvesting
			Observe the condition of the fish before sampling
			Put the harvested fish in tanks separately by sex for conditioning prior to transport to Project Site.

4a. *14*	Compute for genetic parameters (heritability, response to selection)	Inadequate knowledge in SAS, basic quantitative genetics	Assemble all the materials needed in estimating genetic parameters Correct the body weights for the effect of sex prior to estimating heritability and response to selection Run SAS procedures to produce computer outputs

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist		
Duty: Fish breeding	**Completed by:**	**Date:**

Task: 5a. Characterize fish population through morphometry	4. **References/Standards for the task (If any):** Perform with accuracy the step-by-step procedure described in Practical Guide Mod. 5a; Manual on CAMA Version 1.0
1. **Where performed:** Genetics Laboratory	
2. **Triggering Event:** Indication of need to characterize fish to determine genetic variability	
3. **Terminating Event:** Fish stocks/population characterized successfully using morphometrics	

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
5a.1	Plan activities to carry out morphometric measurements	Lack of basic knowledge in morphometry	Review morphometry in general; techniques of truss morphometry
5a.2	Prepare materials for photo documentation	Unfamiliar with the technique	Review technique of photo documentation Assemble the materials, equipment necessary to carry out the task Choose the fish samples to be measured
5a.3	Establish indices by plotting chosen landmark points in fish body	Unfamiliar with the external body morphology of fish	Review external body morphology of fish Identify landmark points in fish body
5a.4	Get photographs of fish samples	Lack of skill in getting photographs, focusing, understanding of lights	Determine the operationalization of a camera Position the camera to take lateral view photos of fish samples for linear measurements

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
5a.5	Collect morphometric data using CAMA	Operationalization of CAMA not known	Determine the operationalization of CAMA Specify the CAMA System requirements Collect morphometric measurements by digitizing the landmark points
5a.6	Analyze morphometric data	Inadequate understanding of performing the Multivariate Statistical Analysis, Discriminant Analysis	Specify the Statistical Analysis to be used Conduct the analysis, Interpret the results Construct the graphs or the graphical illustration to describe the population

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist		
Duty: Fish breeding	Completed by:	Date:
Task: 5b. Characterize tilapia using starch gel electrophoresis	4. References/Standards for the task (If any): According to standard procedures described in "A Practical Laboratory Guide to the Techniques and Methodology of Electrophoresis" by J. Macaranas; Fish Tech. Man. No. 11, Dec. 1991	
1. Where performed: Genetics Laboratory		
2. Triggering Event: Genetic variability of a given fish population not known		
3. Terminating Event: Fish stocks anesthetized successfully		

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
5b.1	Review electrophoresis		Defined electrophoresis
5b.2	Prepare fish samples from field and photo-document for morphometric analysis		Collect fish samples suitable for biochemical characterization
5b.3	Freeze fish samples immediately upon arrival in the laboratory		Photodocument fish samples for morphometric analysis and freeze
5b.4	Prior to electrophoresis, process the tissues from 20-25 fish samples per run		Specify the part of fish body where tissues will be obtained Dissect and extract the tissue Determine procedure on how to properly process the tissues extracted
5b.5	Keep tissue samples in microwell tissue culture plates		Put the microwell tissue culture plates on ice or refrigerated packs to keep the samples cold.
5b.6	Label the plate and store in freezer until tissues are analyzed		Specify correct label for the microwell tissue culture plate

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
5b.7	Prepare starch gel by mixing a buffer with hydrolyzed potato starch		Assemble the materials that will be used in preparing the starch gel Weigh the starch and put in Erlenmeyer flask Cook the starch mixture
5b.8	Release the starch gel from the mold by means of a scalpel blade		Check whether the starch gel is ready for release from the mold
5b.9	Cut the longitudinal section of the starch gel for sample application		
5b.10	Separate the anodal and cathodal portions of the starch gel for sample application		Specify the anodal and cathodal portions of the starch gel
5b.11	Mark the edges and mid portion of the gel with tracking dye		
5b.12	Apply sample from the left to the right end of the gel		
5b.13	Align the anodal and cathodal pieces in preparation for electrophoresis		
5b.14	Set the gel on a cooling plate and apply the filter paper and absorbent sponge cloth on both sides of the gel		
5b.15	Set up the starch gel between the electrode buffer tanks		

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
5b.16	Prepare the starch gel for slicing by putting it on a firm elevated foundation		
5b.17	Slide the gel with a cupping saw fitted with a thin-rusting wire		
5b.18	Cut the gel in two to facilitate the peeling process		
5b.19	Peel the top gel slab with a thin plastic strip		
5b.20	Lift the gel slab with fingers		
5b.21	Transfer the gel slab to the steaming dish		

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist		
Duty: Fish breeding	**Completed by:**	**Date:**
Task: 6a. Maintenance of Live Tilapia Germplasm	**4. References/Standards for the task (If any):** In accordance with the GIFT Project Protocols.	
1. Where performed: Project/Farm Site		
2. Triggering Event: Indication of need to maintain live tilapia germplasm for *ex situ* conservation		
3. Terminating Event: Live tilapia germplasm maintained for specific activities		

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
6a.1	Plan cost-effective strategy for maintaining live fish collections		Define ex-situ conservation of fish Specify purpose/objective of maintaining live collections of fish germplasm Identify cost-effective procedure or means of maintaining live tilapia collection
6a.2	Prepare stocks for maintaining live collections		Identify strains or fish population that will be kept as live collections Pit tag tilapia stocks to differentiate individuals Condition stocks in B-net cages for 14 days to prepare for breeding
6a.3	Breed stock in hapa net cages		Apply procedures in breeding stocks in hapa net cages
6a.4	Collect progeny for use as replacement stocks		Apply procedures in collecting fry Separate 50 fry from each family and stock in 1mx1mx1m hapa net cage

Please turn over

TASK DESCRIPTION FORM			
5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
6a.5	Rear progeny until they can be floy tagged and pit tagged		Separate males and females from each family/strain, flow tagged and stock in B-net cage Apply procedures for rearing until they are ready for pit tagging Transfer pit tagged breeders (replacement stocks) to ponds separated by strain
6a.6	Carry out routine maintenance		Develop a master list of stocks to be maintained in all holding tanks and ponds Organize routine maintenance activities Monitor physico-chemical characteristics of water in ponds and tanks Check feeding requirements of stocks in tanks and ponds Specify amount of fertilizers required in ponds
6a.7	Conduct stocks inventory		Check the number of fish that remain in tanks and ponds Monitor health condition of stocks Check for any unusual behavior or fish mortality
6a.8	Record all observations in data form		Specify pertinent parameters or data to be recorded in form

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist

Duty: Fish breeding | **Completed by:** | **Date:**

Task: 6b. Cryopreservation of Tilapia Spermatozoa

1. **Where performed:** Genetics laboratory

2. **Triggering Event:** Indication of need for *ex situ* conservation of fish germplasm

3. **Terminating Event:** Spermatozoa of fish cryopreserved

4. **References/Standards for the task (If any):**

Manual of Operations of KRYO 10. According to procedures devised by University of Stirling, Scotland

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
6.1	Plan activities prior to cryopreservation activity	Lack of awareness on cryopreservation	Review *ex situ* conservation in general and cryopreservation of the species
6.2	List down resources needed		Identify equipment and materials needed Specify the population or stocks to be cryopreserved
6.3	Prepare extender solution	Lack of knowledge on how to prepare extended solution	Determine appropriate extender to be used in cryopreserving the species Determine dosage of extender solution to be prepared.
6.4	Prepare cryopreservation facility	Unfamiliarity with the use of equipment	Identify the main parts and functions of the KRYO 10 (Cryopreservation Unit) Specify procedures for operating equipment
6.5	Condition and select breeders for milt collection	Possibility of choosing breeders unfit for milt collection	Choose breeders for milt collection
6.6	Prepare materials equipment	Materials incomplete	Assemble all the materials to be used

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
6.7	Collect tilapia milt	Possibility of collecting contaminated milt	Specify procedure for collecting tilapia milt Apply procedure for milt collection
6.8	Estimate sperm density	Inadequate knowledge on how to calculate sperm density Unfamiliarity with the use of haemacytometer	Identify steps for estimating sperm density Identify procedures in counting cell concentration using haemacytometer Use haemacytometer and compound microscope Get average cell count. Convert average cell count into sperm cell concentration
6.9	Prepare milt with extender	Procedure for preparing milt with extenders not clean	Specify procedures for preparing milt with extenders Calculate the amount of methanol to be added to the sperm sample Calculate the number of straws which can be reasonably filled within the time span given
6.10	Fill straws with milt and seal		Assemble the materials for filling straws with milt Assign colors of straw and bead to a particular milt sample of a given strain Gauge proper timing to fill the straw with milt and seal
6.11	Transfer milt samples to KRYO 10 and run program		Specify general safety procedures to be observed Identify protective clothing required for cryopreservation activity

Please turn over

TASK DESCRIPTION FORM			
[5] No.	[6] Sub-Tasks	[7] Performance Difficulties	[8] Summary of S/K/A Requirements
			Specify procedures for operating the equipment Define all signals in the machine Transfer straws containing milt samples and run the program
6.12	Transfer straws from KRYO 10 and store in liquid nitrogen		Gauge proper timing to remove samples from KRYO 10 and transfer in liquid nitrogen Specify general safety procedures when handling liquid nitrogen
6.13	Thaw cryopreserved milt samples		Specify materials/equipment for thawing cryopreserved milt samples Wear proper clothing when opening dewar Immerse cryopreserved milt samples into waterbath
6.14	Check motility of cryopreserved milt samples		Define motility of milt Specify procedures for scoring motility of tilapia milt Estimate motility of tilapia milt
6.15	Check viability of cryopreserved milt samples		Define viability of milt Compare different stages of tilapia eggs
6.16	Record data		Develop cryopreservation record data form Identify cryopreserved milt samples through color of straw, beads and dividers Record pertinent cryopreservation data

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist	
Duty: Fish breeding	**Completed by:** / **Date:**
Task: 7a. Organization and Coordination with Test Stations	4. **References/Standards for the task (If any):** In accordance with the procedures adopted by the GIFT Project
1. **Where performed:** Selected test stations/farms	
2. **Triggering Event:** Need to conduct on-station/farm research experiments	
3. **Terminating Event:** Experiments in test station/farms effectively implemented	

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
7a.1.	Plan/Organize Strategy for conducting on-station/farm experiments	Inadequate planning	Recognize importance of conducting genetic evaluation experiments in selected test stations/farms Determine test environments or culture sites needed Assign overall coordinator for on-station/on-farm Specify schedule of activities Specify protocol for conducting on-farm experiment Survey potential stations/farms
7a.2	Coordinate with test stations/farms	Lack of coordination with heads of stations/farms	Inform station/farm managers about the planned experiment and possibility of considering it as test station/farm
7a.3	Evaluate test stations/farms		Visit the potential study site for survey Determine source of water Specify outlet/inlet of ponds/tanks Evaluate security of the fish in test station/farm

Please turn over

TASK DESCRIPTION FORM

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
7a.4	Arrange meeting with station/farm manager to discuss plans/ results of survey	Possibility of collecting contaminated milt	Inform manager about the study, its objectives, activities Specify requirements for successful implementation of experiment Define roles of station/farm as collaborators/cooperators

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist		
Duty: Fish breeding	Completed by:	Date:
Task: 8a Transport of live fish germplasm	4. References/Standards for the task (If any): ICES/EIFAC Codes of Practice and Manual of Procedures for Transfer of Fish; Live fish germplasm transported with minimal mortality	
1. Where performed:		
2. Triggering Event: Indication of need to transport live fish germplasm		
3. Terminating Event: Live fish germplasm transported successfully		

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
8a.1	Plan/organize strategy for transport of fish germplasm	Lack of awareness on policies/regulations for transfer of fish germplasm	Check policies/regulations for transfer of fish germplasm Specify conditions set by international codes of practice Check protocols set by INGA for importing and exporting live carp and tilapia germplasm Review import and export procedures
8a.2	Contact source of live fish germplasm to be transported		Determine source or place from where fish germplasm will be obtained
8a.3	Secure clearance to import/export		Determine steps in securing clearances and permits from importing/exporting country
8a.4	Prepare holding facilities, transport materials, equipment		Specify all facilities/materials needed for transport Classify the required facilities/materials according to phase of activity

Please turn over

TASK DESCRIPTION FORM			
5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
8a.5	Collect/harvest fish to be transported from the wild/culture sites		Discriminate between desired and undesired stocks for transport
8a.6	Bring collected fish germplasm to the laboratory		
8a.7	Condition stocks in holding tanks		Perform conditioning of stocks
8a.8	Take fish samples for health examination		
8a.9	Submit samples for health examination		Specify fish organs to be examined Make squash preparations from fish organs Examine tissue sample under microscope Identify disease-causing agent or species of parasites present in fish Diagnose findings
8a. 10	Secure quarantine/health certificate		Interpret quarantine/health certificate issued
8a. 11	Pack and load fish to be transported		Determine number of fish to be transported Pack live fish germplasm in plastic bags Examine water quality parameters in transport bag Load transport bags containing fish stocks to be transported

TASK DESCRIPTION FORM

Job: Fish breeder/Aquaculturist			
Duty: Fish breeding		**Completed by:**	**Date:**
Task: 8b. Quarantine and disease diagnosis for new tilapia germplasm		4. **References/Standards for the task (If any):** With consideration of INGA protocols on fish transfer and with minimal mortality after the quarantine period.	
1. **Where performed:** Project/Farm Site			
2. **Triggering Event:** Arrival of newly transported fish germplasm			
3. **Terminating Event:** Newly arrived fish germplasm quarantined			

5. No.	6. Sub-Tasks	7 Performance Difficulties	8 Summary of S/K/A Requirements
8b.1	Plan activities prior to arrival of new fish germplasms	Lack of organization in implementation of transport activities	Organize 'things to do' prior to arrival of new fish germplasm.
8b.2	Disinfect all holding facility (tanks, aquaria) and other quarantine accessories few days before arrival of fish	Possible spread of contaminants	Schedule disinfection of all holding facilities and quarantine accessories.
8b.3	Upon arrival, introduce fish to local water conditions	Stress of fish brought about by difference in water quality	Replace water in plastic bags containing fish with clean water from holding tanks.
8b.4	Stock germplasm in quarantine holding facilities for acclimatization	Low survival of stocks during acclimatization period.	Check fish stocks during acclimatization period of 1-3 days.
8b.5	Conduct routine quarantine procedures after acclimatization	Low survival of stocks during quarantine	Monitor stocks closely during the conduct of routine quarantine procedures.

Please turn over

TASK DESCRIPTION FORM

[5] No.	[6] Sub-Tasks	[7] Performance Difficulties	[8] Summary of S/K/A Requirements
8b.6	Treat fish stocks infected with parasites or diseases	Possibility of disease/ parasite outbreak	Recognize difference between healthy and unhealthy stocks. Examine fish stocks for external signs of diseases and parasites. Diagnose cause of disease or parasite infection. Prescribe treatment to infection.

MODULE TESTS QUESTIONS AND SUGGESTED ANSWERS

PROGRESS TEST - Module 1b

Name: **Date:**

1. Enumerate the major components of research and testing facilities used in GIFT Project.

   Answers:

   (a)______________________________________
   (b)______________________________________
   (c)______________________________________
   (d)______________________________________
   (e)______________________________________
   (f)______________________________________

   (Score : 20 pts.)

2. Enumerate at least 3 important facilities used in the cryopreservation laboratory.

   Answers:

   (a)______________________________________
   (b)______________________________________
   (c)______________________________________

   (Score : 20 pts)

3. What are the examples of facilities used in family testing experiment? (Give 3 examples)

   Answers:

   (a)______________________________________
   (b)______________________________________
   (c)______________________________________

   (Score : 20 pts)

4. State the main purpose of quarantine facilities.

   Answer:

   ____
   ____
   ____

   (Score : 40 pts.)

5. State the next step that should be followed immediately after tagging the fish.

   Answer: Immediately place newly tagged fish in the recovery basin with clean aerated freshwater.

PROGRESS TEST - Module 2c

Name: **Date:**

1. It is important to have a database system in place to have a better understanding and usage of such system. Identify & explain briefly each component of the GIFT database system.

Answer:

____
____
____
____
____

(score: 40 pts.)

2. Database management procedures in the GIFT Project have two phases:

____
____

2.01 Identify the different procedures under these phases

Answer:

____
____
____
____
____

(score: 30 pts.)

3. There are three steps for a database user to access data. This depends however on the ability of the user i.e. knowledge about the data: computer literacy. List at least 2 ways . Indicate what kind of user.

Answer:

____
____
____
____

(score: 30 pts.)

PROGRESS TEST - Module 2c
Suggested Answers

1. It is important to have a database system in place to have a better understanding and usage of such system. Identify & explain briefly each component of the GIFT database system.

Answer:

Database - **Collection of inter-related data designed to meet the information needs of a project**

Data Dictionary - **Detailed listing of the project's database and their data structures. It also includes documentation of the nature/background of data.**

User System Interface - **A computer software w/c contains different application programs written to facilitate access of databases through the database management system.**

DBMS - **A computer software which consists of a set of programs to access the database.**

Database Admin. - **Refers to a group of people responsible for overall direction & implementation of the database system.**

User Groups - **The identified/authorized clients or possible users of the GIFT database, user interface, & data dictionary.**

2. Database management procedures in the GIFT Project have two phases:
Data Processing
Data Maintenance

2.01 Identify the different procedures under these phases
Answer:

i. **Study the data collected**
ii. **Provide appropriate database design**
iii. **Identify software requirements**
iv. **Validate data**
v. **Encode data**
vi. **Edit & update data**

3. There are three steps for a database user to access data. This depends however on the ability of the user i.e. knowledge about the data: computer literacy. List at least 2 ways . Indicate what kind of user.

