Applications of genetics in aquaculture

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Contents

Phenotype and genotype

Phenotype (P) tells how an individual looks like = appearance (e.g. color, body shape, scaled, length, weight, etc.)

Genotype (G) is the genetic make up of an individual (Not seen)

Genetic improving for a given trait is done via working on a hidden element (genetic make up)

Qualitative phenotypes (descriptive)

Qualitative genetics is known after Gregor Mendel who established the mathematical basis of the color inheritance in the petals of green peas

Qualitative traits (Mendelian traits) have a definite appearance and so individual's phenotype is either **this** or **that**; and can be placed in one of discrete classes (discontinuous variations)

Inherited disease (e.g. diabetes), pigmentation, or blood types (groups) of man are examples

Often, each trait is controlled by a **single gene** with two or more alleles

These traits are not influenced by environmental conditions

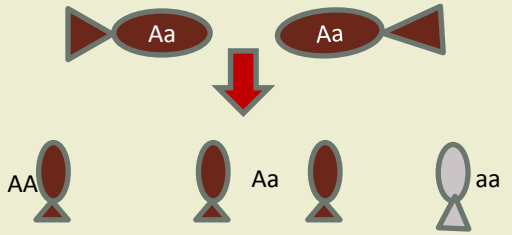
Selection for qualitative traits are designed to **fix** the desired traits and **eliminate** the undesired ones

1822 - 1884

With **some exceptions**, qualitative traits are less important in aquaculture

Qualitative phenotypes (descriptive)

Mendel's Laws of Inheritance Law of:	
Dominance	Some alleles are dominant while others are recessive; an organism with at least one dominant allele will display the effect of the dominant allele
Segregation	During gamete formation, the alleles for each gene segregate from each other so that each gamete carries only one allele for each gene
Independent Assortment	Genes for different traits can segregate independently during the formation of gametes



Example:
Assumption: The dark color is dominant

Analysis: None of the parents is genetically pure

Both parents carry a dominant allele (dark) and a recessive allele (light)

If only one parent is genetically pure, the light color is not produced

Qualitative traits and:
Social & processing considerations

Mirror carp is a strain of common carp with few scales

Less scales is advantageous in regard to processing

No scales may not be accepted in some societies

Naturally fully scaled common carp

Value of qualitative traits in:

Aquaculture

Red tilapia for Sushi dishes

Judging the muscle color in salmonids

Ornamental fish

Color, small size, even deformity are far important traits than weight or FCR

Quantitative traits (measured)

Most productive traits are quantitative: (weight, length, feed conversion "FCR", fecundity,…)

Unlike qualitative traits, Quantitative phenotypes do not show clear cut differences between individuals. Instead, they exhibit **continuous distributions**

Controlled by **several genes** and could be affected by **environmental factors**. Hence: the phenotype: **P = G + E + G-E**

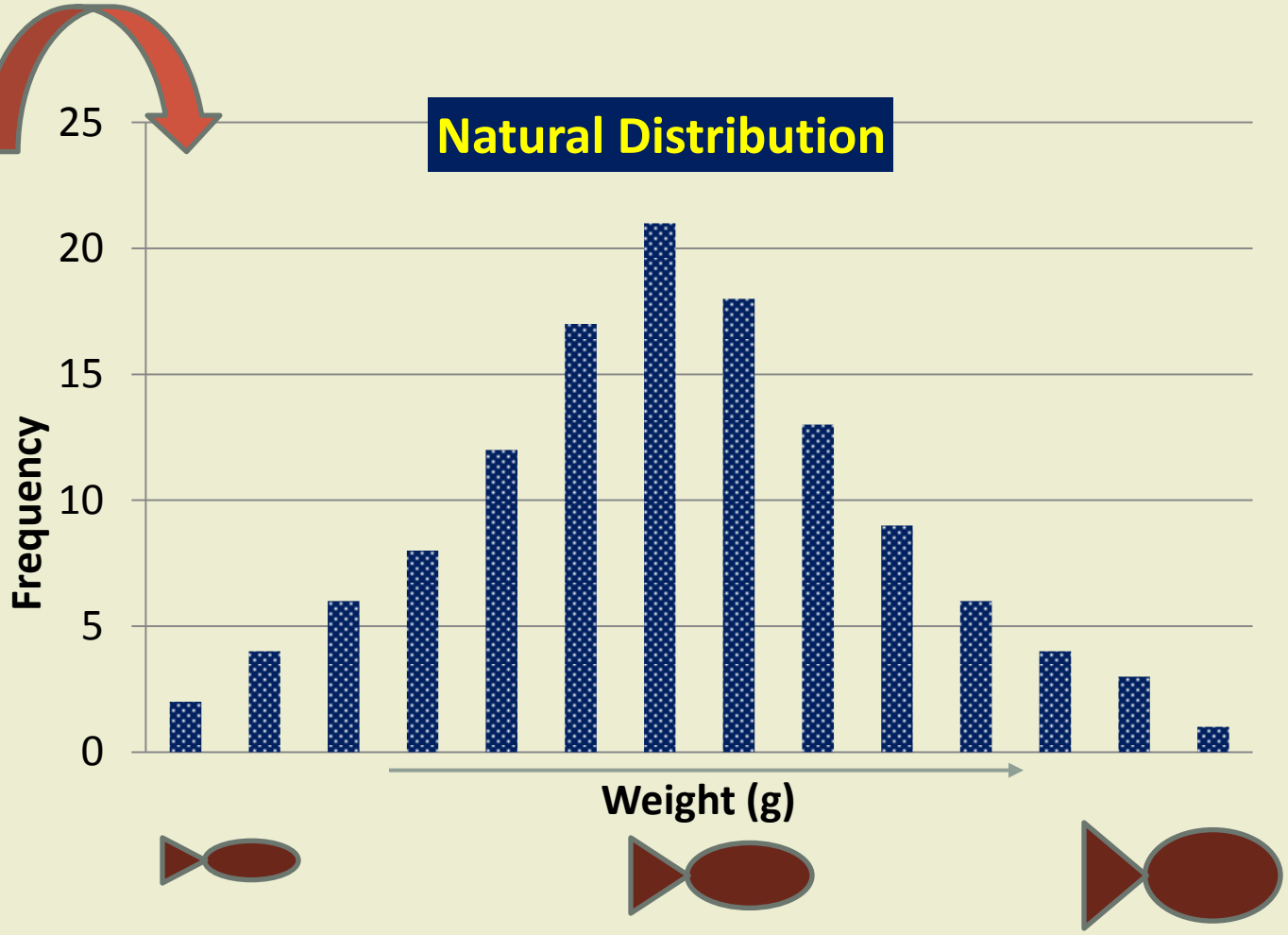
P= phenotype G=genotype E= environmental influence G-E= genetic x environment interaction

In order to improve a quantitative trait, its variance should be analyzed and sorted into heritable and non-heritable components

Heritable component is what breeders are interested in

Quantitative phenotype

Hatched fry of the same age or eggs in the ovary of a given female exhibit natural distribution



Phenotypic and genotypic variance

- There should be a variance in order to attain some improvements through genetics
- If all individual are identical and look the same, there will not be a real chance for their improvement
- Variance in the appearance is phenotypic variance (V_p)
- Phenotypic variance (V_p) is the sum of: genetic variance (V_G), environmental variance (V_E), and the variance resulting from genetic-environmental interaction (V_{G-E})

$$V_P = V_G + V_E + V_{G-E}$$

Environment Variance (V_E)

V_E Has **no genetic basis**. This means that a phenotype could be improved via environment regardless the genetic make up of the organism. Examples are better water quality and/or feed, etc.