Answer:

1. **If user is not knowledgeable of computer operations, database access can be the coursed through the database administration.**
2. **If user is well-versed with the software used, he/she can access data using the user-interface available or**
3. **Using the database management system itself.**

PROGRESS TEST - Module 3a

Name: **Date:**

1. Enumerate the two methods of assessing the tilapia female breeder's readiness to spawn.

   Answer: ____

   ____

   ____

   (Score : 30 pts.)

2. Matching Type. In the following, the morphological characteristics of the tilapia female's genital papilla described in the right-hand column is to be matched with the Code (left column) used to indicate female's sexual maturity, by placing appropriate number in the answer box.

Code	Answer	Papilla condition
Not ready to spawn (NR)	_______	1. Pinkish to reddish, protruding genital pore full opened. Abdomen distended.
Had Spawned (HS)	_______	2. Pinkish/yellowish, genital pore slightly opened. Abdomen slightly distended.
Ready to Spawn (RS)	_______	3. Whitish/clear, genital pore is flat. Abdomen.
Swollen (S)	_______	4. Still reddish, significantly shrunken & compressed abdomen.

(Score : 40 pts.)

3. Estimate the number of days needed before an inspection for possible presence of eggs/fry of tilapia female with 'RS' (ready to spawn) genital papilla can be made.

   Answer: ____

   ____

   ____

   (Score : 30 pts.)

PROGRESS TEST - Module 3a

Suggested Answers

1. Enumerate the two methods of assessing the tilapia female breeder's readiness to spawn.

Answer:

(a) Visual examination of genital papilla
(b) Examination of maturity of eggs

2. Matching Type. In the following, the morphological characteristics of the tilpia female's genital papilla described in the right-hand column is to be matched with the code uses for female's sexual maturity, by placing the morphological characteristics number in the corresponding answer box.

Code	Answer	Papilla condition
Not ready to spawn (NR)	3	1. Pinkish to reddish, protruding genital pore full opened. Abdomen distended.
Had Spawned (HS)	4	2. Pinkish/yellowish, genital pore slightly opened. Abdomen slightly distended.
Ready to Spawn (RS)	1	3. Whitish/clear, genital pore is flat.Abdomen.
Swollen (S)	2	4. Still reddish, significantly shrunken & compressed abdomen.

3. Estimate the number of days needed before an inspection for possible presence of eggs/fry of tilapia female with 'RS' (ready to spawn) genital papilla can be made.

Answer:

In female tilapia with RS papilla condition, inspection for presence of eggs/fry can be made 7-10 days after stocking with male tilapia.

PROGRESS TEST - Module 4a

Name: **Date:**

1. Write True or False

   Consistent ranking of strains in different environments means low GXE____________

   Score: 10 pts.

   Low GXE implies development of specific strain for a particular environment________

   Score: 10 pts.

2. Fill in the blanks.

   a. The ___________value decides on what breeding strategy is to be adopted in a a breeding program.

   Score: 10 pts.

   b. High heterosis level justify______________ strategy

   Score: 10 pts.

   c. Low heterosis level justify______________ strategy

   Score: 10 pts.

3. True or False

   a. Heretability (h^2=1) means that phenotypic variations is all caused by genetic variations ______________

   Score: 10 pts.

   b. Heretability (h^2=0) means that phenotypic variation is all caused by environmental variations ______________

   Score: 10 pts.

   c. Maternal half sib is the most often used to calculate heritability______________

   Score: 10 pts.

4. What is to be avoided in mating of two individuals?

   ____

   ____

   ____

   Score: 20 pts.

PROGRESS TEST - Module 4a

Suggested Answers

1. Write True or False

   Consistent ranking of starins in diff. environments means low GXE ***True***

   Low GXE implies development of specific strain for a particular environment ***False***

2. Fill in the blanks.

   a. The ***heterosis*** value decides on what breeding strategy is to be adopted in a a breeding program.

   b. High heterosis level justify ***cross breeding/hybridization*** strategy

   c. Low heterosis level justify ***pure breeding/selection*** strategy

3. True or False

   a. Heretability (h^2=1) means that phenotypic variations is all caused by genetic variations ***True***

   b. Heretability (h^2=0) means that phenotypic variation is all caused by environmental variations ***True***

   c. Maternal half sib is the most often used to calculate heritability ***False***

4. What is to be avoided in mating of two individuals?

   Mating of related individuals

PROGRESS TEST - Module 5a

Name: **Date:**

(Trainees may be allowed to open book/notes)

1. Enumerate the step-by-step procedures on truss morphometry:
   Answers:
   (a) ____________________
   (b) ____________________
   (c) ____________________
   (d) ____________________
   (e) ____________________
   (f) ____________________
   ____

(Score : 30 pts)

2. Matching type. In the question below, the number of the 'Description' stated in the right-hand column is to be matched with the 'Technique' (left column) corresponding to it, by placing the correct number in the answer box.

Technique	**Answer**	**Description of Technique**
Principal component Analysis	______	1. Uses traditional caliper to measure skeletal structures such as standard length, width & depth.
Conventional Morphometrics	______	2. Uses electronic digitize linked to a computer and a suitable projection to measure distances between homologous pattern of quadrilaterals on cells
Computer aided Monoscopic Analysis	______	3. Multivariate technique for examining relationships among several quantitative variables
Truss Morphometric method	______	4. Computer program used to take linear measurements of objects in photographic image.

(Score : 40 pts.)

3. State briefly the importance of truss morphometry in genetics.
   Answer:

   ____
   ____
   ____

(Score : 15 pts.)

PROGRESS TEST - Module 5a

4. Explain briefly why should there be a logarithmic transformation of raw data prior to multivariate analysis.

Answer:

____

____

____

(Score : 15 pts.)

PROGRESS TEST - Module 5a

Suggested Answers

1. Enumerate the step-by-step procedures on truss morphometry:

   Answers:

   (a) Prepare fish samples
   (b) Mount the camera
   (c) Get photograph of fish samples
   (d) Collect morphometric data using CAMA
   (e) Analyze data
   (f) Interpret results

2. Matching type. In the question below, the number of the 'Description' stated in the right-hand column is to be matched with the 'Technique' (left column) corresponding to it, by placing the correct number in the answer box.

Technique	Answer	Description of Technique
Principal component Analysis	3	1. Uses traditional caliper to measure skeletal structures such as standard lenght, width & depth.
Conventional Morphometrics	1	2. Uses electronic digitizer linked to a computer and a suitable projection to measure distances between homologous pattern of quadrilaterals on cells
Computer aided Monoscopic Analysis	4	3. Multivariate technique for examining relationships among several quantitative variables
Truss Morphometric method	2	4. Computer program used to take linear measurements of objects in photographic image.

3. State briefly the importance of truss morphometry in genetics.

   Answer:

   Truss morphometry is important in detecting subtle difference in shape independent of strains within a given species.

4. Explain briefly why should there be a logarithmic transformation of raw data prior to multivariate analysis.

   Answer:

   Log transformation converts an arithmetic to geometric scale and is necessary to make the distribution normal and make the variance independent of the mean.

PROGRESS TEST MODULE - 5b

Name: **Date:**

Reading of Bands.

1.

2.

3.

4.

Computation of Bands.

Population 1

Population 2

	Population 1	Population 2
LOCUS 1		
A		
a		
LOCUS 2		
B		
b		

PROGRESS TEST MODULE - 5b

Suggested Answers

Reading of Bands.

1.

aa Aa Aa aa AA

2.

aa Aa aa AA AA

3.

Aa AA aa Aa aa AA

Bb BB bb BB BB BB

4.

AA aa aa aa

Bb Bb bb bb

Computation of Bands.

5.

Population 1

AA	AA	AA	AA	AA
		Bb	Bb	
bb	bb			bb

Population 2

Aa	AA			
		aa	aa	aa
BB	Bb			
		bb	bb	bb

	POPULATION 1	POPULATION 2
LOCUS 1		
A	1.0	0.3
a	0	0.7
LOCUS 2		
B	0.2	0.3
b	0.8	0.7

PROGRESS TEST - Module 6a

Name: **Date:**

1. State briefly the importance of maintaining live tilapia germplasm.

   Answer:
   ____
   ____
   ____

   (Score : 20 pts.)

2. Suppose you are planning to replace tilapia broodstocks, how would you go about it?

   Answer:
   ____
   ____
   ____

   (Score : 40 pts.)

3. Enumerate 5 routine maintenance activities for keeping tilapia germplasm.

   Answer: (a) ____________________________________
   (b) ____________________________________
   (c) ____________________________________
   (d) ____________________________________
   (e) ____________________________________

   (Score : 40 pts.)

PROGRESS TEST - Module 6a

Suggested Answers

1. State briefly the importance of maintaining live tilapia germplasm.

   Answer: Keeping live germplasm is important because of the present limitation of cryopreservation to preserve only the spermatozoa of fish germplasm. *Ex situ* conservation of fish is also necessary for future use in breeding and evaluation of other important economic traits such as disease resistance, carcass quality and maturation.

2. Suppose you are planning to replace tilapia broodstocks, how would you go about it?

   Answer: Breed broodstock at one pair of breeders per hapa, one strain of broodstock per pond. When first filial stocks of different strains are produced, separate 50 fry from each family and stock in 1 m^3 fine mesh hapa. Once fish reach sexable size of 20 g, select four males and four females from each family/strain and tag it with fingerling floy tag. Stock fish in B-net cages until they reach 80 g size and are ready for pit tagging.

3. . Enumerate four routine maintenance activities for keeping tilapia germplasm.

   Answer:
   (a) Check physico-chemical characteristics of ponds/reference tanks
   (b) Fertilize ponds regularly
   (c) Feed stocks as necessary
   (d) Monitor stocks daily
   (e) Conduct regular inventory of stocks

PROGRESS TEST - Module 6b

Name : **Date :**

(Trainees may open manual/notes)

1. State briefly the importance of gene or sperm bank.

   Answer: ____________________

   ____

   ____

   ____

   (Score : 10 pts)

2. Fill in the blank. ____________ is a suitable extender found for tilapia.

   (Score : 10 pts)

3. Enumerate the steps (in chronological order) involved in the cryopreservation of tilapia spermatozoa

   Answer:

   (a) ____________________

   (b) ____________________

   (c) ____________________

   (d) ____________________

   (e) ____________________

   (f) ____________________

   (g) ____________________

   (h) ____________________

   (i) ____________________

   (Score : 30 pts)

4. Calculate for the amount of milt, methanol and modified fish ringer to come up with 12 straws of 0.5 and 0.25 ml.

   Answer: ______________________________
   ____
   ____

   (Score : 25 pts)

5. Assuming the average number of cell count is 120, what is the sperm cell concentration per ml.

   Answer:
   ____
   ____
   ____

   (Score : 25 pts.)

PROGRESS TEST - Module 6b

Suggested Answers

1. State briefly the importance of gene or sperm bank.

   Answer:

   It is essential to a selective breeding program because it affords storage of important genetic material for future reference, as well as easy access and distribution of this genetic material to national breeding programs.

2. Fill in the blank. ***Modified Fish Ringer*** is a suitable extender found for tilapia.

3. Enumerate the steps (in chronological order) involved in the cryopreservation of tilapia spermatozoa

   Answer:

   (a) Prepare Extenders

   (b) Collect Milt

   (c) Check collected milt if contaminated or not

   (d) Label straws for identification (Straws should contain informations such as divider number, fish origin, Pit tag no. and freezing date)

   (e) Get cell count (sperm density)

   (f) Prepare Milt with Extenders + MeOH

   (g) Transfer sample to Kryo 10 and Run Programme

   (h) Transfer frozen sample in dewar storage containers filled up with LN2

   (i) Check viability of frozen milt by using it to fertilize eggs.

PROGRESS TEST - Module 6b

Suggested Answers

4. Calculate for the amount of milt, methanol and modified fish ringer to come up with 12 straws of 0.5 and 0.25 ml.

Answer: For 0.5 ml straw you will need 6250 µl of sample (250 µl is the allowance for checking pre-freezing motility score)

(a) divide 6250 by 20 to come up with the amount of milt needed

6250/20=312.5 µl milt

(b) multiply 6250 by 10 to come up with the amount of methanol to be added

6250 x .10 = 625 µl methanol

(c) add amount of milt and methanol and subtract this with 6250 to come up with the amount of Modified Fish Ringer.

312.5 µl milt
\+ 625 µl methanol
937.5
\- 6250
5312.5 µl Modified Fish Ringer

d) add amount of milt, methanol and Modified Fish Ringer. This should tally with the amount of sample needed to fill up 12 straws of 0.5 ml.

312.5 µl milt
625 µl methanol
\+ 5312.5 µl Modified Fish Ringer
6250.0 µl sample

PROGRESS TEST - Module 6b

Suggested Answers

Answer: *For 0.25 ml straw, you will need 3250 µl of sample (250 µl is the allowance for checking pre-freezing motility score).*

a) divide 3250 by 20 to come with the amount of milt needed

3250/20 = 162.5 µl milt

b) multiply 3250 by 10 to come up with the amount of methanol to be added

3250 x .10 = 325 µl methanol

c) add amount of milt and subtract this with 3250 to come up with the amount of Modified Fish Ringer.

```
   162.5 µl milt
+  325   µl methanol
   487.5
-  3250.0
   2762.5 µl Modified Fish Ringer
```

d) add amount of milt, methanol and Modified Fish Ringer. This should tally with the amount of sample needed to fill up 12 straws of 0.25 ml.

```
   162.5 µl milt
   325   µl methanol
+  2762.5 µl Modified Fish Ringer
   3250.0 µl sample
```

5. Assuming the average number of cell count is 120, what is the sperm cell concentration per ml?

Answer:

average cell count	=	*120*
dilution number	=	*500*
constant	=	*5,000 then*
sperm cell concentration (no./ml)	=	*120 x 500 x 5,000*
	=	3.0×10^8

PROGRESS TEST - Module 7a

Name: **Date:**

1. Describe through a flow chart the protocols in organizing and coordinating on-station/on-farm trial.

   (pls. use extra sheet of paper for this question)

   (score: 50 pts.)

2. During appraisal/evaluation of research facilities, what are the things to be inspected?

   Answer:

   1. ______________________________
   2. ______________________________
   3. ______________________________
   4. ______________________________
   5. ______________________________
   6. ______________________________
   7. ______________________________

   (score: 25 pts.)

3. On fish monitoring, what important parameters should be checked? How often a farm should be visited?

   Answer:

   1. ______________________________
   2. ______________________________
   3. ______________________________
   4. ______________________________
   5. ______________________________

   (score: 25 pts.)

PROGRESS TEST - Module 7a

Suggested Answers

1. Describe through a flow chart the protocols in organizing and coordinating on-station/on-farm trial.

   (pls. use extra sheet of paper for this question)

2. During appraisal/evaluation of research facilities, what are the things to be inspected?

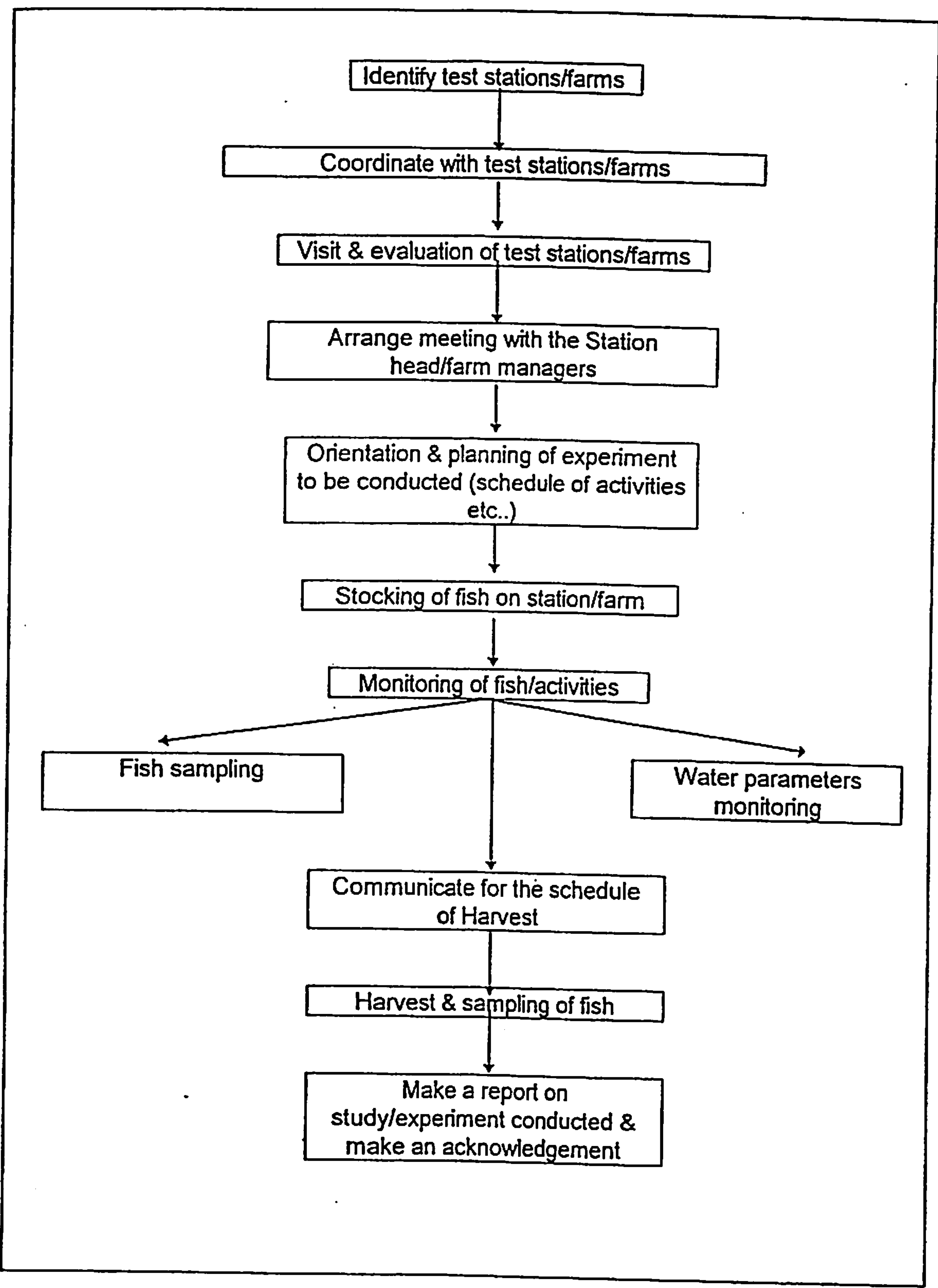
   Answer:

   1. Area of the site
   2. Security of the fish
   3. Inlet/Outlet of water
   4. Source of the water
   5. Location
   6. Facilities
   7. Accessibility of transportation

3. On fish monitoring, what important parameters should be checked? How often a farm should be visited?

Answer:

1. Water quality (parameters - pH, D.O., temperature, water depth, transparency
2. Fish- body weight, body depth, standard length and condition of fish
3. Farms/stations should be visited twice a month.


Identify test stations/farms
Coordinate with test stations/farms
Visit & evaluation of test stations/farms
Arrange meeting with the Station head/farm managers
Orientation & planning of experiment to be conducted (schedule of activities etc..)
Stocking of fish on station/farm
Monitoring of fish/activities
Fish sampling
Water parameters monitoring
Communicate for the schedule of Harvest
Harvest & sampling of fish
Make a report on study/experiment conducted & make an acknowledgement

PROGRESS TEST - Module 8a

Name: **Date:**

1. Identify the important documents needed when importing or exporting fish germplasm

   Answers:

   (a) ____________________

   (b) ____________________

   (c) ____________________

   (Score : 10 pts.)

2. Enumerate the basic steps in packing of tilapia germplasm prior to transport

   Answers:

   (a)____________________

   (b)____________________

   (c)____________________

   (d)____________________

   (e)____________________

   (Score : 20 pts.)

3. Indicate on the opposite column whether the task listed below is the responsibility of the exporting country or importing country

TASK	Indicate whether exporting country or importing country
1. Provide information on fish requested	a ____________________
2. Certify health of fish	b. ____________________
3. Quarantine newly arrived fish	c ____________________
4. Advise on status of fish received	d ____________________
5. Disinfect fish prior to transport	e ____________________

(Score : 40 pts.)

4. State briefly the main reason why laboratory fish health examination is necessary prior to transport of fish to other countries.

Answers:

____

____

____

(Score : 30 pts)

PROGRESS TEST - Module 8a

Suggested Answers

1. Enumerate the important documents needed when importing or exporting fish germplasm

   Answers:

   (a) Importation permits issued by requesting country

   (b) Permit to export issued by source country

   (c) Health certificate issued by country of origin/source of shipment.

2. Enumerate the basic steps in packing of tilapia germplasm prior to transport

   Answers:

   (a) Fill plastic bag with unchlorinated water

   (b) Transfer the appropriate number of fish per plastic bag

   (c) Introduce oxygen to plastic bag

   (d) Fasten upper end of plastic bag with rubber band

   (e) Place plastic bag either in styropore box or in buri bag

3. Indicate on the opposite column whether the task listed below is the responsibility of the exporting country or importing country

TASK	Indicate whether exporting country or importing country
1. Provide information on fish requested	a Exporting country
2. Certify health of fish	b. Exporting country
3. Quarantine newly arrived fish	c Importing country
4. Advise on status of fish received	d Importing country
5. Disinfect fish prior to transport	e Exporting country

4. State briefly the main reason why laboratory fish health examination is necessary prior to transport of fish to other countries.

   Answers:

   Health Examination is absolutely necessary prior to transport of fish germplasm to prevent the introduction of any disease or parasite to existing fish stocks in the country of destination.

PROGRESS TEST - MODULE 8b

Name: **Date:**

1. Enumerate two main reasons why quarantine is important
   Answer:
   (a) ______________________________
   (b) ______________________________

   (Score : 20 pts.)

2. Enumerate at least three practical ways of avoiding contamination of stocks when incidence of during or parasitic infection is observed during quarantine period.
   Answer:
   (a) ______________________________
   (b) ______________________________
   (c) ______________________________

   (Score : 20 pts.)

3. Give at least 3 pointers to be considered when treating infected fish.
   Answer:
   (a) ______________________________
   (b) ______________________________
   (c) ______________________________

   (Score : 20 pts.)

4. Given a 1 kg weight of fish to be treated for 3 days and using a feeding rate of 3%, compute for the total amount of feed needed for the entire duration of treatment (Note the amount of food given to fish during normal feeding is reduced).
   Answer:

$$\text{total amount of feed} = 1000g \times \frac{2.5}{100} \times 3 = 750g$$

5. Complete the diagram below by filling out the blank boxes with the appropriate step

(Score : 40pts.)

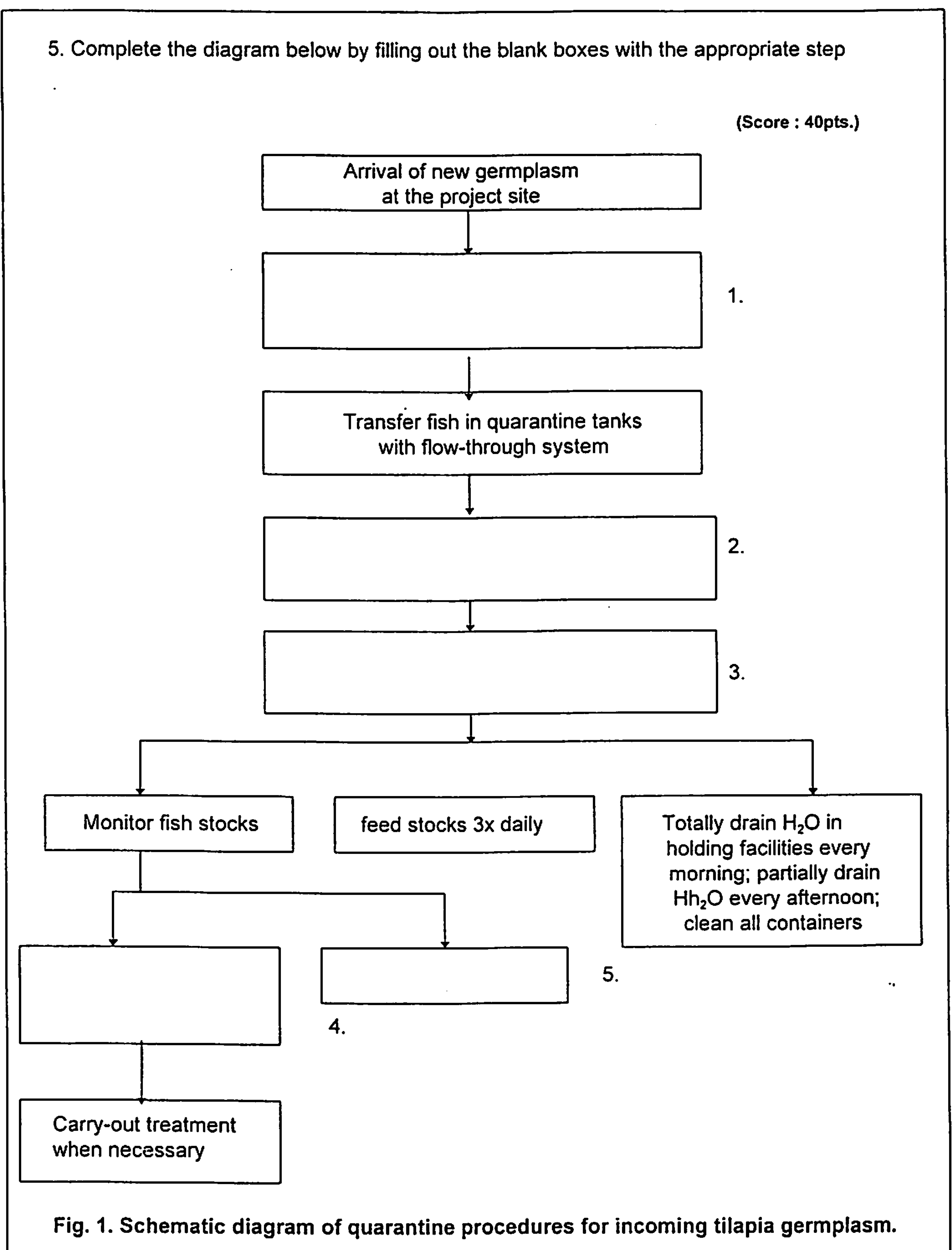


Fig. 1. Schematic diagram of quarantine procedures for incoming tilapia germplasm.

PROGRESS TEST - MODULE 8b

Suggested Answers

1. Enumerate two main reasons why quarantine is important

Answer:

(a). There is risk of spreading diseases and parasites during international exchange of fish germplasm.

(b). The health status of the fish is not fully known .

2. Enumerate at least three practical ways of avoiding contamination of stocks when incidence of disease or during parasite infection is observed quarantine period.

Answer:

(a) Avoid transfer of stock from one container to another

(b) Use siphon and scoop nets only to containers/holding facility assigned to it.

(c) Thoroughly wash and disinfect nets, and holding facility after each use.

3.. Give at least 3 pointers to be considered when treating infected fish.

Answer: (Anyone of the answers below is correct).

(a) Sort fish by degree of infection. Segregate those which are severely affected from those which are less affected.

(b) Before giving any therapeutic or prophylactic treatment, test it first on a few fish. Observe treated fish for 12-24 hours before treating the rest.

(c) Treatment of fish is done best early in the morning or when water temperature is low.

(d) Calculate the dosage carefully before administering treatment.

(e) Repeat treatment only if absolutely necessary and after 30 hours of the first treatment.

(f) Starve infected fish for 12-24 hours prior to treatment.

4. Given a 1 kg weight of fish to be treated for 3 days and using a feeding rate of 3%, compute for the total amount of feed needed for the entire duration of treatment (Note the amount of food given to fish during normal feeding is reduced).

Answer:

$$\text{total amount of feed} = 1000\text{g} \times \frac{2.5}{100} \times 3 = 750\text{g}$$

5. Complete the diagram below by filling out the blank boxes with the appropriate step

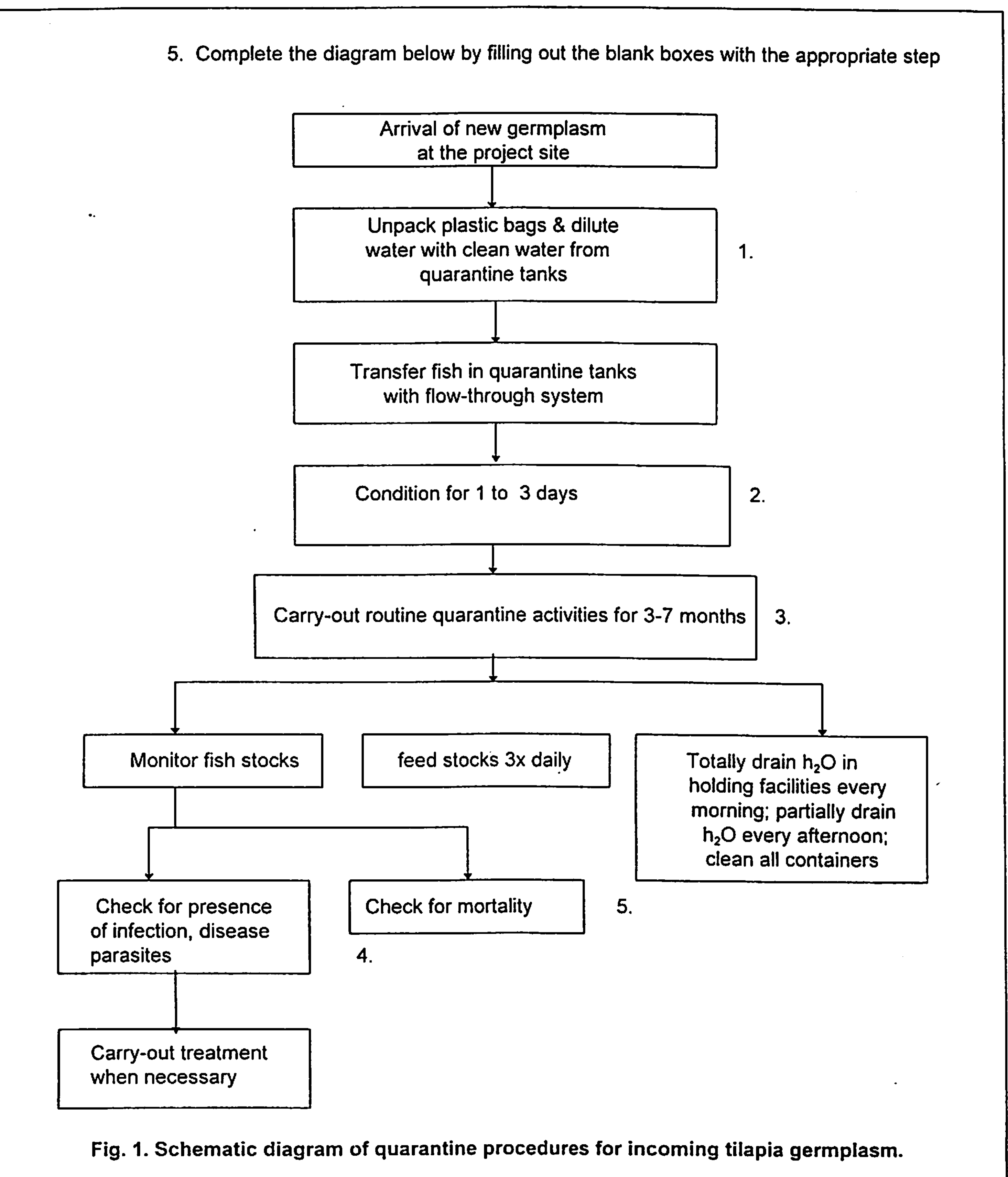


Fig. 1. Schematic diagram of quarantine procedures for incoming tilapia germplasm.

MASTERY TEST QUESTIONS/SCORING KEY

MASTERY TEST - Module 1b

Name : **Date :**

1. As a trained staff, your supervisor is requesting you to handle strategic research in multiplying your few selected breeders, how will you identify the minimum facility requirement to implement the required activities :

   Manpower
   Selection
   Operating funds
   Technical capability

2. Which of the facility discussed in the Module will be used to differentiate fish population & why?

3. You have encountered problems of survival among your selected male broodstocks, which facility is helpful to overcome this decreasing number of live population? And why?

MASTERY TEST - Module 1b

(Suggested Answers)

1. As a trained staff, your supervisor is requesting you to handle strategic research in multiplying your few selected breeders, how will you identify the minimum facility requirement to implement the required activities :

    Manpower
    Selection
    Operating funds
    Technical capability

Answer:

It is important to determine first the required manpower, how much is the available operating funds, and what is the technical capability of the staff to perform activities under selection work. Based on these findings, the trained staff will list down only the facility required to implement the activity given the present constraints/limitations.

2. Which of the facility discussed in the Module will be used to differentiate fish population & why?

Answer:

Digitizer
Vernier caliper method
Equipment for Electrophoresis

These equipment are used to get morphometric and biochemical differences of the fish population.

3. You have encountered problems of survival among your selected male broodstocks, which facility is helpful to overcome this decreasing number of live population? And why?

Answer:

Quarantine
Cryopreservation

MASTERY TEST - Module 2a

Name : **Date :**

1. Given the attached data forms (Forms 1-4) and the necessary sampling materials, trainees will record all pertinent data corresponding to the following activities :
   a. Sampling prior to stocking of tilapia breeders in breeding hapa for spawning.
   b. Sampling during fry collection.
   c. Sampling during transfer of post-fry to B-net cage.
   d. Sampling prior to distribution of tagged fingerlings to different test stations/environments.

2. Trainees should follow the standard sampling and recording procedures developed by the GIFT Project (see Practical Guide on Module 2a for details) in performing the activity.