If V_E is neither controlled nor quantified or got confound with V_G, it will not be possible to evaluate the genetic improvement and this can ruin a breeding program

V_E **is not** transmitted from parents to offspring

Environmental variance V_E (examples)

Shooting: This phenomenon was found in common carp, *Cyprinus carpio*. It is defined as a sudden and dramatic growth of the shoot individuals (shooters; jumpers)

Shooting have been attributed mainly to the competition for food and/or space. This could result of high stocking density & insufficient natural food, or due to the inadequate size of feed particles

If shooters are selected as broodstock, no progress should be expected

Environmental variance (examples- Cont.)

Egg size (Maternal effect): This occurs when a pronounced effect is found of egg size on the growth rate of early life of fish and when this effect disappears as fish gets older. In Nile tilapia, the effect was virtually gone by 20 days. Thus, **selection should not be carried out before such effect disappears**

Because egg size could be influenced by environmental factors (e.g. nutrition) in addition to genetics, it is crucial to use females of same age and comparable size in evaluation programs, otherwise, detected differences in their progeny may be due to mother's age, size, or diet, and not due to genetic merit

Heritability (h^2)

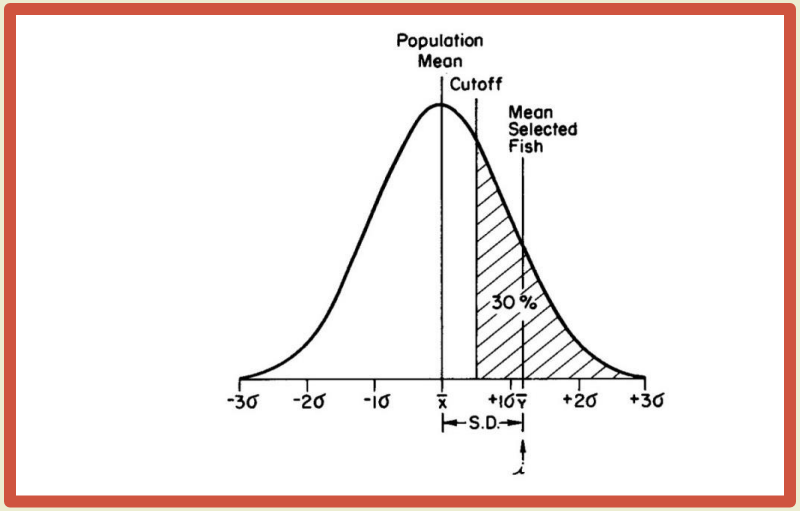
Heritability (h^2) is the proportion of variation in a quantitative phenotype trait that is caused by additive genetic variation among individuals

$$h^2 = V_A/V_p$$

The remaining phenotypic variation is usually attributed to environmental factors

Heritability coefficient (h^2) ranges from **0 – 1** whereas zero refers to the non-genetic reason behind phenotypic variation while theoretical value "$h^2=1$" means that all of phenotypic variation is attributed only to genetics

“Realized Heritability” and selection



Given information (Example):

Mean weight of population = 100 g

Mean of selected population = 140 g

Selection differential = 140-100 = 40 g

Assuming h^2 for growth rate = 25%

Response to selection:

40 x 25% = 10 g/generation

Response to selection/generation R = Heritability h^2 x Selection Differential D

In selection programs, the response to selection could be adequately estimated

Inbreeding

Inbreeding is the mating of individuals that are related to each other relatives through one or more common ancestors.

If harmful & recessive genes that are hidden in the heterozygous state are paired through the mating of relatives, their expression may produce abnormal phenotypes as in a form of deformity, poor performance, less viability and could lead to mortality “inbreeding depression”

Although, detrimental recessive alleles can be also paired and expressed when unrelated fish mate, but such incidence is much less

Higher possibility of inbreeding problems occur upon reaching higher levels (about 15%) and in smaller mating populations

Inbreeding and Effective Breeding Number – **will follow**

Genetic management of broodstock
Effective Breeding Number (N_e)

N_e is the best term describing the population size (from genetics point of view). Since population of fish is finite, it is better to describe it by N_e rather than absolute number.

Effective breeding number depends on the number of breeding individuals, sex ratio and mating system (random; pedigreed)

$$N_e = \frac{4(\#\text{ females})(\#\text{ males})}{\#\text{ females} + \#\text{ males}}$$

In random mating

Effective Breeding Number (N_e)

Why important?

Tendency to keep less males due to:

Females are the spawners not the males

Males compete for space/feed

A Male can mate with many females

However, skewed sex ratio may lower the N_e than targeted

Genetic value (N_e) of **20 M + 80 F** = **64**

15 M + 85 F = **51**

Absolute number is 100; N_e varies

When value of numbers are not the absolute numbers?

Genetic value of 100

Could be obtained from:

50 M + 50 F = 100 OR

31 M + 130 F = 161 OR

Other M & F combinations

Low N_e = High inbreeding

Effective breeding number (inbreeding – generations)

Number of generations:

The largest number of generations in a breeding program – the highest N_e required

Remember: inbreeding accumulates generation after another

Maximum inbreeding level allowed:

The lowest the inbreeding permitted, the highest the N_e required

Effective breeding number needed in relation to generations & inbreeding

No. generations	Maximum inbreeding allowed		
	5%	08%	15%
	Required N_e		
4	40	25	14
8	80	50	27
15	150	94	50
20	200	125	67

Source: FAO, 1999. Inbreeding and brood stock management

Generation

Generation is the average time interval between the birth of parents and the birth of their off spring

A generation may be also defined as the replacement of brood fish by their offspring

In regard to farmed fish, generation intervals are about 6 months for Nile tilapia, 3 years for trout, 4 years for salmon and much longer for sturgeon

The shorter generation interval will be advantageous in selection gain (R) while be **disadvantageous** in the accumulation of inbreeding

Enhancing the Effective Breeding Number

Sufficient number of spawners

The number of spawners should be sufficient to produce the targeted N_e

This often comes into conflict with the tendency to reduce production cost via the spawning the fewest number sufficient to produced the target number of fry especially with highly fecund fish

If produced eggs/fingerlings are in excess of hatchery needs, an equal random sample from each spawn is kept; extras should not be considered as future broodstock

Shifting to pedigreed mating

Pedigrees helps avoid the mating of relatives

The N_e in pedigreed mating:

$N_e = 16(F)\,(M)/3\,(F)+(M)$ or $(F) + 3(M)$

more females more males

A greater N_e is produced for the same number of spawners/sex ratio than in random mating

Fewer number of spawners and/or a more skewed sex ratio could be used and still produce targeted N_e in pedigreed mating.