3. Trainees will be graded following the criteria/scoring key indicated in the attached.

CRITERIA/SCORING KEY FOR MASTERY TEST 2a (Sampling and Recording of Phenotypic Traits)

Mastery test 1 (Breeding and Fry collection - sampling)

<u>Scoring Key</u>		<u>Points</u>
1. Accuracy	....	40%
2. Speed	....	20%
3. Sequence	....	20%
4. Handling	....	20%
Total Score	....	**100%**

Mastery test 2 (Sampling of Fish transfer to B-net - during fry rearing)

<u>Scoring Key</u>		<u>Points</u>
1. Accuracy	....	40%
2. Speed	....	20%
3. Sequence	....	20%
4. Handling	....	20%
Total Score	....	**100%**

Mastery test 2 (Sampling of Fish transfer to B-net - during fry rearing)

<u>Scoring Key</u>		<u>Points</u>
1. Accuracy	....	40%
2. Speed	....	20%
3. Sequence	....	20%
4. Handling	....	20%
Total Score	....	**100%**

BREEDING AND FRY COLLECTION

____________________ GENERATION

HAPA NO.	SEX	TAG NO.	PIT TAG NO.	BEFORE SPAWNING					AFTER SPAWNING				FRY COLLECTION								
				BDWT	STDL (cm)	BDPTH (cm)	CFB	MATY CONDN.	BDWT (g)	STDL (cm)	BDPTH (cm)	CFB	BATCH	DATE COLL	DATE STOCKED	ALIVE	DEAD	TOTAL	B_WT (g)	M_WT (g)	HAPA SIZE
	M																				
	F_1																				
	F_2																				
	M																				
	F_1																				
	F_2																				
	M																				
	F_1																				
	F_2																				

HAPA NO. =hapa number or family number
PIT TAG NO. =Pit tag number
M =male breeder
F1 =female number 1 or dam 1
F2 =female number 2 or dam
BDWT =body weight (g)
BDPTH =body depth (cm)

CFB - number of caudal fin bars
MATY CONDN. - maturity condition (NR/RS/HS)
BATCH - number of times the female spawn
B_WT - bulk weight of fry (g)
M_WT - mean weight of fry (g)
HAPA SIZE - size of nursery hapa

SUMMARY ON FISH TRANSFER TO B-NET/ SUMMARY RECORD ON SAMPLING BEFORE TAGGING

Date of Fry Collection__________

Date of Transfer to B-net__________

HAPA NO.	FINE MESH		SURVIVAL REPLICATES						TOTAL NO.	HAPA SIZE 2
	HAPA SIZE 1	PREV. NO.	A	B	C	D	E	F		

HAPA NO. - Hapa number or family number
PREV. NO. - Number of fry at stocking in nursery hapa
SURVIVAL - Survival of fry from nursery hapa up to transfer in B-net (for each REPLICATE)
HAPA SIZE 1 - Size of nursery hapa
HAPA SIZE 2 - Size of B-net

SAMPLING RECORD ON FISH TRANSFER TO B-NET/ SAMPLING RECORD BEFORE TAGGING

______________________ GENERATION

Date of Stocking in Nursery hapa:__________

Date of Transfer to B-net :__________

FAM NO.____ REP NO.____ Bdwt (g)	FAM NO.____ REP NO.____ Bdwt (g)	FAM NO.____ REP NO.____ Bdwt (g)	FAM NO.____ REP NO.____ Bdwt (g)	FAM NO.____ REP NO.____ Bdwt (g)	FAM NO.____ REP NO.____ Bdwt (g)

FAM. NO. - family number or hapa number

REP. NO. - replicate number for each family (i.e., A,B,C,D, etc.)

Bdwt - individual body weight of sampled fish (g)

GROW-OUT DATA
_____ GENERATION

CULTURE SYSTEM:_______ CAGE/POND NO. :________
PLACE OF STUDY :_______ DATE: ________

SAMPLE NO.	TAG NO.	TAG COLOR R	P	Y	W	LB	O	SEX	BDWT (g)	STDL (cm)	BDPTH (cm)	MAT'Y COND'N	REMARKS

Remarks:
Water Temperature °C: _______
General Observations on water condition:_________

Name of person
Weigh/measure :____________
Record :____________

TAG NO =Tag Number of individual
TAG COLOR =R=red, P=Pink, Y=yellow, B=Blue and O=Orange
SEX =M=for male; F=for female
BDWT =Body weight (g)
STDL =Standard length (cm)
BDPTH =Body depth (cm)
MAT'Y. COND'N. =Maturity condition (NR=not ready to spawn, S=Swollen, RS=Ready to spawn, and HS=had spawned).

MASTERY TEST - Module 2a(Attachment)
(Anesthetization of tilapia)

Name : **Date :**

1. At the end of the exercise, the task is for the trainees to performindividually the anesthetization of tilapia fingerlings. the anesthetized tilapia after exposure in recovery water should have minimal stress and regain 100% mobility after a designated period. Trainees should refer to Practical Guide on Module 2a for detailed procedures on anesthetization.

2. Each trainee will be graded using the criteria/scoring key in the attached.

CRITERIA/SCORING KEY FOR MASTERY TEST 2a (Anesthetization of tilapia)

Mastery test (Anaesthetization of tilapia)

<u>Scoring Key</u>	<u>Points</u>
1.1 Revival - 5	
1.2 Speed - 5/10	
10/10	**100%**
9/10	**95%**
8/10	**90%**
7/10	**85%**
6/10	**80%**
5/10	**75%**
4/10	**70%**
3/10	**65%**
2/10	**60%**
1/10	**55%**

Mastery test Sampling tilapia fingerlings

<u>Scoring Key</u>	<u>Points</u>
2. TIME	
= 60 seconds =	**100%**
Beyond 60seconds	
1-5	**99%**
6-10	**98%**
11-15	**97%**
16-20	**96%**
21-25	**95%**
26-30	**94%**
31-35	**93%**
36-40	**92%**
41-45	**91%**
46-50	**90%**
51-55	**89%**
56-60	**88%**
61-65	**87%**
66-70	**86%**
71-75	**85%**
76-80	**84%**

81-85	....	**83%**
86-90	....	**82%**
91-95	....	**81%**
96-100	....	**80%**

MASTERY TEST - Module 2b

Name : **Date :**

1. At the end of the exercise, the task is for each trainees to perform the marking procedures as indicated step-by-step un the Practical Guide on Module 2b. Each trainee will be given the required set of material for each marking technique and will perform the following:

   (a) fix and prepare floy tags and plastic disc tag;
   (b) floy tag and plastic disc tag at least 20 tilapia fingerlings;
   (c) PIT tag 10 fish breeders;
   (d) fin-clip 15 fish breeders (pectoral, pelvic and dorsal spine uprooting).

2. Individual trainee will be graded using the criteria/scoring key in Attached.

CRITERIA/SCORING KEY FOR MASTERY TEST 2b (Marking of tilapia)

Mastery test 1 (Fixing of Floy fingerling & Plastic Disc Tags)

Scoring Key		Points
1. Efficiency in knot making	....	50%
2. Efficiency in arranging the tags in sequence	....	5%
3. Efficiency in preparing the board	....	40%
4. Time/speed	....	5%
Total Score	....	**100%**

Mastery test 2 (Floy fingerling & Plastic Disc tagging)

Scoring Key		Points
1. Speed/time	....	20%
2. Making three loophole knot	....	20%
3. Length of the thread	....	10%
4. Insertion site (between the 6th and 7th dorsal spine, above lateral line)	....	10%
5. Insertion through the tissue (no bleeding should occur)	....	20%
6. Mortality/survival after tagging	....	20%
Total Score	....	**100%**

Mastery test 3 (Pelvic/Pectoral Fin Clipping)

Scoring Key		**Points**
1. Manner of clipping (cut from the base and with slight bleeding)	....	60%
2. Speed/time	....	20%
3. Positioning of the fish	....	20%
Total Score	....	**100%**

Mastery test 4 (Dorsal Spine Uprooting)

Scoring Key		**Points**
1. Positioning of the fish	....	10%
2. Manner of uprooting (should be uprooted from the base not cut)	....	50%
3. Speed/time	....	10%
4. Survival/Mortality after tagging	....	30%
Total Score	....	**100%**

Mastery test 5 (Dorsal Spine Uprooting)

Scoring Key		**Points**
1. Positioning of the fish	....	**10%**
2. Location of the insertion site (left ventral side of the fish adj. anus)	....	**20%**
3. Manner of insertion (insert only the tip of the needle, touching only the visceral wall of the fish)	....	**20%**
4. Speed/time	....	**10%**
5. Readability of the tag after injection	....	**20%**
6. Survival/mortality after tagging	....	**20%**
Total Score	....	**100%**

MASTERY TEST - Module 3a

Name : **Date :**

1. Given the materials for assessing/scoring the sexual maturity of tilapia breeders, the tasks are the following:

    a. Select matured male tilapia breeders.
    b. Examine the genital papilla condition of female tilapia breeders and classify whether RS, S, NR or HS (refer to Table 1 of Module 3a for detailed description).
    c. Stock the selected male and female tilapia breeders in breeding hapas.

2. Refer to Practical Guide on Module 3a for the step-by-step procedure.

3. Performance of each trainee will be evaluated/graded by using the criteria/scoring key for Mastery Test 3a (see attached).

CRITERIA/SCORING KEY FOR MASTERY TEST 3a (Techniques in Synchronization of Tilapia Spawning)

Mastery test

Scoring Key		**Points**
1. Papilla grading (selection of 4 different kinds of papilla based on maturity condition)	....	**30%**
2. Setting on breeding (at least 2 males, females should be selected as 'RS' and 'S')	....	**25%**
3. Success of spawning 9 check maturity at the time of inspection)	....	**25%**
4. Breeding data (accuracy of recording the data during stocking, spawning & fry collection)	....	**20%**
Total Score	....	**100%**

MASTERY TEST - Module 4a

Name : **Date :**

1. Given the background information on the subject, sample data sets (see attachment 1,2) trainees (in groups of 2) will compute for :

   a. heritability; and
   b. response to selection using calculator and SAS procedure

   (Note: Body weight correction for the sex effects should be done prior to computation)

2. Each trainee will be graded using the criteria/scoring key indicated in the attached.

CRITERIA/SCORING KEY FOR MASTERY TEST 4a (Breeding Strategies)

Mastery test (Breeding Strategies)

<u>Scoring Key</u>		<u>Points</u>
1. Results of computations (answers to computation using hand calculator ans SAS procedures must be the same)	....	**90%**
2. Speed/time	....	**10%**
Total Score	....	**100%**

TRAINEE # 1

(OBS 1-50 FOR HERITABILITY ESTIMATION)
(OBS 1-81 FOR RESPONSE TO SELECTION ESTIMATION)

OBS	TAGNO	DAM	SEX	BW	SIRE	GROUP
1	F001-016	1	M	169.7	1	SELECTED
2	F001-017	1	F	63.1	1	SELECTED
3	F001-018	1	F	95.3	1	SELECTED
4	F001-019	1	M	131.1	1	SELECTED
5	F001-020	1	F	52.8	1	SELECTED
6	F002-016	2	F	68.4	1	SELECTED
7	F002-017	2	M	133.8	1	SELECTED
8	F002-018	2	M	166.4	1	SELECTED
9	F002-019	2	F	81.2	1	SELECTED
10	F002-020	2	F	117.0	1	SELECTED
11	F003-016	1	M	142.0	2	SELECTED
12	F003-017	1	F	69.8	2	SELECTED
13	F003-018	1	F	79.8	2	SELECTED
14	F003-019	1	M	132.5	2	SELECTED
15	F003-020	1	M	54.2	2	SELECTED
16	F004-016	2	M	101.3	2	SELECTED
17	F004-017	2	F	91.3	2	SELECTED
18	F004-018	2	M	104.4	2	SELECTED
19	F004-019	2	F	70.1	2	SELECTED
20	F004-020	2	F	61.5	2	SELECTED
21	F005-016	1	M	107.9	3	SELECTED
22	F005-017	1	M	88.5	3	SELECTED
23	F005-018	1	F	97.8	3	SELECTED
24	F005-019	1	M	69.9	3	SELECTED
25	F005-020	1	M	102.7	3	SELECTED
26	F006-016	2	F	51.1	3	SELECTED
27	F006-017	2	M	127.2	3	SELECTED
28	F006-018	2	M	117.2	3	SELECTED
29	F006-019	2	F	58.3	3	SELECTED
30	F006-020	2	M	39.8	3	SELECTED
31	F007-016	1	M	40.5	4	SELECTED
32	F007-017	1	M	79.5	4	SELECTED
33	F007-018	1	M	85.7	4	SELECTED
34	F007-019	1	F	56.8	4	SELECTED
35	F007-020	1	F	45.0	4	SELECTED
36	F008-016	2	M	156.4	4	SELECTED
37	F008-017	2	M	132.2	4	SELECTED
38	F008-018	2	F	42.8	4	SELECTED
39	F008-019	2	F	70.0	4	SELECTED
40	F008-020	2	M	110.5	4	SELECTED

TRAINEE # 1

(OBS 1-50 FOR HERITABILITY ESTIMATION)
(OBS 1-81 FOR RESPONSE TO SELECTION ESTIMATION)

OBS	TAGNO	DAM	SEX	BW	SIRE	GROUP
41	F009-016	1	F	85.2	5	SELECTED
42	F009-017	1	F	73.2	5	SELECTED
43	F009-018	1	M	150.2	5	SELECTED
44	F009-019	1	M	136.5	5	SELECTED
45	F009-020	1	F	54.8	5	SELECTED
46	F010-016	2	M	163.8	5	SELECTED
47	F010-017	2	F	48.8	5	SELECTED
48	F010-018	2	F	79.2	5	SELECTED
49	F010-019	2	M	45.4	5	SELECTED
50	F010-020	2	F	53.1	5	SELECTED
CONTROLS						
51	G4-456	1	M	104.0	0	ISRAEL
52	G4-459	1	M	47.3	0	ISRAEL
53	G4-460	1	M	83.6	0	ISRAEL
54	G4-461	1	M	82.2	0	ISRAEL
55	G4-462	1	M	79.1	0	ISRAEL
56	G4-463	1	M	74.7	0	ISRAEL
57	G4-464	1	M	116.9	0	ISRAEL
58	G4-466	1	F	41.9	0	ISRAEL
59	G4-A1-010	1	M	42.5	0	AVERAGE
60	G4-A1-011	1	F	31.7	0	AVERAGE
61	G4-A1-013	1	M	64.5	0	AVERAGE
62	G4-A1-014	1	F	48.7	0	AVERAGE
63	G4-A1-015	1	M	77.4	0	AVERAGE
64	G4-A1-016	1	F	48.0	0	AVERAGE
65	G4-A3-028	1	F	29.9	0	ISRAEL
66	G4-A3-034	1	F	20.7	0	ISRAEL
67	G4-A3-037	1	F	38.7	0	ISRAEL
68	G4-A3-049	1	M	39.7	0	ISRAEL
69	G4-A3-050	1	M	40.6	0	ISRAEL
70	G4-A3-051	1	F	26.0	0	ISRAEL
71	G4-A3-054	1	F	29.8	0	ISRAEL
72	G4-C2-009	1	F	49.9	0	AVERAGE
73	G4-C2-010	1	M	99.7	0	AVERAGE
74	G4-C2-011	1	F	52.9	0	AVERAGE
75	G4-C2-012	1	F	50.6	0	AVERAGE
76	G4-C2-016	1	F	53.0	0	AVERAGE
77	G4-E2-010	1	M	66.6	0	AVERAGE
78	G4-E2-011	1	F	33.6	0	AVERAGE
79	G4-E2-013	1	M	69.2	0	AVERAGE
80	G4-E2-014	1	F	75.5	0	AVERAGE
81	G4-E2-015	1	F	44.4	0	AVERAGE

MASTERY TEST - Module 5a

Name : Date :

1. At the end of the exercise for each major activity, the task is for eacj trainee to perform the step by step procedures on Photodocumentation, Collection of data (Vernier Caliper/Digitizer), Management of data, size correction and individual assessment of data, as described in Practical Guide on Module 5a.

2. Trainees will be graded using the criteria/scoring key described in Attachment 1.

(Note : Use designated forms (see Attachment 2) during collection of data.

CRITERIA/SCORING KEY FOR MASTERY TEST 5a (Truss Morphometry)

Mastery test 1 (Photodocumentation)

Scoring Key		Points
1. Focusing	....	25%
2. Checking of light & speed	....	25%
3. Clarity of Image	....	25%
4. Positioning of sample	....	25%
Total Score	....	**100%**

Mastery test 2 (Vernier Caliper Method)

Scoring Key		Points
1. Data collected by Caliper Method	....	100%
Total Score	....	**100%**

Mastery test 3 (Data Management)

Scoring Key		Points
1. Print-out data (from .DBF to . SAS)	....	100%
Total Score	....	**100%**

Mastery test 4 (Size Correction)

Scoring Key		Points
1. SSD data used to establish standard size	....	100%
Total Score	....	**100%**

Mastery test 5 (Individual Assessment - Details of Using SAS)

Scoring Key		Points
1. Opening of Program	....	20%
2. Viewing of Data	....	20%
3. Saving Data	....	20%
4. Retrieving Programs	....	20%
5. Printing data	....	20%
Total Score	....	100%

MASTERY TEST - (MODULE5a)
(Truss Morphometry)

PART 1: FEMALE GROUP

NAME: DATE:

INDICES	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
MTDS										
MTPM										
MTDL										
PMDS										
PMDS										
PMLL										
PMPC										
PMPV										
DSL										
LPF										
LCPF										
DPF										
PSAS										
PFDR										
DSAS										
DSR										
DRAS										
ASTC										
DRBC										
DRTC										
TCBC										

PART 2: MALE GROUP

INDICES	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
MTDS										
MTPM										
MTDL										
PMDS										
PMDS										
PMLL										
PMPC										
PMPV										
DSL										
LPF										
LCPF										
DPF										
PSAS										
PFDR										
DSAS										
DSR										
DRAS										
ASTC										
DRBC										
DRTC										
TCBC										

LEGEND:

mouth tip-1st dorsal spine (MTDS)

mouth tip-premaxilla (MTPM)

mouth tip-dorsal line (MTDL)

premaxilla-dorsal spine (PMDS)

premaxilla-lateral line (PMLL)

premaxilla-pectoral fin (PMPC)

premaxilla-pelvic fin (PMPV)

dorsal spine-lateral line (DSL)

lateral line-pectoral fin (LPF)

pectoral fin-pelvic fin (PCPF)

dorsal spine-pelvic fin (DPF)

pelvic fin-anal spine (PSAS)

pelvic fin-dorsal ray (PFDR)

dorsal spine-anal spine (DSAS)

dorsal spine-dorsal ray (DSR)

dorsal ray-anal spine (DRAS)

anal spine-top caudal peduncle (ASTC)

dorsal ray-bottom caudal peduncle (DRBC)

dorsal ray-top caudal peduncle (DRTC)

top caudal-bottom caudal peduncle (TCBC)

bottom caudal peduncle-anal spine (BCAF)

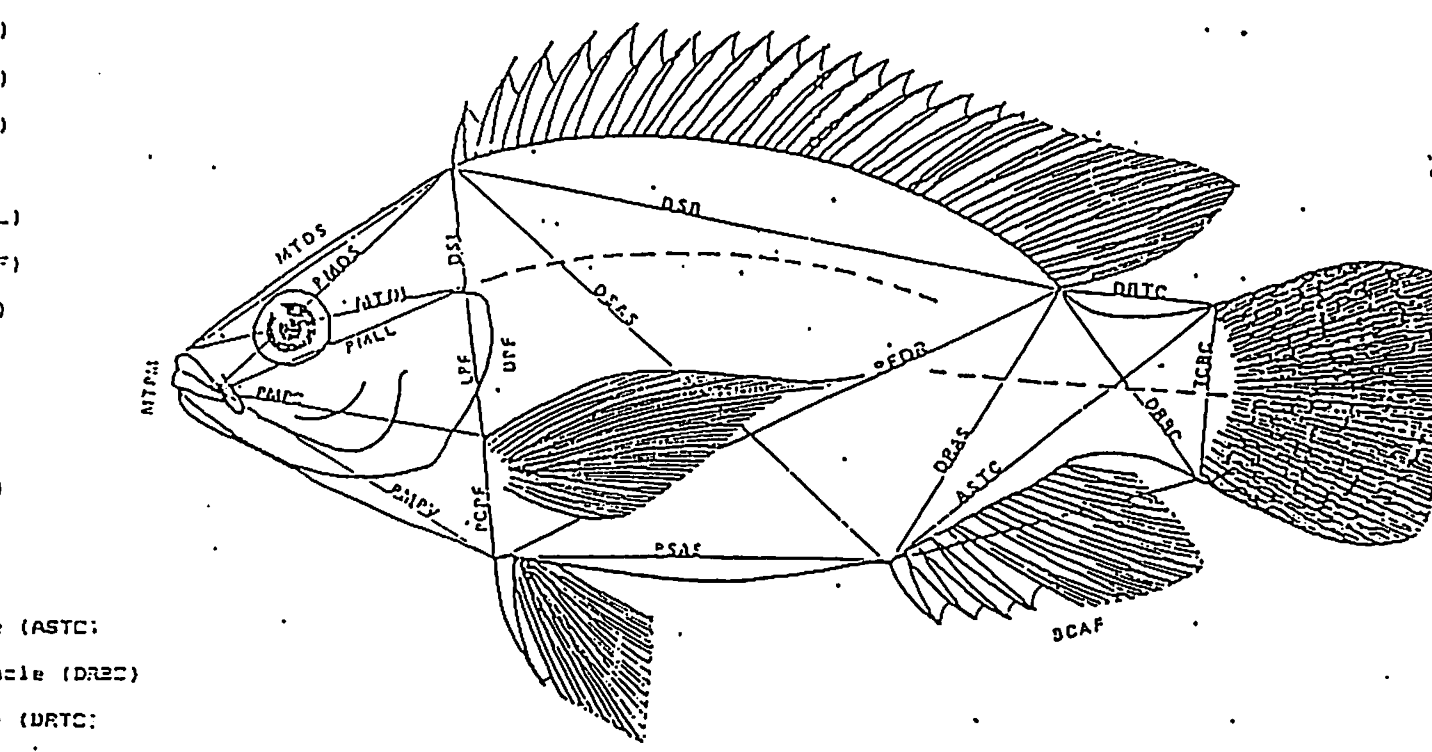


Figure 1. Truss network of 21 landmark points on the body outline, measured during morphometric characterization of eight test strains of *Oreochromis niloticus*.

MASTERY TEST - Module 5b

Name : **Date :**

1. At the end of the exercise, trainees will be grouped in pairs. The test is for each pair of participants to perform electrophoresis on tilapia samples and interpret the information gathered from the gels.
(Note : Trainees will refer to Practical Guide and Manual on Electrophoresis for details of the procedures).

2. Trainees will be graded based on the Scoring key/criteria for Mastery test 5b (see attached).

CRITERIA/SCORING KEY FOR MASTERY TEST 5b (Starchgel Electrophoresis)

Mastery test (Starchgel Electrophoresis)

<u>Scoring Key</u>		**<u>Points</u>**
1. Attention	....	**33.33%**
2. Participation	....	**33.33%**
3. Practicals	....	**33.33%**
Total Score	....	**100%**

MASTERY TEST - Module 6a

Name : **Date :**

1. At the end of the exercise, the task is for each trainee to conduct inventory of stocks. Each trainee will record 5 to 10 individual fish mark (PIT tag, floy tag, fin clip etc.). While recording, trainee will check against the masterlist and will examine the health condition of the fish. Overall performance of inventory will be based on the accuracy of the task performed and the general health condition of the fish within 5 hours after conducting the inventory.

2. Trainees (individual) will be graded based on the criteria/scoring key indicated in the Attachment 1.

(Note : Trainees should record data in the designated form (see Attachment 2.).

CRITERIA/SCORING KEY FOR MASTERY TEST 6a (Maintenance of tilapia Germplasm)

Mastery test

Scoring Key		**Points**
1. Accuracy in reading & recording of PIT tag number	....	25%
2. Check health condition	....	25%
3. Check against masterlist	....	25%
4. Check condition of the fish after inventory (w/o mortality & w/ minimal stress)	....	25%
Total Score	....	**100%**

MASTERY TEST - MODULE 6a
(Maintenance of Tilapia Germplasm)

NAME: DATE:

REC NO.	ID_NO	STRAIN	GROUP	SEX	HOLDING FACILITY	DATE	REMARKS
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

MASTERY TEST - Module 6b

Name : Date :

1. At the end of the exercise, the task are for the individual trainee to perform the following using the step-by-step procedures described in the Practical Guide on Module 6b.
   a) Collect uncontaminated samples of tilapia milt and score sperm motility.
   b) Estimate sperm density (cell concentration per ml) of collected spermatozoa
   c) Fill up dewar with liquid nitrogen and check program installed in KRYO 10
   d) Label straws and goblets, prepare sperm samples, fill-up straws and freeze sperm samples using KRYO 10.
   e) Thaw cryopreserved samples and after freezing, compute the sperm motility score.
   f) Check viability of cryopreserved milt by using this to fertilize tilapia eggs.

2. Trainees (individual) will be graded based on the criteria/scoring key indicated in the Attached.

(Note : Trainees should record data in the attached Cryopreservation form).

CRITERIA/SCORING KEY FOR MASTERY TEST 6b (Cryopreservation of tilapia Spermatozoa)

Mastery test 1 (Collection & Motility scoring of milt)

Scoring Key		Points
1. Know the pre-collection preparations	....	20%
2. Able to collect uncontaminated sample	....	20%
3. Know post-collection stage of sperm	....	20%
4. Can score sperm motility	....	25%
5. Know sequential steps of milt collection & motility scoring	....	20%
Total Score	....	**100%**

Mastery test 2 (Estimation of Sperm Density)

Scoring Key		Points
1. Know sequential steps in estimating sperm density	....	30%
2. Know how to use Haemacycometer counter	....	20%
3. Know how to dilute samples	....	25%
4. Can compute cell concentration per ml.	....	25%
Total Score	....	**100%**

Mastery test 3 (Check program KRYO 10)

Scoring Key		**Points**
1. Know sequential steps in checking program	....	80%
2. Familiar with the display features of KRYO 10	....	20
Total Score	....	100%

Mastery test 4 (Preparation of samples for Cryopreservation)

Scoring Key		**Points**
1. Know sequential steps of preparing/mixing sample for preservation	....	20%
2. Can properly label straw	....	15%
3. Able to calculate amount of sperm suspension needed	....	25%
4. Can fill and seal straw	....	20%
5. Accuracy in measurements	....	20%
Total Score	....	100%

Mastery test 5 (Thawing of straws after freezing)

Scoring Key		**Points**
1. Know sequential steps of thawing cryopreserved sample	....	**30%**
2. Aware of the required temperature	....	**20%**
3. Can score sperm motility	....	**25%**
4. Aware of the precautionary measures such as wearing mask & gloves.	....	**25%**
Total Score	....	**100%**

Mastery test 6 (Check viability of cryopreserved milt)

Scoring Key		**Points**
1. Sequential step in checking viability of cryopreserved milt	....	**30%**
2. Able to fertilize eggs using cryopreserved milt	....	**25%**
3. Identify morula stage of tilapia egg development	....	**25%**
4. Compute fertility rate	....	**20%**
Total Score	....	**100%**

GENETIC IMPROVEMENT FOR FARMED TILAPIA

CRYOPRESERVATION RECORD

FISH DETAILS — SPECIES: — NO. OF STRAWS:

MALE/FEMALE: — ORIGIN: — TAGNO:

LOCATION — DEWAR: — STRAW SIZE:

CAN NUMBER: — SUCCESS: PF-

CAN POSITION: — AF-

	Red	Yel	Pink	Purp	Clear	Blue	Grey	Black	Brown	Orange	Green	Silver	Cream
STRAW													
POWDER/ BEAD													
DIVID													
DIVID W/ LINE													

DIVIDER No.

PRESERVATION DETAILS

DATE. — DILUTION:

DENSITY: — COOL RATE:

FREEZE METHOD:

CRYOPRESERVATIVE: — REMARKS:

MASTERY TEST - Module 7a

Name : **Date :**

1. The task is for the trainees to carry out, in groups of two, the step-by-step procedures in organizing equipments and coordinating with test station/farms. Trainees will coordinate, conduct an ocular inspection to the site and interview the farm/station manager, following the protocols indicated in Practical Guide on Module 7a. Each group will prepare a report on the farm visit and this will be presented before the class for discussion.

2. Performance of the trainees will be graded based on the criteria/scoring key for Mastery test 7a (see attached).

CRITERIA/SCORING KEY FOR MASTERY TEST 7a (Organization & Coordination with Test Stations)

Mastery test (Organization & Coordination w/ Test Stations)

Scoring Key		**Points**
1. Interview/Participation	....	**30%**
2. Station/farm evaluation efficiency	....	**10%**
3. Awareness of the on-farm/on-station coordination procedure	....	**20%**
4. Technique of coordination	....	**20%**
5. Report presentation	....	**20%**
Total Score	....	**100%**

MASTERY TEST - MODULE 7a
(ORGANIZATION & COORDINATION WITH TEST STATIONS)

POND SOIL CHARACTERIZATION

Name: Date:

DATE	POND	pH	OM	N (ppm)	P (ppm)	Al (ppm)	Ca (ppm)	Mn (ppm)	Fc (ppm)	REMARKS

MASTERY TEST - MODULE 7a
(ORGANIZATION & COORDINATION WITH TEST STATIONS)

WATER CHARACTERISTICS

Name: Date:

DATE	POND/ CAGE	WATER DEPTH	DO	TEMP.	pH	WATER TRANS-PARENCY	COLOR OF WATER	ALKALI-NITY	AMMONIA (NH4)	NITRATE (NO2)	PHOS-PHORUS	NITRO-GEN	REMARKS

ATTACHMENT - 4
MTQ - 7a

MASTERY TEST - MODULE 7a
(ORGANIZATION & COORDINATION WITH TEST STATIONS)

BIOLOGICAL PARAMETERS

Name:

Date:

DATE	POND/CAGE	PHYTOPLANKTON COMPOSITION	ZOOPLANKTON	BENTHOS	PLANKTON BIOMASS	SEDIMENT RESPIRATION	REMARKS

ATTACHMENT - 5
MTQ - 7a

MASTERY TEST - MODULE 7a
(ORGANIZATION & COORDINATION WITH TEST STATIONS)

GENETIC EVALUATION
(Growth Performance Monitoring)

Name: Date:

POND/ CAGE	AREA	NO. OF STOCKS	INITIAL SIZE OF STOCK				SAMPLING DATA					REMARKS
			BODY DEPTH	BODY LENGTH	BODY WIDTH	WEIGHT	DATE	BODY DEPTH	BODY LENGTH	BODY WEIGHT	WEIGHT	

MASTERY TEST - MODULE 7a
(ORGANIZATION & COORDINATION WITH TEST STATIONS)

OTHER FARMING DATA

Name: Date:

DATE	POND/ CAGE	OPERATION ACTIVITY	FARM INPUTS			LABOR INPUTS		WAGES
			QTY.	UNIT PRICE	VALUE	FAMILY LABOR	HIRED LABOR	

MASTERY TEST - Module 8a

Name : **Date :**

1. At the end of the exercise, the task is for the trainees (in two groups) to apply the basic steps in transporting live tilapia germplasm. Group 1 will perform the packing/loading procedures of live tilapia for transport to other country. Group 2 will perform the packing/loading and transport to test station (e.g. Tayag's Tilapia Farm in Arayat, Pampanga).
Trainees will refer to Practical Guide on Module 8a for details of procedures.

2. Trainees in group will be graded using the Criteria/Scoring key in Attachment 1.

(Note : Attachment 2 will be used only by the group who will transport fish to other country).

CRITERIA/SCORING KEY FOR MASTERY TEST 8a (Transport of Fish Germplasm)

Mastery test

Scoring Key		Points
1. Accuracy in packing (Note the following: survival of fish condition of fish after transport fish handling)	....	60%
2. Participation in practicum session	....	10%
3. Efficiency in executing transport procedure	....	20%
4. Presentation of Report	....	10%
Total Score	....	**100%**

SAMPLE OF CERTIFICATION ISSUED BY THE EXPORTING COUNTRY

Date Issued:

CERTIFICATION

TO WHOM IT MAY CONCERN:

This is to authorize the ______________________ to transport ________ pieces of ______________________ to ______________ on ______________________ .

The fingerlings will be utilized for ______________________ .

This is to certify that the fish to be transported are apparently healthy. Attached is the result of the parasitological examination conducted by our fish health laboratory.

Signature by: ______________

____
Director of Fisheries
Country

HEALTH CERTIFICATE

We certify that cultured *Orechromis niloticus* (GIFT Strain) from GIFT project, BFAR, NFFTRC, Muñoz, Nueva Ecija, Philippines were found to be apparently healthy. We conducted external and internal parasitological examination of the samples (n = 30; mean weight = 0.25g; mean standard length = 1.25 cm) and found the following:

Strain	Parasite Species	Site	Prevalence (%)
GIFT	*Cichlidogyrus schlerosus*	gills	10.0
	intensity	(parasite/fish)	1.0

The sample did not show any overt signs of possible Bacterials Hemorrhagic Septiceamia (*Aeromonas hydrophila)* of finfish which are reported to be a major disease problem in Indonesia (ADB/NACA 1991):

Furthermore, the sample did not show overt signs of viral diseases.

Examined by:

Research Associate
Fish Health Section

Research Assistant
Fish Health Section

MASTERY TEST - Module 8b

Name : **Date :**

1. At the end of the exercise, each trainee will be given live tilapia specimens, trainees will prepare the required $KMnO_4$ solution (20 ppm) and then administer the prophylactic treatment to tilapia fingerlings. For details of procedures, trainees will refer to Practical Guide on Module 8b.

2. Individual trainee will be graded using the criteria/scoring key in the attached page.

CRITERIA/SCORING KEY FOR MASTERY TEST 8b (Prophylactic Treatment of tilapia)

Mastery test

Scoring Key	Points
1. Attainment of Objective	**30%**
2. Accuracy of Measurement/time	**15%**
3. Participation/Interaction	**10%**
4. Fish survived after treatment	**25%**
5. Sequencing of steps	**20%**
Total Score	**100%**

MASTERY TEST - Module 8b
(Postmortem examination)

Name : Date :

1. At the end of the exercise, the task is for each trainee to conduct post mortem examination of tilapia following the standard procedure developed by BFAR Fish Health Unit (please refer to Practical Guide on Module 8b. for details). Each trainee will be given sample specimen of infected tilapia (adult size) for the examination.

2. Trainees (individual) will be graded using the criteria/scoring key indicated in the attached page.

CRITERIA/SCORING KEY FOR MASTERY TEST 8b (Post Mortem Examination)

Mastery test

Scoring Key		Points
1. Gross Examination	....	20%
2. Dissection	....	20%
3. Smear Preparation	....	20%
4. Squash Preparation	....	20%
5. Microscopy	....	20%
Total Score		100%

TRANSPARENCIES

TITLE OF MODULE

RESEARCH AND TESTING FACILITIES

RESEARCH AND TESTING FACILITIES

OBJECTIVES

- Enumerate the major components of research and testing facilities used in the GIFT Project.
- Determine the minimum facility requirement to run a given tilapia research activity.

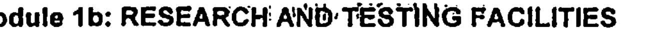
RESEARCH AND TESTING FACILITIES

The Research and Testing Facilities used in the genetic improvement program comprise various components, each designed for a specific activity.

MAJOR COMPONENTS OF RESEARCH AND TESTING FACILITIES USED IN GIFT PROJECT:

FACILITIES FOR:

- Quarantine of Fish Germplasm
- Genetic Characterization (Morphometry) of tilapia
- Maintenance of Live Fish Germplasm
- Maintenance of Cryopreserved Tilapia Germplasm
- Breeding
- Rearing Families to Tagging Size
- Grow-out Experiments
  (Group and Family Testing)



QUARANTINE FACILITY:

- used to hold incoming or outgoing fish germplasm until free of harmful diseases/parasites and completely safe for transfer to culture environment

e.g. fry troughs, glass aquaria flexitubs

Facility for Truss Morphometric Characterization of Tilapia

e.g. Digitizing tablet, computer unit with CAMA Program, Slide Projector, Camera

Facility for Maintenance of Cryopreserved Tilapia Germplasm :

- used for long-term preservation and maintenance of tilapia spermatozoa

e.g. KRYO 10, storage dewars, incubation system

Facility for Maintenance of Live Fish Germplasm :

- holding facilities used for maintaining representative samples of selected tilapia germplasm

e.g. reference collection tanks, fishponds

Facility for Tilapia Breeding

e.g. nursery hapas

Facility for Rearing Families to Tagging Size:

e.g. incubating trays, nursery hapas

Facility for Grow-Out

e.g. ponds, cages, rice paddies

FACILITIES FOR MASS PRODUCTION OF BROODSTOCK FOR HATCHERY OPERATIONS

e.g. breeding pond, nursery pond, rearing pond & conditioning pond

TITLE OF MODULE

SAMPLING AND RECORDING OF PHENOTYPIC TRAITS

OBJECTIVES

- *Specify procedures in sampling and recording of important phenotypic traits*
- *Given sample data forms, materials, perform with accuracy the step-by-step procedure in gathering and recording of important phenotypic traits*

WHAT ARE PHENOTYPIC TRAITS?