Pedigreed mating requires marking in order to identify fish in individual families.

Sex ratio	Ne Random	Ne Pedigreed
30 M + 70 F	84	140.0
20 M + 80 F	64	98.5

Enhancing the Effective Breeding Number

Egg fertilization practices

Fish hatcheries adopt any of the following in egg fertilization:

- Stripping milt from a live male directly over the egg mass
- Using pooled milt collected from several males or stripping several males in a sequential manner

Notes:

- The use of pooled milt is expected to lower N_e due to the competition among the sperm with differences in their potency leading to a disproportion fertilization
- Similarly, if milt is added in a sequential manner, the sperm from the first male will have a time advantage and hence fertilize most of the eggs compared to subsequent males

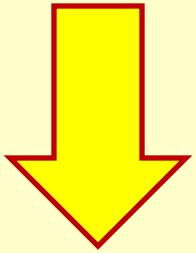
Recommendation:

- In order to maintain the N_e level, it is advised to fertilize eggs from each female with a sperm from a single male. If the intention is to use sperm from several males, the egg mass is subdivided and each portion is fertilized by a single male

Enhancing the Effective Breeding Number
Stretching generations

Generation intervals can be stretched if brood fish are allowed to spawn for extra time before they are replaced by their offspring

This approach may be adopted to slow down the inbreeding accumulation over a fixed period of time (longer generation intervals – fewer generations/specific time frame – lower accumulation of inbreeding



BUT the longer the generation, the lower the selection response over a fixed period of time

Genetic consideration in broodstock management

Hatchery broodstock from the wild:

- For first time ever
- For stock enhancement programs
- For species which cannot mature in captivity

From other hatchery/farm:

- Proven efficiency under particular environments (GxE)
- Not passing through genetic bottlenecks (e.g. tilapia from Brazil to USA)
- From maximum number of spawns

Genetic bottleneck: If N_e drops – because of whatever reason- below the desired value for a single generation, a genetic bottleneck is developed along with its permanent and irreversible genetic damage.

Causes of N_e decline could be mortality caused by adverse environmental conditions, diseases, etc.

In selective harvesting accompanied by catching the fastest growing individuals, choosing broodstock from the residual population means the selection of slower growing population

Genetic-environment interaction (GxE)

Does Exist <u>When</u>:

Various genotypes perform differently in different environments

<u>But</u> if compared genotypes maintain their rank in various environments ⇨ **No G x E interaction**

Example: Common carp strains (Chinese and Polish) in:

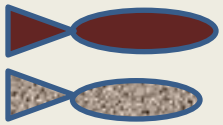
Different stocking densities

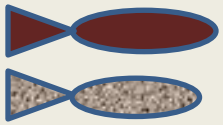
Different feeding regimes (fertilization and artificial feed)

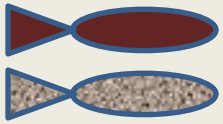
GXE (Example)

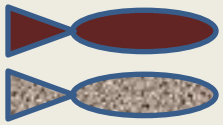
	Environment A	Environment B	
Strain A	80	60	No interaction
Strain B	60	45	
Strain A	80	60	Strong interaction
Strain B	60	70	

Genetic Environment Interaction (GXE) in fish







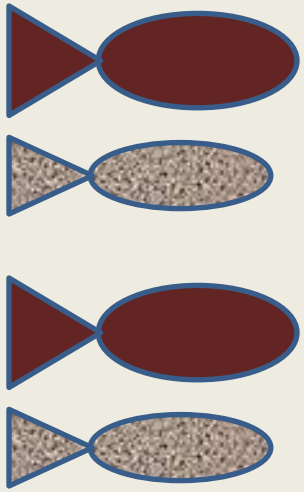


Genetic x environment does exist when evaluated strains perform differently in different farming environments

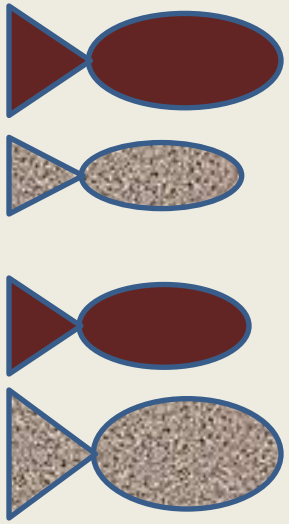
Environment A: earthen ponds; nutrition relies more on organic manure & supplemented by artificial feed

Environment B: earthen ponds; aeration; higher stocking density; complete artificial feed is the only source of nutrition

The data should be statistically analyzed and result of well-designed experiment starting with fish of the same size/age



NO GXE



YES GXE

Applications of genetics

Genetic enhancement approaches

Traditional Approaches

- Selection
- Hybridization

Advanced approaches

- Ploidy induction
- Gynogensis - Androgensis
- Genetic engineering

Selection – Selective breeding

Selection is the oldest approach for genetic improvement

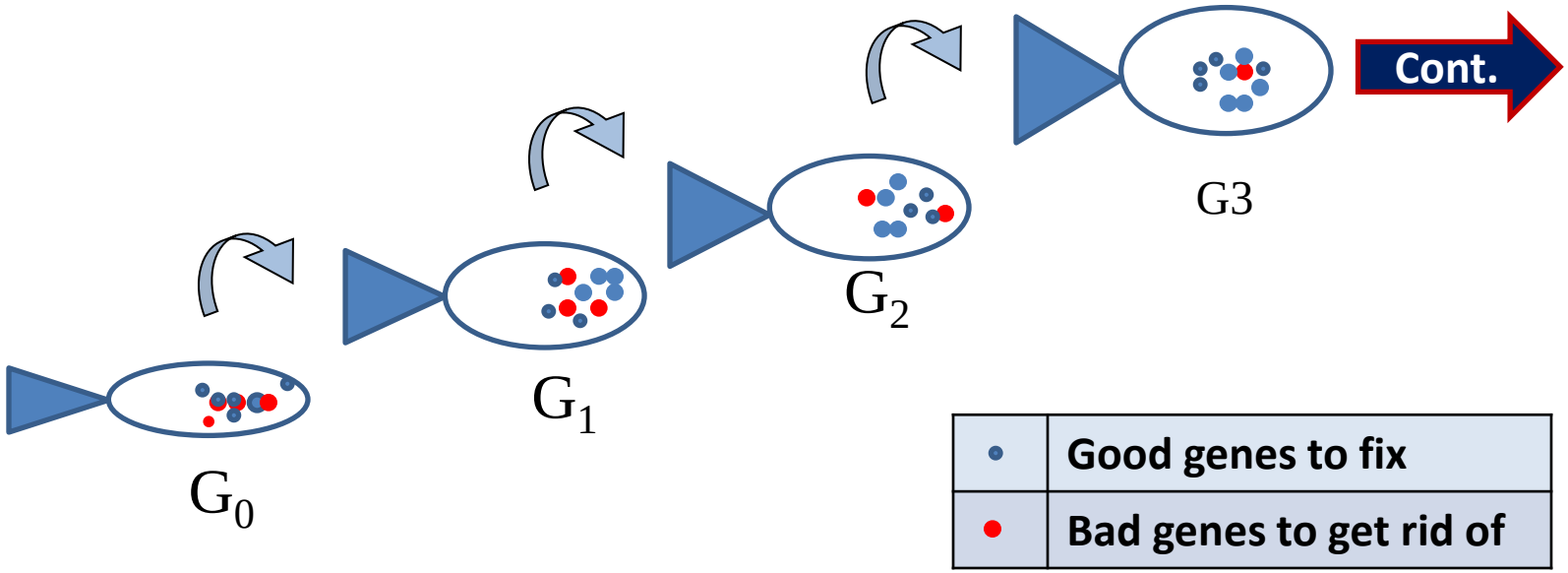
It is simply a choosing the parents of coming generations (positive selection) and through culling (negative selection)

As long as V_A exists, selection response accumulates over generations (variation is the raw material for selection)

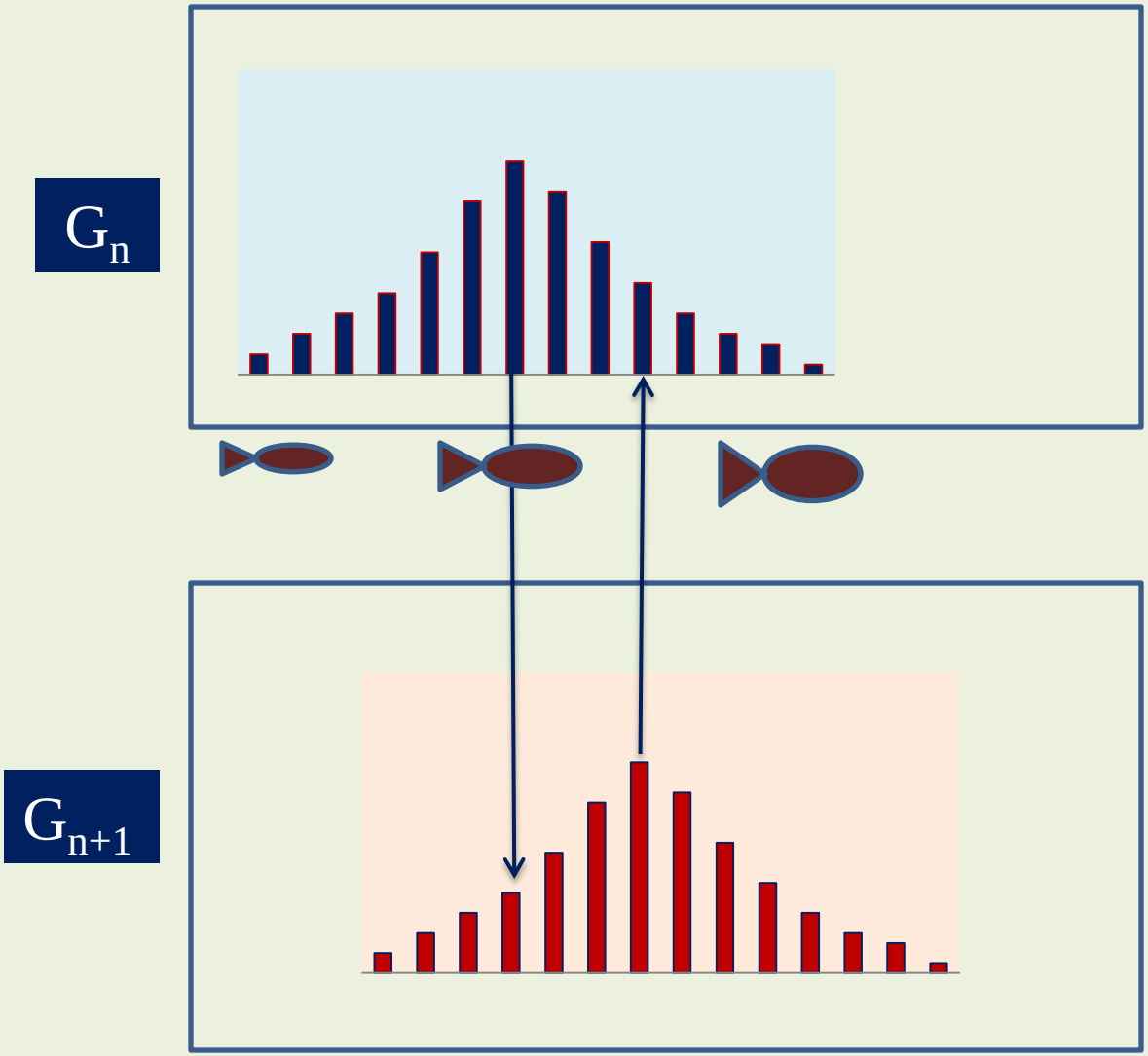
Selection plateau is reached when genetic variation V_A is consumed – **No more progress**. In such case, an external interventions will be needed to create variations (e.g. mutation)

Selection (Selective breeding)

The concept: Continue choosing the "best" individuals to be the breeders



Selection - moving the mean



The bell-shape curve remains

But
Population mean moves rightward

Accumulated selection gain

The response to selection continues in each generation until the genetic variation is consumed up