- *Phenotypic traits are those traits of fish which are observable and measurable.*

Examples: *fish body weight, body length, sexual maturity conditions, no. of fry produced, etc.*

The Following Questions Should Be Asked Prior to Sampling and Recording of Data:

- What is the objective of breeding experiment ?
- What are the pertinent data which need to be collected?
- How often one should gather these data?
- How data should be collected ?
- Who should collect data ?

BREEDING AND FRY COLLECTION

______________________ *GENERATION*

HAPA NO.	SEX	TAG NO.	PIT TAG NO.	BEFORE SPAWNING					AFTER SPAWNING				FRY COLLECTION								
				BDWT	STDL (cm)	BDPTH (cm)	CFB	MATY COND'N	BDWT (g)	STDL (cm)	BPDTH (cm)	CFB	BATCH	DATE COLL	DATE STOCKED	ALIVE	DEAD	TOTAL	B_WT (g)	M_WT (g)	HAPA SIZE
	M																				
	F_1																				
	F_2																				
	M																				
	F_1																				
	F_2																				
	M																				
	F_1																				
	F_2																				

HAPA NO. =hapa number or family number
PIT TAG NO. =Pit tag number
M =male breeder
F1 =female number 1 or dam 1
F2 =female number 2 or dam
BDWT =body weight (g)
BDPTH =body depth (cm)

CFB - number of caudal fin bars
MATY COND'N. - maturity condition (NR/RS/HS)
BATCH - number of times the female spawn
B_WT - bulk weight of fry (g)
M_WT - mean weight of fry (g)
HAPA SIZE - size of nursery hapa

Sampling & Recording ...

- Planning Specific Activities
- Identify pertinent data which needs to be collected
- How & who should gather the data
- Prepare data sheets form
  - Familiarization to the data forms
  - Recording Information (Accurate)
    - Before spawning
    - After spawning
    - During fry collection
    - During transfer of post fry from nursing hapa to B-net cage
    - Before tagging
    - At stocking, regular sampling & harvesting

TITLE OF MODULE

ANAESTHETIZATION OF TILAPIA

OBJECTIVES

- *to specify and apply the step-by-step procedure in anaesthetization of tilapia*

WHAT IS AN ANAESTHETIC?

- *a chemical which when applied to an aquatic organism such as fish, produces anaesthesia by preventing the initiation and conduction of nerve impulses.*

Examples of Anaesthetic

- *Chlorobutanol*
- *MS - 222*
- *Quinaldine*

STEP 1: Prepare the materials needed in the application of anaesthesia to tilapia.

STEP 2: **With a spatula, take a small amount of MS-222 powder and weigh this on a weighing balance.**

STEP 3: Dissolve a known amount of MS-222 powder in the water (desired concentration for tilapia = one (1) gram of MS-222 powder for every three (3) liters tapwater).

STEP 4: Immerse the fish in anesthetic solution; after 2-3 minutes or the moment it loses equilibrium, remove fish from solution.

STEP 5: Perform the activity desired for the anaesthetized fish (e.g. weigh fish).

STEP 6: After weighing or performing the activity desired for anaesthetized fish, immediately put fish in a recovery basin to regain consciousness.

SUMMARY

- Purpose/Importance
- Suitable Anaesthetic
- Materials Needed
- Step-by-Step Procedure

TITLE OF MODULE

MARKING OF TILAPIA

OBJECTIVES

- specify the kind of tag suitable for fish;
- fix and prepare Floy Fingerlings and plastic disc tags;
- apply the step-by-step procedures in marking of tilapia (Floy Fingerling tagging, PIT tagging, fin clipping and uprooting of dorsal spine)

FIXING FLOY FINGERLING TAGS

1) Cut several pieces of 6-inch vinyl thread
2) Make single knot on one end of the thread
3) Put the needle and plastic disc

MATERIALS FOR TAG INSERTION

- Floy fingerling tags
- Vinyl threads
- Needles
- Plastic disc
- card board
- paste
- pentel pen
- scissors / scotch tape
- Masterlist form

TYPES OF MARKING

- Fin clipping - removal of fins or spines
- Tagging - insertion, attachment or injection of foreign object

TYPES OF MARKING

✪Fin clipping

1. pelvic fin-clipping - group marking
2. pectoral fin-clipping - group marking
3. dorsal spine uprooting - group marking

✪Tagging

1. Floy fingerling tagging - individual marking
2. Plastic disc tagging - group marking
3. PIT tagging - individual marking

WHAT IS FISH MARKING?

- *it is an efficient method of identifying or distinguishing group or individual fish*

IMPORTANCE OF FISH MARKING

- *proper evaluation of genetic parameters (e.g. growth, survival, mortality, etc.)*
- *tracing pedigrees because each generation of fish is identified based on unique tags or marks*
- *identify strains, broodstocks, families and individuals of tilapia in breeding program*

PROCEDURES
PIT TAGGING

1. prepare materials
2. anesthetize
3. load identity tag in the needle
4. position the fish
5. locate insertion site
6. insert needle - underneath the scale
7. check the efficiency of implantation
8. put in a recovery basin

PROCEDURES FLOY TAGS

1. prepare materials
2. anesthetize
3. position the fish - swimming position facing your left hand
4. locate insertion site - between 6th & 7th dorsal spine
   - above lateral line
5. insert needle - underneath one scale
6. put round plastic disc
7. make 3 loopholes knot
8. cut thread - 1-2 inches
9. put in recovery basin

MATERIALS FOR DORSAL SPINE UPROOTING

- Towels
- Anesthesia
- Basins
- Strainers
- Aerators
- Scoop nets
- Surgical scissors
- Tubs
- Clean fresh water
- Dissinfectant
- Cotton ball / buds
- Hand gloves
- Tissue paper
- Masking tape
- Net covers
- Twissors
- Record sheets

MATERIALS FOR FIN-CLIPPING
(pelvic & pectoral fin clipping)

- Towels
- Anesthesia
- Basins
- Strainers
- Aerators
- Scoop nets
- Surgical scissors
- Tubs
- Clean fresh water
- Dissinfectant (merthiolate)
- Hand gloves
- Tissue paper
- Masking tape
- Net covers
- Tally counters
- Record sheets

PROCEDURES
DORSAL SPINE UPROOTING

1. prepare materials
2. anesthetize
3. position the fish
4. locate the spine for uprooting
5. rip of tear the membrane
6. break the spine
7. uproot the spine
8. disinfect the wound
9. put in recovery basin

MATERIALS FOR PIT TAGGING

- Identity tags
- Implanter (modified syringe)
- Identity tag reader
- Basins
- Anaesthesia
- Aerators
- Towels
- Strainers / scoop nets

PROCEDURES FOR FIN CLIPPING

(pelvic & pectoral fin clipping)

1. prepare materials
2. anesthetize
3. position the fish
4. cut the pelvic fin
5. disinfect the wound
6. put in a recovery basin

TITLE OF MODULE

DATABASE MANAGEMENT

OBJECTIVES

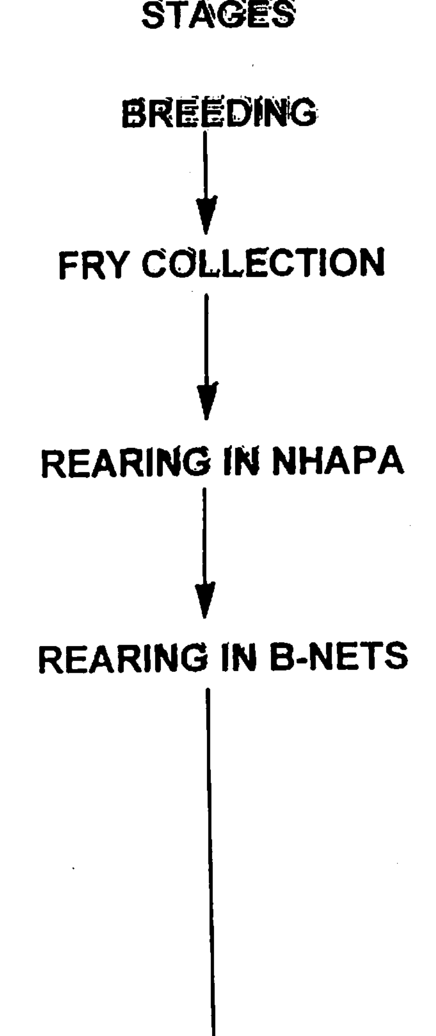
- ✪ *Specify and apply procedures for database management.*

INTERMEDIATE OBJECTIVES

- *define database system*
- *identify and explain components of database system*
- *identify procedures for data validation*
- *identify & apply procedures for data processing*
- *identify & apply procedures for data maintenance*
- *identify & apply procedures for data retrieval*

WHAT IS A DATABASE?

- *A database is a shared collection of interrelated data designed to meet the information needs of a project.*


STAGES
BREEDING
FRY COLLECTION
REARING IN NHAPA
REARING IN B-NETS
GROW OUT

G# BREED

G# FRIES

G# ESNH — Survival in N-Hapa

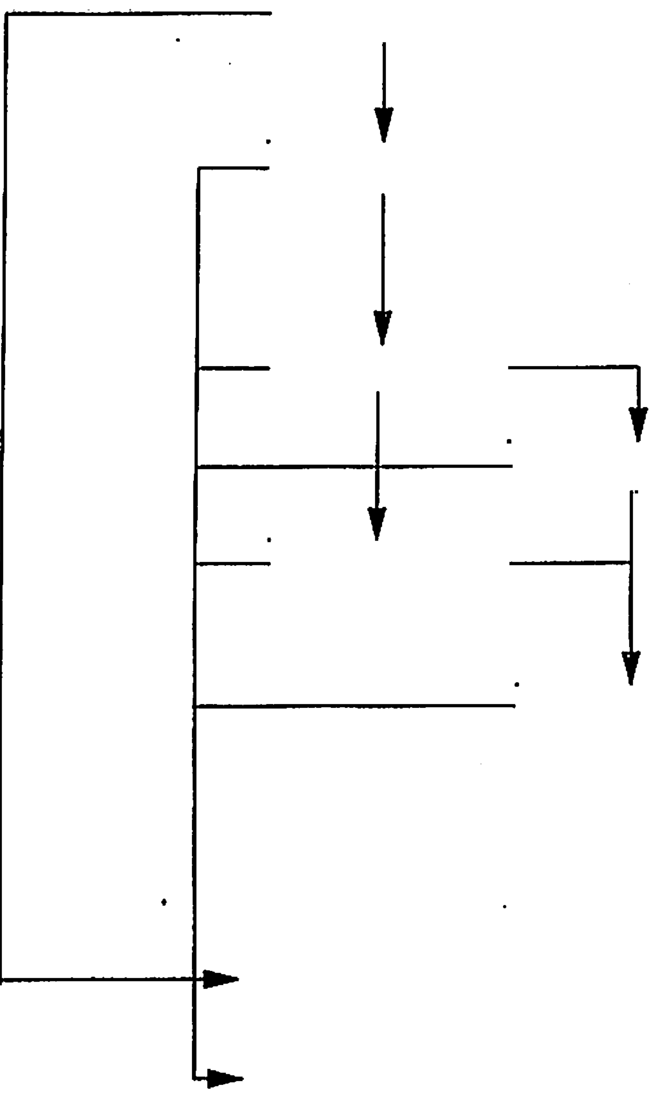
G# EGNH — Growth in N-Hapa

G# ESBN — Survival in B-net

G# EGBN — Growth in B-net

G# ENVR1
G# ENVR2
.
.
G# ENVRN

Transparency 2c.6



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FAMNO

FAMNO

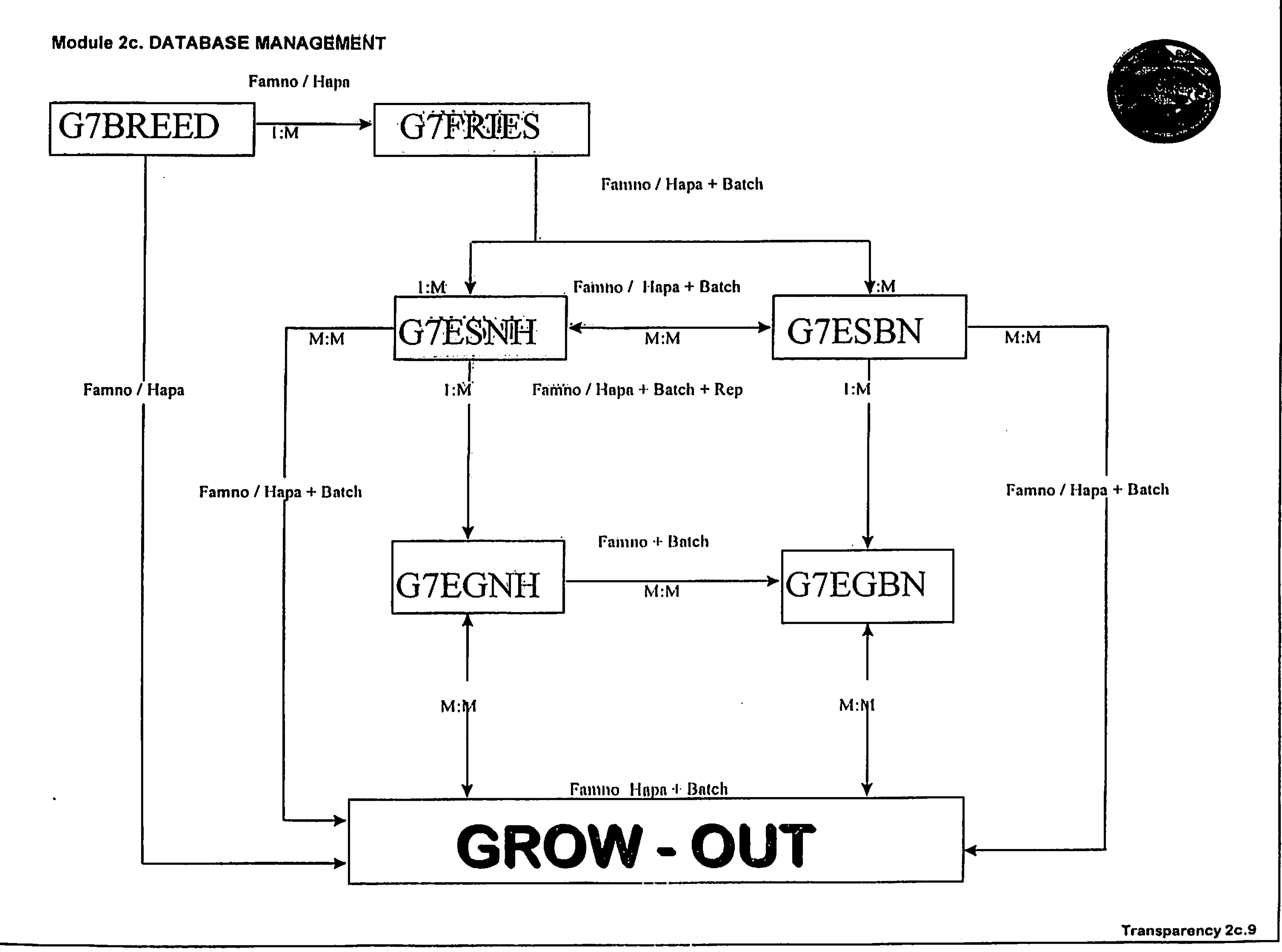
FAMNO + BATCH

FAMNO + BATCH

FAMNO + BATCH

FAMNO + BATCH

FAMNO + BATCH



Module 2c. DATABASE MANAGEMENT
Famno / Hapa
G7BREED
1:M
G7FRIES
Famno / Hapa + Batch
1:M
Famno / Hapa + Batch
1:M
G7ESNH
M:M
G7ESBN
M:M
M:M
Famno / Hapa
1:M
Famno / Hapa + Batch + Rep
1:M
Famno / Hapa + Batch
Famno / Hapa + Batch
Famno + Batch
G7EGNH
M:M
G7EGBN
M:M
M:M
Famno Hapa + Batch
GROW - OUT
Transparency 2c.9

Components of GIFT Database Environment

- *Database*
- *Data Dictionary*
- *User-system interface*
-

TITLE OF MODULE

TECHNIQUE IN SYNCHRONIZATION OF NATURAL SPAWNING IN NILE TILAPIA

CONDITIONING OF BREEDERS

1 - 2 weeks before setting-up of breeding experiment enough time for the breeders to be prepared in spawning

Feeding

Morning and afternoon

3 to 5% body weight

GENERAL BREEDING CHARACTERISTICS

Sexual Maturity

- 2-3 months
- at smaller size
- (from 15g to 20g body weight)

Spawning

- began early from 3-6 months
- frequency - variable

Problem

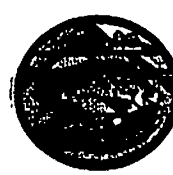
asynchronous breeding

produced different age & size of fry

confounding effect of initial age and size

OBJECTIVES

- Specifi technique in synchronization of natural spawning in Nile Tilapia
- Categorize sexual maturity condition of female tilapia based on genital papilla



HOLDING OF BREEDERS

Fin clipping

dorsal spine uprooting / cutting

Stocking

separate by sex

at 3 - 7 fish per cage

Feeding

2 - 3% body weight

Fertilization

100 to 150 kg 16-20-0

2500 to 3000 kg C.M.