⇩

Selection plateau

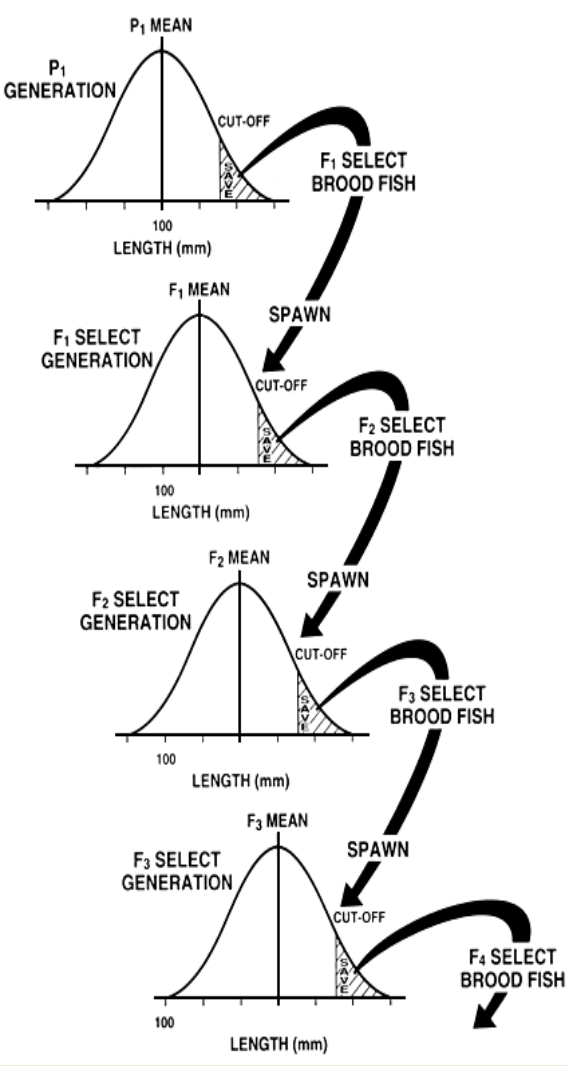
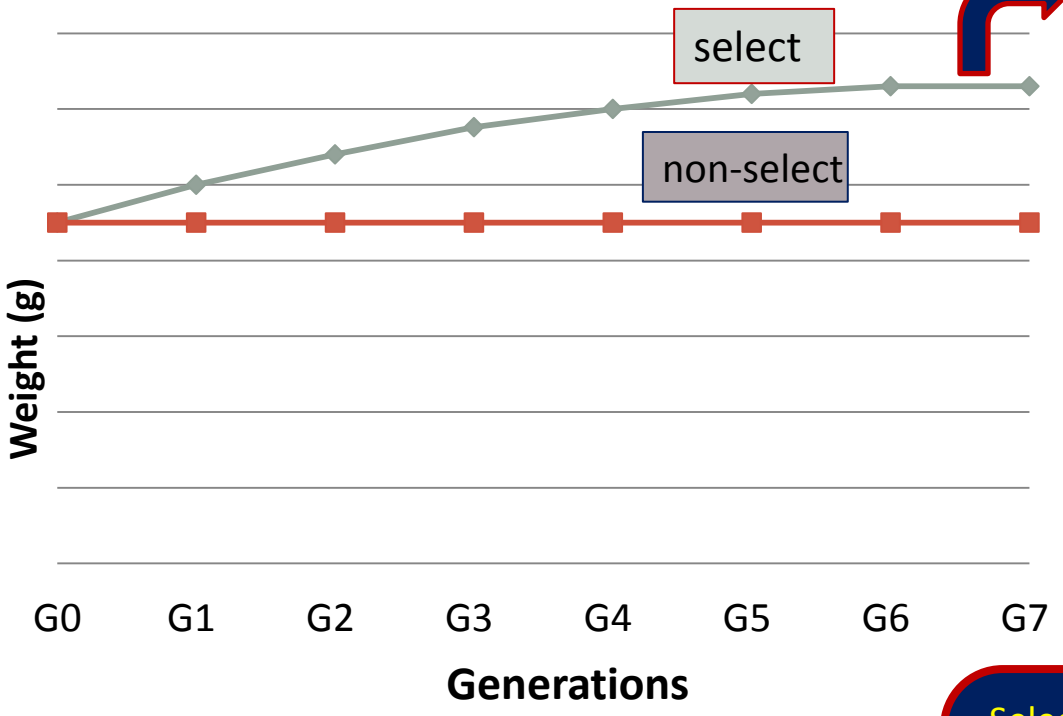


Diagram source: FAO

Fish selection and selection plateau



Assumptions:
Weight of non-select remains constant over the generations

Observations:
Selected populations continued to enjoy selection responses over generations

However, the response to selection occurred at a declining rate till the plateau is reached

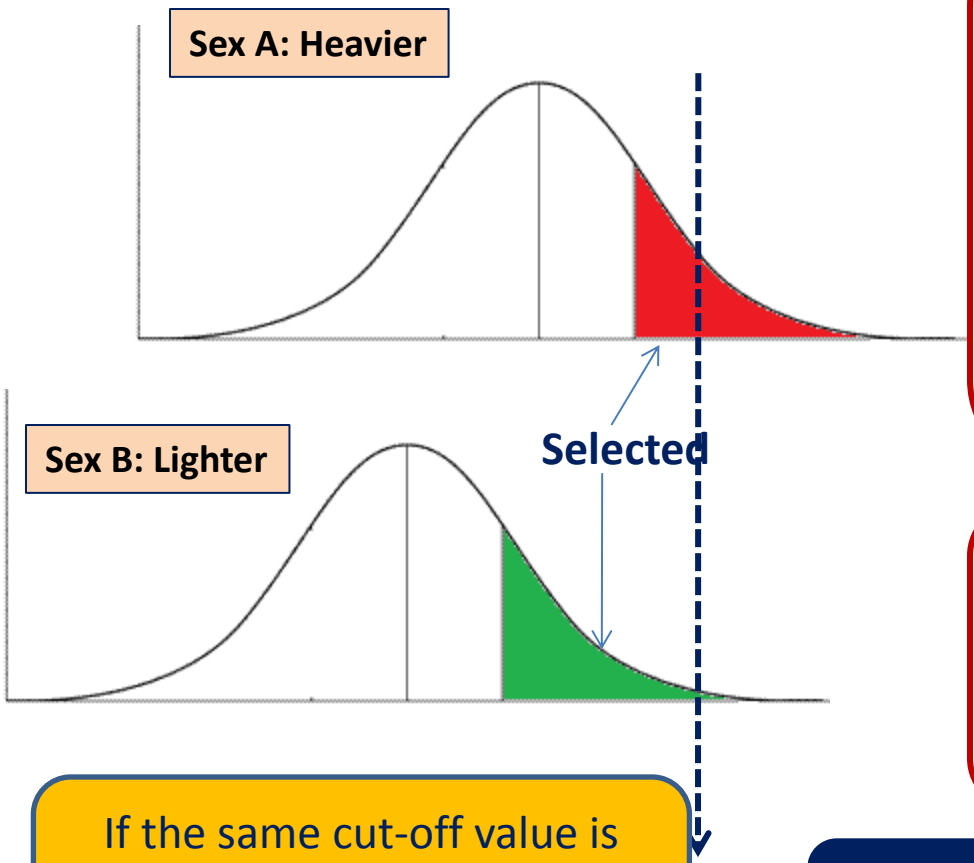
The response to selection (R) is an accumulated gain every generation
R = heritability coefficient (h^2) x selection differential (SD)
Even h^2 is constant, the decrease in R results from the declining of SD – till reaching the plateau

Selection plateau is reached when selection variation is consumed

Selection plateau once reached, no gain could be achieved **unless:**

Variance is again created whether naturally or artificially (mutation)

Selection and sexual dimorphism



Sexual dimorphism: phenotypic differences (qualitative or quantitative) for some traits between individuals of different sex in the same species.

In regard to size, females of eels grow to larger size than males of the same age

In contrary, males of Nile tilapia are larger than females of same age

Sexual dimorphism for size should be considered in selection programs otherwise most of selected fish would come from the heavier sex

If the same cut-off value is used for both sexes in case of dimorphism

How to determine the selection cut-off value for each sex?

Cut-off values when sexual dimorphism exists

Determining the cut-offs:

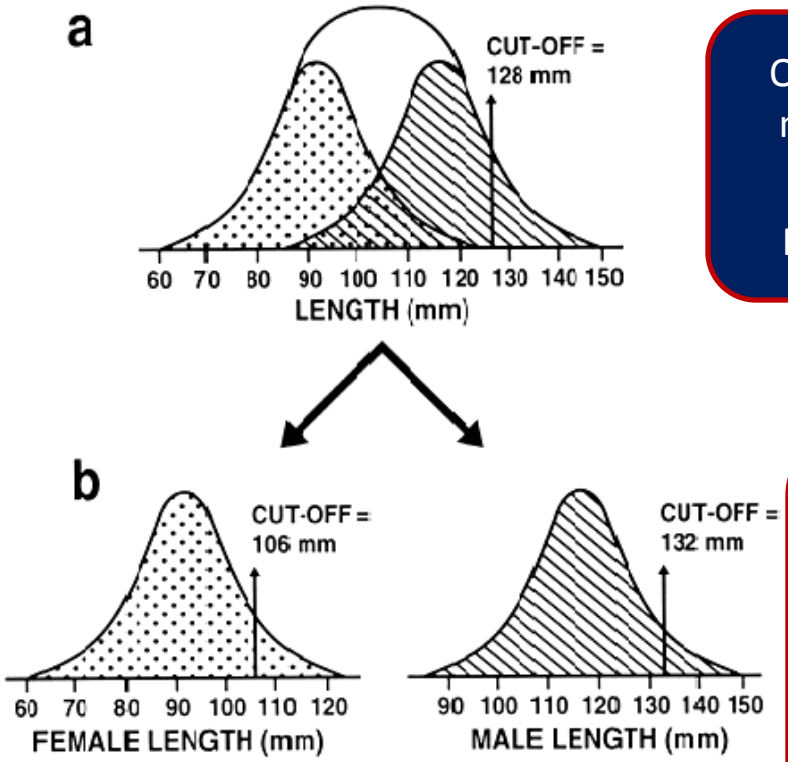
Begin with random sample

Separate the sexes

Get individual values for the trait

List the values in descending order

Scroll-down till reaching the planned number of fish to be selected – value against it is the cut-off value



One cut-off for both male & female (128 mm)

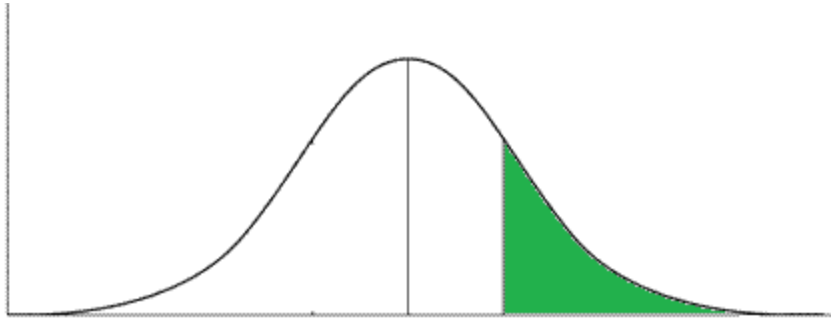
No female selected

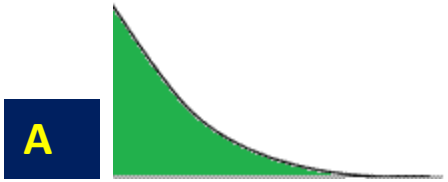
Independent cut-off for each sex (106 for female and 132 mm for male)

Both sexes are equally selected

Diagram source: FAO

Selection intensity - selection differential & the response to selection





Theoretically:

The smaller the selected portion, the largest the selection differential (SD) as in B

The largest the selection differential (SD), the highest will be the response to selection

$R = SD \times h^2$

(assuming the heritability coefficient h^2 is constant)

The question will remain how small the selected portion that leads to largest selection gain without **inbreeding** problems

Selection strategies

Individual (mass) Selection: Choose the best

When h^2 for selected traits is high

Easy to conduct (methodology, facilities and recording)

Requires high heritability ≥ 0.25

Not favored by many fish breeders

Similar age of evaluated strains is a must

A day difference can result in faulty outcomes

Family Selection (select or reject the whole family)

When h^2 for selected traits is lower

Require more tagging & recording

Is a must for traits such as meat quality & dressing percentage

Superior individuals may be discarded because of low family credit

To correct for losing superior individuals in rejected families:

Within Family selection

Saves superior individuals through keeping the best of each family regardless the family status

Multiple-traits selection

Tandem selection

Traits are selected one at a time; only after achieving the desired level of performance in the first trait, the selection of the second trait begins

Correlation between trait should be considered (especially negative correlation)

More traits targeted for selection = more difficult will be the program

Independent culling

Individuals are either selected or culled based on determined cut-off values

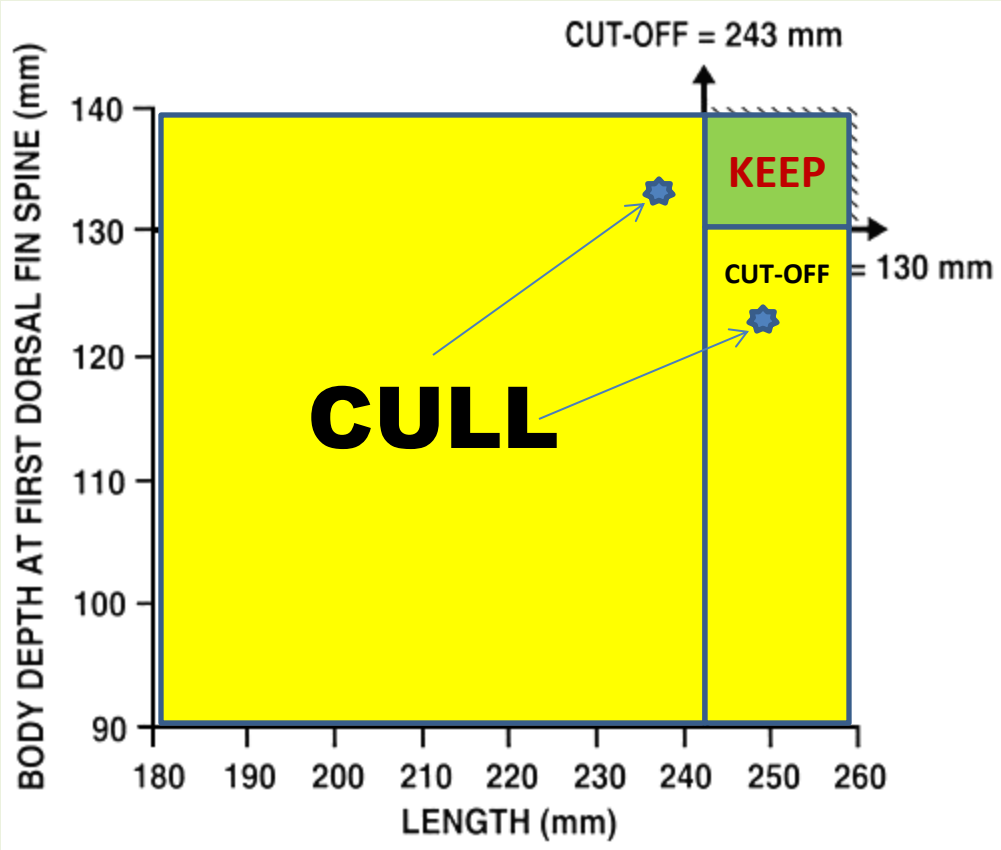
May restrict the size of selected population (depending on the cut-off values

Multiple Trait Selection
Independent Culling

Only individuals with 243 mm length and above & with body depth of 130 mm and above **are kept**

Possible loss of superior individuals because a shortage in another trait.