CONDITIONING OF BREEDERS

1 - 2 weeks before setting-up of breeding experiment enough time for the breeders to be prepared in spawning

Feeding
morning and afternoon 3 to 5 % body weight

STOCKING IN BREEDING HAPAS

* based on the prepared mating plan

200 females * will be selected for breeding

100 males * male are stocked in breeding hapas along with females preciously recorded as much "ready to"

Table 1. Coding of females sexual maturity based on their genital papilla conditions

Code	Papilla Condition	Probable Onset of Spawning
Ready to Spawn (RS)	Pinkish to reddish, protruding; genital pore fully opened; Abdomen distended.	3-7
Swollen (S)	Pinkish/yellowish, genital pore slightly opened; Abdomen slightly distended	5-10
Not Ready to Spawn (NR)	Whitish/clear, genital pore is flat; abdomen	21-30
Has Spawned	Still reddish, significantly shrunken and compressed abdomen	15-30

TITLE OF MODULE

MORPHOMETRIC CHARACTERIZATION OF TILAPIA

OBJECTIVES

- Specify and apply step-by-step procedure of characterizing strains within the same species of fish through:
  - photodocumentation
  - digitization
  - photointerpretation
  - manipulation of datasets
  - statistical analyses and reporting

WHAT IS MORPHOMETRY?

- a tool used by systematists to describe groups of fish by measurements or counts of morphological characters or variables

TRUSS VS CONVENTIONAL MORPHOMETRIC METHOD

- Truss morphometric method - measures distances between homologous points along fish body; uses electronic digitizer linked to a computer and projection of specimen onto a flat surface

TRUSS VS CONVENTIONAL MORPHOMETRIC METHOD

- Conventional morphometric method - measures distances between homologous points along fish body; uses electronic digitizer linked to a computer and projection of specimen onto a flat surface

WHY TAKE FISH MEASUREMENTS ?

- *Quantify shape similarities & differences*
- *Find out how they are related*

CLASSES OF MEASUREMENTS

- *Distance*
- *Coordinates*

INSTRUMENT FOR MEASURING DISTANCE

- *Calipers - distances across a form*

INSTRUMENT FOR COORDINATE DATA

- *Digitizing pad (graphic tablets) device for getting positional information into a computer with cursor (resembles a mouse) with cross-hair*

TRUSS MORPHOMETRICS

- body of fish divided into series of "cells"
- cell or quadrilateral contain six lines (2 diagonals, 2 lengths, 2 breadths)

LANDMARKS

- homologous points along the body (Fig. 1 and Fig. 2)
- antero-dorsal measurements (10 distances)
- length and body measurements (6 distances)
- caudal measurements (5 distances)

Fig. 1 Nile tilapia external morphology showing landmark points for plotting.

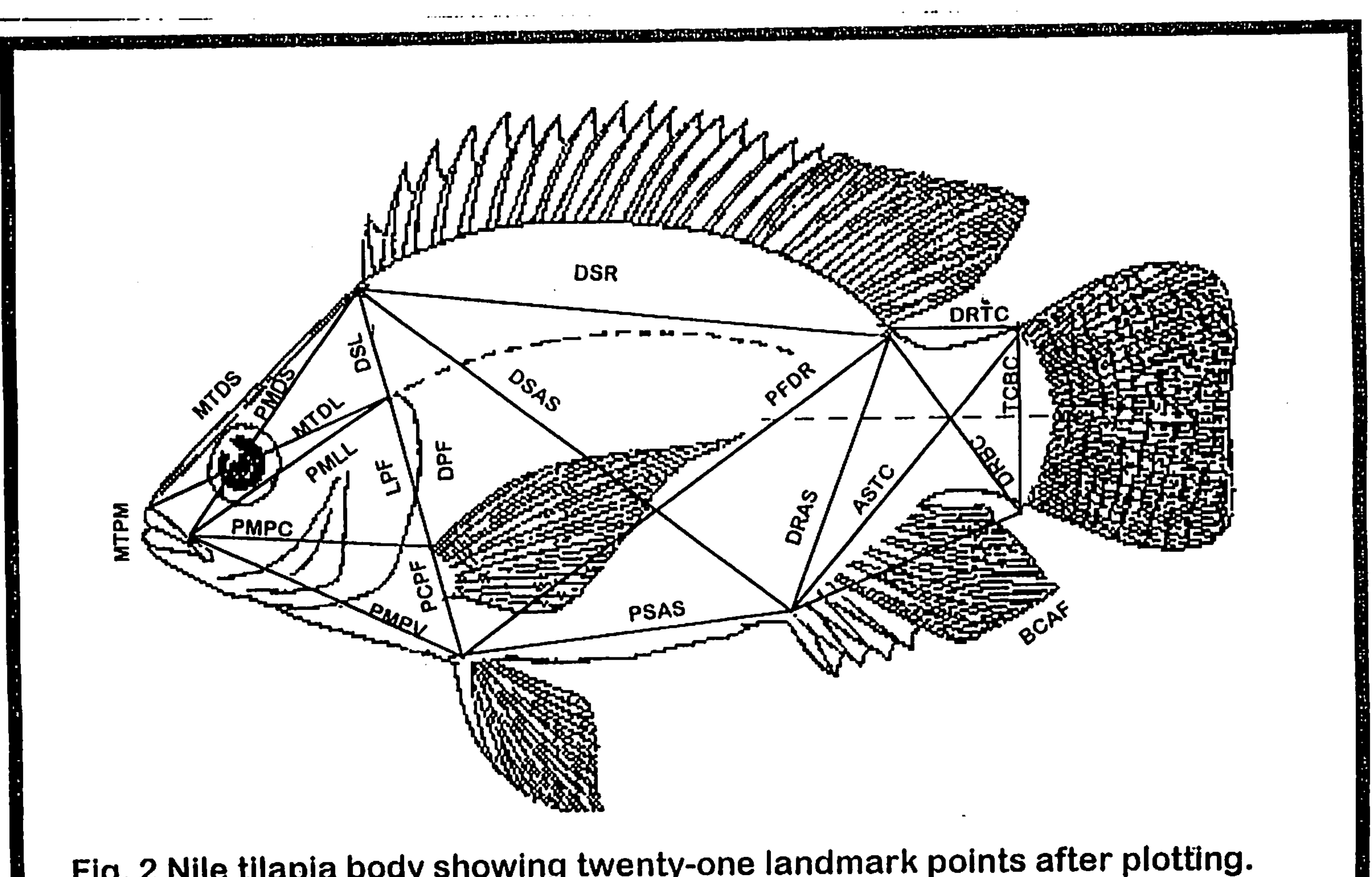


Fig. 2 Nile tilapia body showing twenty-one landmark points after plotting.

A. *Photodocumentation*

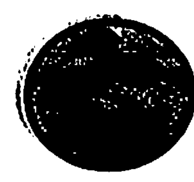
- camera (50 mm lens; aperture setting, 5,6,; speeds, 1/30 to 1/60)
- slides in kodalith form (ASA set at 25)
- copy stand (mounted 50 cm above board)
- foot rule (12 inch ruler)
- illustration boards (1x2 inch) as identification marker
- styropor board (12 x 18 inches)

B. *Preparation of Samples*

- collect at random 30-45 pieces samples (approximately 100 g in average weight)
- immerse fish samples in anesthetic solution (0.3 g MS222 to 1 liter water)
- lay fish samples on a 12 x 18 inch styropor board. With dissecting needles, fasten the left uppermost side of each fin down.

C. Photointerpretation

- CAMA program
- plug digitizer to 110 volts
- check perpendicularity of projection (positioner slides)
- open menu-driven program
- calibrate standard length on image projected
- measure distances
- save to file and print



DATA MANAGEMENT

- import saved file into Lotus worksheets
- translate into logarithmic form
- Quatro software to translate lotus to .DBF file
- translate .DBF to SSD file (SAS file)
- file 24 columns (21 distances, strain, sex, id codes)

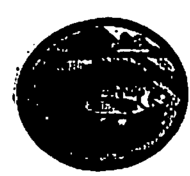
STATISTICAL ANALYSIS

Multivariate Techniques

- multiple measurements never contain less information than single measurements

Multivariate Software Programs

- Statistical Analysis System



MODULE IN SAS

Principal Component Analysis

- summarizes variance of a set of points in multi-dimensional space along new synthetic axes that are orthogonal to each other.
- new variable related to the old variables along synthetic axes or principal components.
- each variable has a loading on the new axis expressing its correlation to the synthetic variable.

DEFINITION OF TERMS

Orthogonal	=	eigenvectors are at right angle
Loading	=	popular term for coefficients scaled in one another way; the correlation between original variables and the scores
Principal Components	=	variables whose values called principal component scores represent linear combinations of the original variables.
Eigenvalues	=	are principal component variances or variances of the scores.
Eigenvectors	=	are the weights or coefficients (f values)
f-values	=	are the co-sines of the angles by which the axes are rotated.

INTERPRETATION OF PCS

1st principal component or major axis (PC1)

- maximum variance
- all coefficients have same sign and magnitude
- size component

2nd principal component or minor axis (PC2)

- reflect contrasts of measurement ("-" and "+")
- shape component

Any component with the highest value of coefficient for a given variable may indicate:

- length component
- eye region component

GRAPHICAL INTERPRETATION

Plotting of two adjacent components detect patterns of data to give graphical separation of strains.

Module 5B:

Characterization of Tilapia Populations Using Starch Gel Electrophoresis

STARCHGEL ELECTROPHORESIS

OBJECTIVES

- ☞ To appreciate the need to evaluate genetic variation and characterize tilapia stocks by relating its applications to current research activities

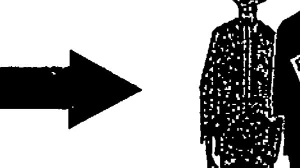
- ☞ To increase interest to develop skills in the use of starch gel electrophoresis

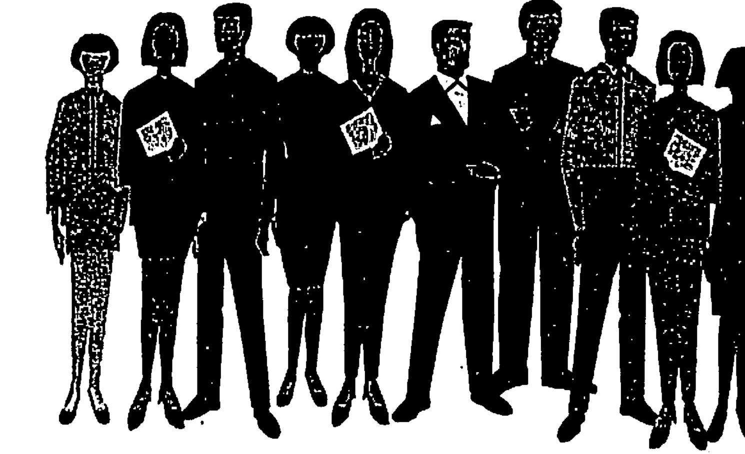
DEFINITION OF TERMS

- Gene
- Allele
- DNA
- Chromosomes
- Locus
- Protein patterns

STARCHGEL ELECTROPHORESIS

THE NATURE OF VARIATION





VARIATION AT THE POPULATION LEVEL

QUANTIFYING VARIATION

- Morphological Variations
- Performance Variations
- Genetic Markers

STARCHGEL ELECTROPHORESIS

APPLICATIONS

- Stock identification
- Hatchery management
- Conservation

GENOTYPE VS. PHENOTYPE

	BY	By	bY	by
BY	BBYY	BBYy	BbYY	BbYy
By	BBYy	BByy	BbYY	Bbyy
bY	BbYY	BbYy	bbYY	bbYy
by	BbYy	Bbyy	bbYy	bbyy

	BY	By	bY	by
BY				
By				
bY				
by				

NON-OVERLAPPING GENERATION MODEL

Birth Maturation Reproduction Death I		Birth Maturation Reproduction Death II		Birth Maturation Reproduction Death III

RANDOM MATING SYSTEM

Choice of mate is independent of genotype and phenotype

Other possible mating systems:

- Positive or Negative Assortative Mating
- Inbreeding

HARDY-WEINBERG EQUILIBRIUM

	A	a
A	**AA** p^2	**Aa** pq
a	**Aa** pq	**aa** q^2

ASSUMPTIONS OF HARDY-WEINBERG EQUILIBRIUM

- Sexually reproducing diploid organism
- Non-overlapping generations
- Random mating
- Large population size
- Negligible migration or mutation
- Natural selection does not affect the trait considered

TITLE OF MODULE

MAINTENANCE OF TILAPIA GERMPLASM

OBJECTIVES

- Specify routine procedures on how to maintain important live fish germplasm.
- Conduct efficient inventory of fish stocks without mortality and minimal stress on the fish.

IMPORTANCE

- *Ex-situ* conservation
  - future use in breeding
  - evaluation of other economic traits (disease resistance, carcass quality, sexual maturation, survival)
- Limitation of cryopreservation technique

LIVE TILAPIA STOCKS MAINTAINED IN GIFT PROJECT

a) Eight founderstocks and their replacement stocks

Egypt Strain
Ghana Strain
Senegal Strain
Kenya Strain
Israel Strain
Singapore Strain
Taiwan Strain
Thailand Strain

b) Top ranking crosses

c) Base Population

d) Selected Lines

Procedure in Stock Replacement

- Setting up of breeding
  - pairwise mating
- Collection of fry
- Nursing of fry
- Rearing to B-net cage until tagging size

Fish Marks used in Live Tilapia Germplasm

- PIT tags
- Floy tags
- Fin clip

Facilities used to Maintain Stocks

- Concrete Tanks
- Nursery Ponds
- Rearing Ponds
- Breeding Ponds

Table 1. A Sample of Masterlist File

GROUP	PIT TAG	OLD TAG	GENERATION	SEX	HOLDING FACILITY
G1_FS_EGYPT	000*000*126	G1-A1-934AB	G1	F	TANK4B
G1_FS_ISR	000*000*272	G1-P1	G1	F	NP4
G1_FS_ISR	000*000*284	G1-P1	G1	F	NP4
G2_SELECT	000*000300	G2-A3P4-384	G2	F	NP7
G2_SELECT	000*000368	G2-A1A4-347	G2	F	NP7

Inventory of Stocks

- Importance of conducting inventory of stocks
  - Check the health condition of fish
  - Check the survival of the stocks
- Time of conducting inventory of stocks
  - early morning
  - late in the afternoon

Routine Maintenance Activities

- Supplemental Feeding

  Ponds
  2-3% fish body weight

  Tanks
  ad libitum

  Cages
  3-5% fish body weight

Routine Maintenance Activities

- Pond fertilization

  Chicken manure

  2500-3000 kg/ha/mo.

  Ammonium phosphate (16-20-0)

  100-150 kg/ha/mo.

Routine Maintenance Activities

- Daily inspection in all holding facilities
- Daily monitoring of stocks
- Daily recording of activities and observation in logbook

Monitor Water Quality

Tanks

- Daily flushing of tanks with freshwater
- Avoid overfeeding
- Regular Cleaning

Maintain Good Water Quality

Ponds

- Regular adding of water (overflowing if needed)
- Observe color and odor of water
- Monitor water level

Maintain Good Water Quality

Tanks/Ponds

Check -chemical parameters (DO, temperature, pH) of water

SUMMARY

- Importance of maintaining live fish germplasm
- Routine Maintenance
- Importance of conducting inventory of stocks

Masterlist is prepared and kept after tagging of breeders for future use, especially during verification and editing.

PREPARATION OF MASTERLIST

A Masterlist form contains record on the following :

(a) Group or generation of selection\

(b) PIT tag no. of individual fish

(c) Old tag no.

(d) Sex of fish

(e) Remarks

TITLE OF MODULE

CRYOPRESERVATION OF TILAPIA SPERMATOZOA

OBJECTIVES

- *Specify and apply the step-by-step procedure for cryopreservation of tilapia spermatozoa*

Record Form for Cataloguing Cryopreserved Tilapia Sperm

FISH DETAILS

MALE/FEMALE: NO. OF STRAWS:

SPECIES: TAGNO:

ORIGIN:

LOCATION

DEWAR: STRAW SIZE:

CAN NUMBER: SUCCESS: PF-

CAN POSITION AF-

	RED	YEL	PINK	PURP	CLEAR	R'BLUE	GREY	BLACK	ORANG	GREEN	SILVER
STRAW											
POWDER/BEAD											
DIVID											
DIVID W/ LINE											

PRESERVATION DETAILS

DATE: DILUTION:

DENSITY: COOL RATE:

CAN POSITION:

FREEZER METHOD:

CRYOPRESERVATIVE: REMARKS:

Incubate fertilized egg in cryopreservation of tilapia spermatozoa.

After 2 hours of fertilization, check tilapia egg development under an inverted microscope.

Collect unfertilized eggs by stripping female tilapia broodfish.

Cut end of straw containing thawed tilapia milt sample and empty sperm suspension over egg aliquots.

Thaw cryopreserved milt samples in water bath at 40-41 °C for 8 seconds.

Transfer frozen milt samples to storage container (Dewar) filled with liquid nitrogen.

Fill straws with prepared milt and ready all straws containing samples for freezing: freeze samples using programmable KRYO 10.

PROGRAM KRYO 10

- Start temperature at 20-25 ° C
- Set cooling rate at -5 ° C/minute
- Target temperature -60 ° C
- Hold step here for 2 minutes

Straw Filling - Note the hand holding the straw

Prepare tilapia milt for cryopreservation; mix extender (modified Fish Ringer), cryoprotectant (MeOH) and milt using a micropipette.

Label straws for identification of tilapia milt (note: straw contains information on strain code, Pit tag no., freezing date and straw number).