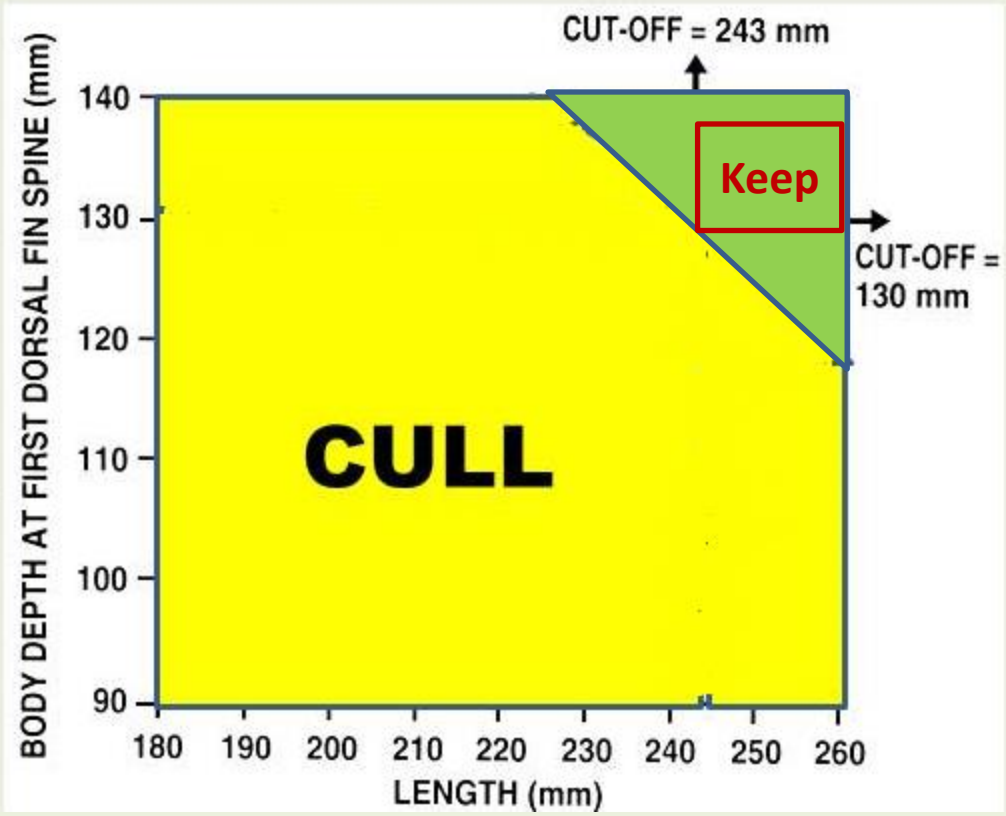
Some modification was felt needed



Source: FAO

Multiple Trait Selection
Independent Culling (Modified)

Relaxing the cut-off value for a trait has saved superior individuals for the second trait



Source: FAO

Multiple trait selection
Selection index

- Index selection has advantages of improving target traits simultaneously with placing differential importance of target traits.
- Selecting or culling is based on the individual's cumulative values of the traits
- The selection index allows for a superior level of performance in one trait to compensate for acceptable deficiencies in other traits.
- only traits of economic importance are included in selection objectives
- The industry needs are well considered in this approach (**tilapia**: growth, cold tolerance/late maturation; **shrimp:** growth, disease resistance)
- Selection index has been applied to key finfish (e.g. Atlantic salmon), and currently applied for Pacific white shrimp, *Litopenaeus vannamei*

Relative importance of traits is considered in this program

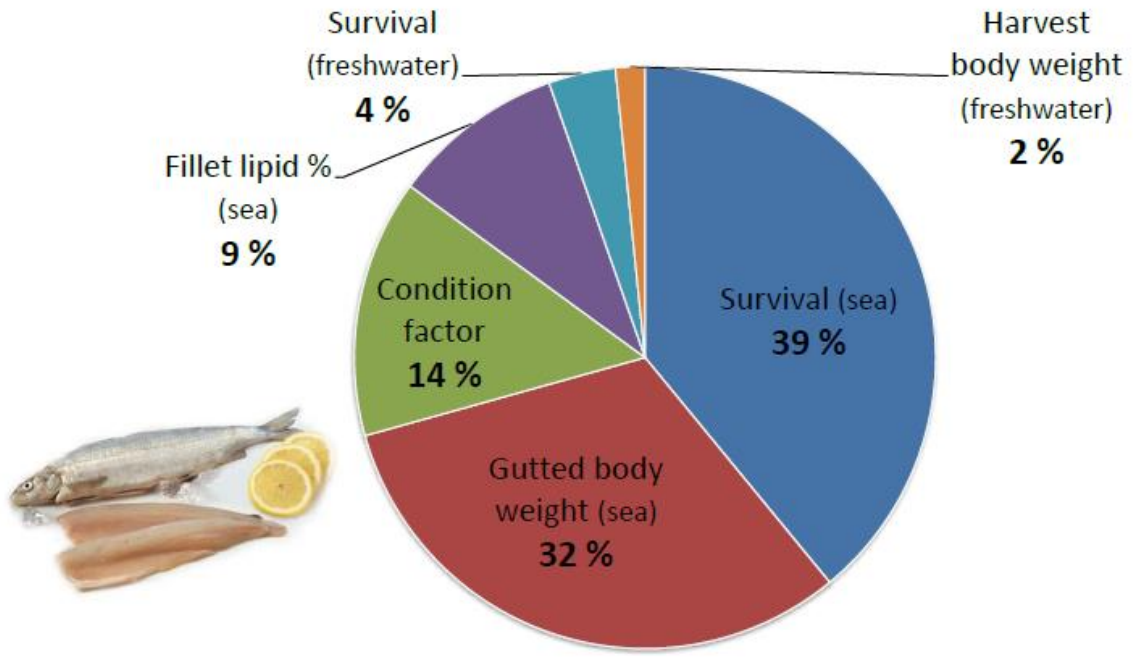
Adding few grams to the weight may be less important than adding 1-2 degree centigrade in the tolerance to cold which means life or death

Selection index
Relative importance

Often varies among species/groups and/or locations

Species	Selected economic traits				
	1	2	3	4	5
Salmonids	Growth/weight	Survival	Color score	Disease resistance	Feed conversion
Tilapia	Growth/weight	Survival	Cold tolerance	Late maturation	Feed conversion
Source: Defining Breeding Objective for Nile Tilapia (*Oreochromis niloticus)* Fish under Low-Input Smallholder Production in Kenya (A wish list as developed by farmers)					
Growth/ fry & fingerlings	Growth (Table size)	Survival	Late maturation	Feed conversion	Pink eye
Length	Height	Thickness	Parasite resistance	Only five of the above traits have been included in the selection index)	

Relative importance (RI) of traits in the selection index for European whitefish



This is a short list of the 13 identified traits contributing to supply-chain profitability

Compare the RI of survival in sea water and freshwater

Source: Finnish national breeding program for European whitefish (*Coregonus lavaretus*)

Relative importance of productive traits (examples)

Marron crawfish (Australia)

Crawfish farmers can produce high yields (in regard to biomass)

However, much of the production after 12 months was below market size. Another year of grow-out was required

Target of selective breeding program: Increasing growth and reducing the size variation

This leads to that the majority of animals are above market size & allowing farmers to move from a 24-month to a 12-month production cycle

TSV and Pacific white shrimp

An important trait in regard to survival and production; far important for particular species & disease (e.g. Taura syndrome virus "TSV" in Pacific white shrimp; L. vannamei).