Diagram 1

Diagram 2

PROCEDURE

1. Count the no. of sperm in 5 of the squares marked with an x on diagram (1)
2. Each of the squares are divided into 16 diagrams (2)
3. This procedure should be repeated 3 times with different groups of 5 squares
4. Calculate average = Total no. (numbers from the 3 different counts) divided by 3
5. Use this figure in the above formula to give cell conc./ml

Sperm concentration per ml = average cell count x dilution number
(volume of sperm suspension x constant)

Example: If average cell count = 200
dilution number = 500
constant = 50,000

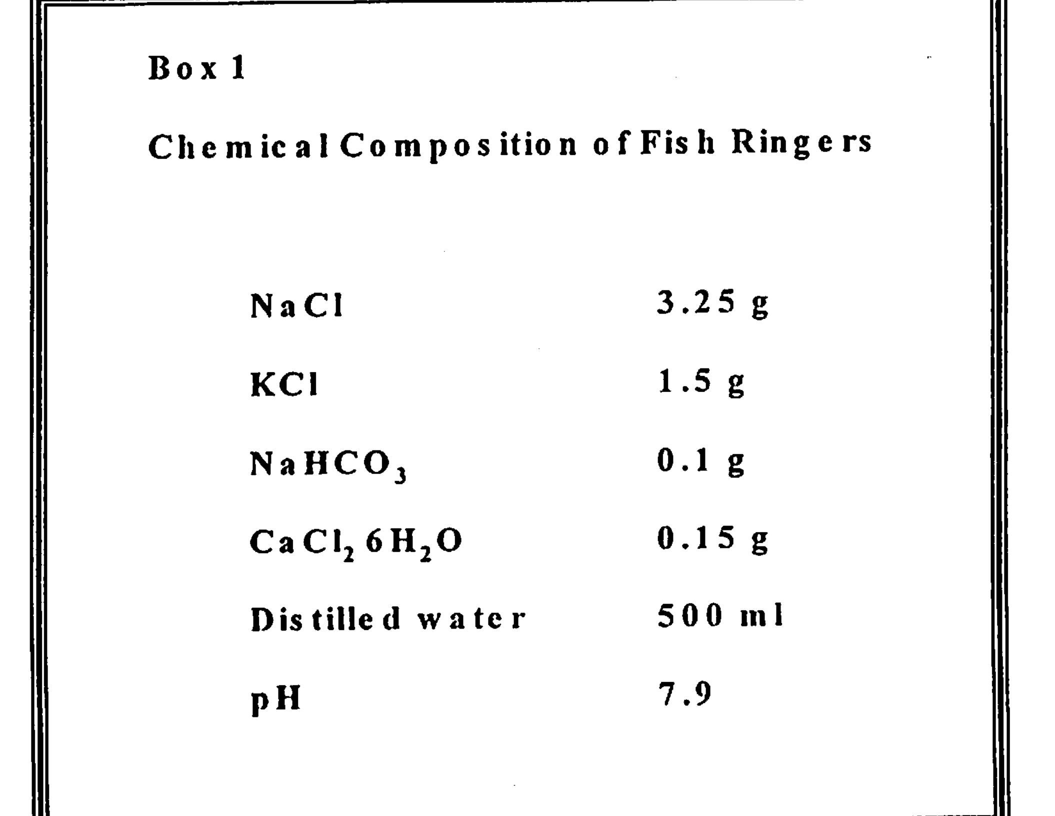
sperm cell concentration (no./ml) = 200 x 500 x 50,000 = 5.0×10^9

Collect milt from tilapia male broodfish

Check motility of tilapia sperm under an inverted microscope; estimate sperm density using a Neubeur counter haemacytometer

MATERIALS/EQUIPMENT NEEDED DURING MILT PRESERVATION:

- fin pipette tips
- goblets
- straw
- beads
- styropor box w/ cracked ice
- Appendorf tubes
- Haemacytometer counter
- methanol
- modified fish ringer
- thermometer
- scissors
- Petri disc
- stop watch
- face visor, protector gloves



Box 1

Chemical Composition of Fish Ringers

Component	Amount
$NaCl$	3.25 g
KCl	1.5 g
$NaHCO_3$	0.1 g
$CaCl_2\,6H_2O$	0.15 g
Distilled water	500 ml
pH	7.9

Box 1. Chemical Composition of Fish Ringers

Fig. 1. Summary flowchart of protocol for cryopreservation of tilapia milt

- Preparation of Modified Fish Ringer — 1
- Fill Dewar and Programme Kryo 10 — 4
- Collection of Milt — 2
- Check Milt
  - Bad (Motile 10%) → Discard
  - Good (Non Motile) ↓
- Store in Fridge (14°C) — 3
  - → Cell Count
  - → Label Cryo Straws (→ Straw Filling and Sealing)
- Preparation of Milt with Extender + MeOH — 5
  - Equilibratio (Max 30 min.)
- Straw Filling and Sealing — 6
- Transfer to Kryo 10 and Run Programme
- Transfer of Straws and Storage in LN_2 — 7
- Thawing Procedure — 8
  - → Cut Straw and Activate under Microscope. Score Motility
  - → Cut Straw over Eggs and Flush Contents of Straw (← Collect underfertilized egg (100-200/Petri dish))
    - Add equal quantities of tap water
    - Rinse after 5 minutes Incubate and monitor Eyed Embryo Survival

Rinse after 5 minutes Incubate and monitor Eyed Embryo Survival

What is a genebank ?

- it is a maintained collection of spermatozoa from various stocks of fish.

Why is genebank important?

- it affords storage of important genetic material for future reference
- for easy access and distribution of genetic material to national breeding programs, following protocol set by ICES/EIFAC International Codes of Practice.

What is Extender?

- solution of organic and inorganic chemicals
- resembles blood or seminal plasma

WHAT IS CRYOPRESERVATION?

- *Cryopreservation is the long-term preservation of gametes (fish spermatozoa) through freezing in liquid nitrogen using a cryoprotectant and an extender.*

TITLE OF MODULE

ORGANIZATION AND COORDINATION WITH TEST STATIONS/FARMS

OBJECTIVES

- *Specify the step-by-step procedures in coordinating with test stations based on GIFT Project experience.*
- *Perform under simulated conditions the above step-by-step procedures.*

- *On-farm/on-station trial is conducted to test or evaluate the animal (specifically strain of fish) and the technology in comparison with farmer's stocks and culture practices.*

Why is effective organization/ coordination with test stations important in the conduct of genetic experiments?

- *For rigorous strain evaluation experiments under different culture systems and geographic locations, there is a need to select test stations farms and culture systems suitable for conducting the experiments.*

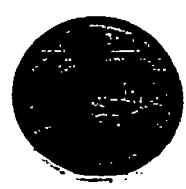


Table 1. List of Test Stations/Institutions/Organization that participated/assisted the GIFT Project in conducting studies.

Station/Institution/ Organization	Address	Activity Involvement
Freshwater Fisheries Research Outreach Station	San Mateo, Isabela	Test station in pond for family testing and farmer coordination (cage culture)
International Institute for Rural Reconstruction, (IIRR)	Sto. Domingo, Albay and Silang, Cavite	Farmer coordination for on-farm trial
Bicol Integrated Agriculture-Research Center. Dept. of Agriculture	Regional Office Pili, Camarines Sur	Farmer coordination for on-farm trial
Regional Freshwater Freshwater Fisheries Center Department of Agriculture	Bula, Camarines Sur	Test station in pond
Research Outreach Station La Trinidad Fish Farm	La Trinidad, Benguet	Test station in pond family/ group testing for cold environment
DA-BFAR Research Outreach Station	Hanga, Hagonoy Bulacan	Test station in pond for group testing for brackish-water environment
DA-BFAR, National Brackishwater Fisheries Research and Training Center	Pagbilao, Quezon	Test station in pond for family/ group testing for brackishwater environment
Pantabangan Fish Cage Operator Cooperative	Pantabangan, Nueva Ecija	Test environment for family group testing and on-farm trial
Seven Lakes Fish Cage Operator Cooperative	San Pablo, Laguna	Test environment for family and on farm trial

Fig. 1. Flow Chart for On-Farm Coordination

COORDINATION WITH BFAR ROS STATION OR INSTITUTION WORKING

LIST POTENTIAL FARMER COOPERATOR RECOMMENDED IN

CONDUCT FARM VISIT AND FARM

ORIENTATION OF FARMERS THAT WILL BE INVOLVED FOR ON-FARM

PLANNING AND SCHEDULE OF STOCKING AND

STOCKING OF FISH FOR

MONITORING OF ON-FARM

FISH SAMPLE

WATER PARAMETERS

COMMUNICATION FOR HARVEST

HARVEST AND FISH SAMPLING

Fig. 1. Flow Chart for On-Station Coordination

COORDINATION WITH THE STATION HEAD

↓

STATION VISIT AND EVALUATION

↓

ORIENTATION AND PLANNING OF EXPERIMENT TO BE CONDUCTED IN THE STATION

↓

STOCKING OF FISH IN THE STATION

↓

MONITORING OF ON STATION EXPERIMENT

→ FISH SAMPLING

→ WATER PARAMETERS MONITORING

→ COMMUNICATION FOR HARVEST SCHEDULE

↓

HARVEST AND FISH SAMPLING

TITLE OF MODULE

TRANSPORT OF LIVE FISH GERMPLASM

INGA (International Network on Genetics in Aquaculture) Member Countries:

- Bangladesh
- China
- Cote d'Ivoire
- Egypt
- Fiji
- Ghana
- India
- Indonesia
- Malawi
- Philippines
- Thailand
- Vietnam

Fig. 2. Flow of activities prior to and during transport of live tilapia germplasm from Africa to the Philippines

Holding of collected fish at NARS laboratory (Africa)

↓

Conditioning of fish for 2 days prior to transport

↓

Packing of fish in oxygenated plastic bags placed inside styrofoam boxes

↓

Fish transport (from Laboratory to airport cargo section)

↓

Presentation of certificate and documents at the airport fish quarantine and customs section (Certificate to transport fish from Africa to Phil., Health Certificate, etc.)

↓

Loading and transport by air

to Germany

↓

Brief (2-3 days) stage point at the University of Hamburg, Germany → Get samples of collected fish for serological examination

to Philippines

↓

Loading and transport by air

Collection of fish samples for health examination Fish Health Laboratory (NARS institution, Africa)

↓

Issuance of fish certificate (NARS institution, Africa)

- - → (to Presentation of certificate and documents)

OBJECTIVES

- Specify detailed procedures in importing and exporting live tilapia germplasm.
- Apply the basic steps in transporting live tilapia germplasm with consideration of the ICES/EIFAC Codes of Practice and Manual of Procedures for transfer of fish.

IMPORTANT DOCUMENTS NEEDED WHEN IMPORTING OR EXPORTING FISH GERMPLASM:

- Importation permit issued by the requesting country
- Permit to export issued by the source country
- Health certificate issued by country of origin/source of shipment

Fig. 1. Schematic diagram of procedures followed during germplasm collection in Africa

GIFT Project expedition team

Prepare materials for collection and transport

Locate germplasm collection site

Contact reliable fishermen for each site identified

Collect pure strains of *O. niloticus*

Load fish and pact containers for land transport

Transport of fish to laboratory

Laboratory

PROGRESS TEST - Module 1b

Suggested Answers

1. Enumerate the various components of research and testing facilities used in GIFT Project.

   Answers: (a) Facilities for quarantine of fish germplasm
   (b) Facilities for maintenance of live fish germplasm
   (c) Facilities for breeding
   (d) Facilities for rearing families to tagging size
   (e) Facilities for grow-out

2. Enumerate at least 3 important facilities used in the cryopreservation laboratory.

   Answers: (a) Kryo 10 programmable cryo chamber
   (b) Olympus inverted microscope
   (c) Storage dewars

3. What are the examples of facilities used in family testing experiment? (Give 3 examples)

   Answers: (a) Ponds
   (b) Cages
   (c) Rice paddies

4. State the main purpose of quarantine facilities.

   Answer: Quarantine facilities are used to hold imported or newly arrived fish germplasm until they are free of harmful diseases or parasites and are completely safe for transfer to their intended culture environment

PROGRESS TEST - Module 2a

Name: **Date:**

1. Enumerate the important question to be answered prior to gathering and recording of data

   Answer:

   ____

   ____

   ____

   ____

   Score : (25 pts)

2. How do you differentiate the sexes of male and female tilapia?
   Describe the distinct and important morphological features of the genital papillae.

   Answer:

   ____

   ____

   ____

   ____

   Score : (25 pts)

3. Construct sample data forms for strain evaluation experiment *Score (50 pts)*

PROGRESS TEST - Module 2a

Suggested Answers

1. Enumerate the important questions to be answered prior to gathering and recording of data.

Answer:

(a). **What are the data to be collected?**
(b). **How often one should collect the data?**
(c). **How are data to be collected?**
(d). **Who should collect the data?**

2. How do you differentiate the sexes of male and female tilapia? Describe the distinct and important morphological features of the genital papillae.

Answer:

The male tilapia has two openings, the lagre opening or anus and the smaler opening or urogenital pore. In females, the genital papillae is usually smaller and has three openings; the anus, the oviduct (where eggs are released) and the urethra.

3. Construct sample data forms for strain evaluation experiment

Answer: (see attached forms)

GROW-OUT DATA FORM

PLACE OF STUDY : ____________________

CAGE NUMBER : ____________________

DATE OF SAMPLING : ____________________

SAMPLING NUMBER : ____________________

SAMPLE NUMBER	STRAIN CODE	SEX		BODY WEIGHT (g)	STANDARD LENGTH (g)	REMARKS
		M	F			

SURVIVAL AT HARVEST

CAGE NUMBER : ______________________________

DATE OF STOCKING : ______________________________

DATE OF HARVEST : ______________________________

STRAIN CODE	NO. OF FISH STOCKED	NO. OF FISH RECOVERED	PERCENTAGE RECOVERY

PROGRESS TEST - Module 2a

(Anesthetization of tilapia)

Suggested Answers

1. Fill in the blank. The *MS - 222 powder* has been found an effective anesthetic used by the GIFT Project for tilapia fingerlings and breeders.

2. Enumerate at least 4 materials that should be prepared prior to anesthetization of tilapia (Any of the following answers is correct):

Answer:
- **(a) Anesthetic (Ms - 222)**
- **(b) Weighing Balance**
- **(c) spatula**
- **(d) Petri dish**
- **(e) Plastic basin**
- **(f) Tap water**
- **(g) Stirring rod**

3. State briefly the main purpose of applying anesthetic or tranquilizer to fish.

Answer:
The application of an anesthetic eliminates the stress experienced by the fish during handling, for example during tagging and sampling.

4. Enumerate the step-by-step procedures of applying anesthetic to tilapia.

Answers :
- **(a) Prepare the materials needed.**
- **(b) With a spatula, take a small amount of MS - 222 powder and weigh this on a weighing balance.**
- **(c) Dissolve a known amount of MS-222 powder in water.**
- **(d) Immerse the fish in anesthetic solution.**
- **(e) After 2-3 minutes or the moment the fish losses its equilibrum, remove fish from solution.**

PROGRESS TEST - Module 2b

Suggested Answers

1. What is the importance of tagging in a fish breeding program? (Underline the correct answer below)

    (a) It ensures improved fish productivity
    (b) It is used in marking or identifying the strains, broodstocks, families and individual fish in a breeding program
    (c) It is used in characterizing tilapia populations

2. What are the two types of tags used by the GIFT Project?

    Answer: (a) Tagging
    (b) Fin clipping

3. Differentiate the use of floy fingerling tags and plastic disc tags/fin clips

    Answer: Floy fingerling tags are used for individual marking of tilapia while Plastic disc tags and fin clips are for marking groups of tilapia.

4. State whether the following statements are true or false:

    a. Passive Integrated Transponder (PIT) tag is suitable only for smaller fish or tilapia with an average weight of 3-5 g.

    Answer: False

    b. The insertion site for PIT tag is on the left ventral side of the tilapia adjacent to the anus.

    Answer: True

    c. The insertion site for the floy fingerling tag is between the 6th and 7th dorsal spine above the lateral line.

    Answer: True

PROGRESS TEST - Module 2a
(Anesthetization of tilapia)

Name: **Date:**

1. Fill in the blank. The _____________ has been found an effective anesthetic used by the GIFT Project for tilapia fingerlings and breeders.

(Score : 10 pts)

2. Enumerate at least 4 materials needed to perform anesthetization of tilapia :

Answer: (a) ______________________________

(b) ______________________________

(c) ______________________________

(d) ______________________________

(Score : 20 pts.)

3. State briefly the main purpose of applying anesthetic or tranquilizer to fish.

Answer: ______________________________

____

____

____

(Score : 20 pts.)

4. Enumerate in chronological order the step-by-step procedures of applying anesthetic to tilapia.

Answer: (a) ______________________________

(b) ______________________________

(c) ______________________________

(d) ______________________________

(d) ______________________________

(Score : 50 pts)

PROGRESS TEST - Module 2b

Name: **Date:**

1. What is the importance of tagging in a fish breeding program?
   (Underline the correct answer below)

   (a) It ensures improved fish productivity
   (b) It is used in marking or identifying the strains, broodstocks, families and individual fish in a breeding program
   (c) It is used in characterizing tilapia populations

   (Score : 20 pts)

2. What are the two types of tags used by the GIFT Project?

   Answer: (a) ______________________________
   (b) ______________________________

   (Score : 20 pts.)

3. Differentiate the use of floy fingerling tags and plastic disc tags/fin clips.

   Answer: ______________________________
   ____
   ____

   (Score : 10 pts.)

4. State whether the following statements are true or false:

   a. Passive Integrated Transponder (PIT) tag is suitable only for smaller fish or tilapia with an average weight of 3-5 g.
   Answer: ______________________________

   (Score : 10 pts.)

   b. The insertion site for PIT tag is on the left ventral side of the tilapia adjacent to the anus.
   Answer: ______________________________

   (Score : 15 pts.)

   c. The insertion site for floy fingerling tag is between the 6th and 7th dorsal spine above lateral line.
   Answer: ______________________________

   (Score : 15 pts.)

5. State the next step that should be followed immediately after tagging the fish.

   Answer:

   ____

   ____

   (Score : 10 pts.)