The sufficient additive genetic variation enabled a significant improvement in the resistance of selected shrimp of about 18% higher after a TSV-challenge test.

Measuring selection response can be straight forward (e.g. weighing) or may require standardized procedures (e.g. challenge test)

Response to selection – weight gain

Species	Gain per generation %	Number of generations	References
Coho salmon	10.1	4	Hershberger *et al*., 1990
Rainbow trout	10.0	3	Kincaid *et al.*, 1977
Rainbow trout	13.0	2	Gjerde, 1986
Atlantic salmon	14.4	1	Gjerde, 1986
Atlantic salmon	12.0	6	Gjerde and Korsvoll, 1999
Atlantic salmon	12.5	1	Flynn *et al.*, 1999
Channel catfish	12.0-18.0	1	Dunham, 1987
Channel catfish	20.0	1	Bondary, 1983
Nile tilapia	15.0	5	Rye and Eknath, 1999
Rohu carp	17.0	2	Mahapatra *et al*., 2000

Traditional approaches
Hybridization

Species purity and hybridization barriers:

Purity of species in nature is maintained through hybridization barriers:

- Biological: (number of chromosomes)
- Spawning seasons
- Reproduction requirements

In general, hybridization in nature is minimum while in captivity, chances may increase

Hybridization could be:

- Intergeneric (among genera)
- Interspecific (among species)
- Intraspecific (strains within species- cross breeding)

Traditional approaches
Hybridization

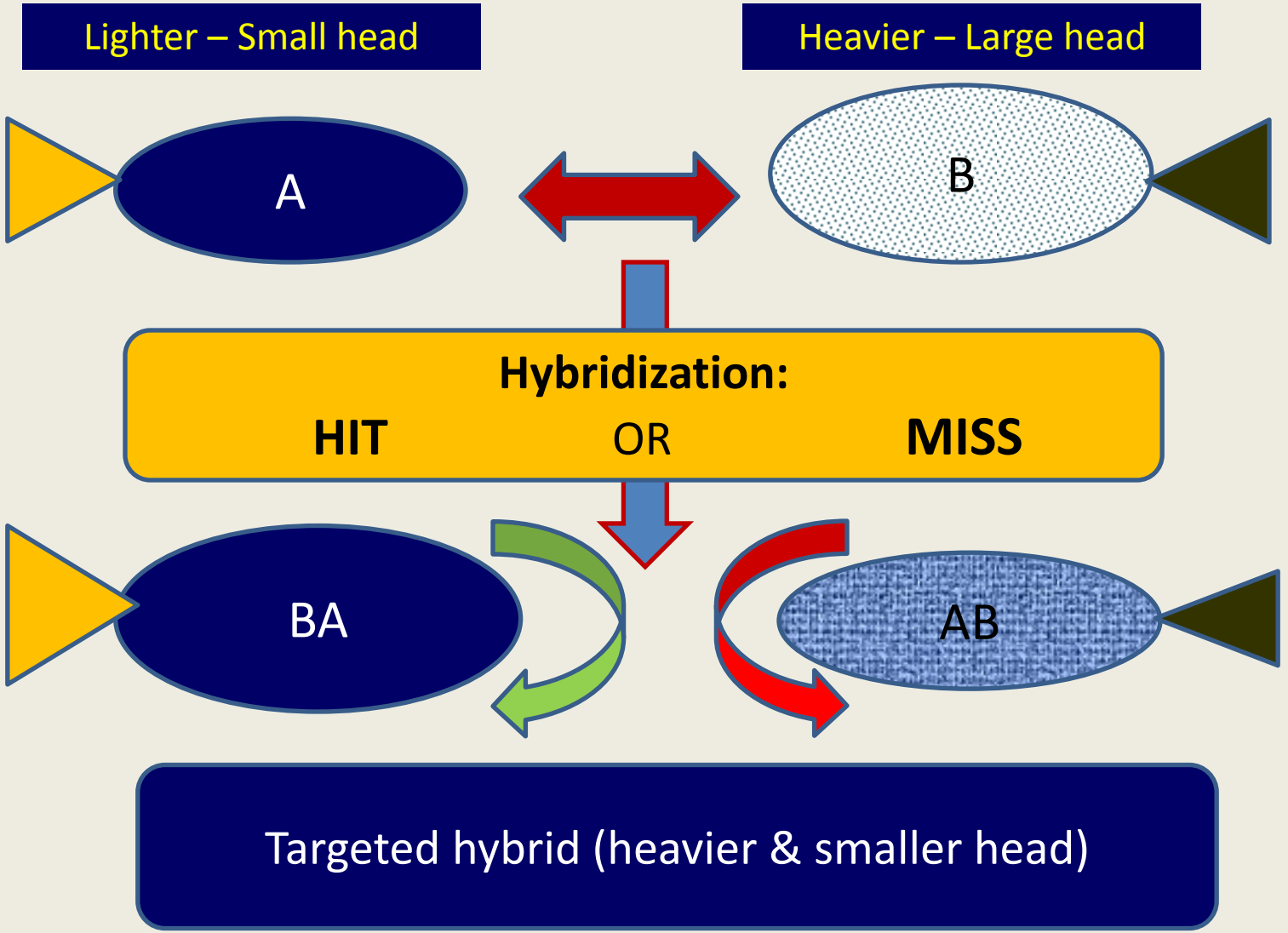
Hybridization is carried out for:

Hybrid Vigor: The tendency of hybrids to grow faster, get larger, tolerate more to specific conditions, or better dressing than their parents. Also called heterosis.

Sterility

Production of uniform progeny

Famous hybrids:
Mule: male donkey x female horse
Plants: hybrid corn
Poultry: broilers – layers
Fish: to follow


Lighter – Small head
Heavier – Large head
A
B
Hybridization:
HIT
OR
MISS
BA
AB
Targeted hybrid (heavier & smaller head)

Interspecific hybridization (e.g. tilapia)

Two species of tilapia can be crossed to yield all-male offspring

Male *O. Hornorum* or *O. aureus* tilapia can be hybridized with the female of Nile tilapia (*O. niloticus*) to produce all-male offspring (**theoretically**) Why not 100% males produced?

Tilapia hybrid is **fertile** and can backcross with parent species which could **upset** the purity of parent species (Nile tilapia in Lake Victoria)

It is not recommended to carry out tilapia hybridization in Africa (the home of tilapia)

Interspecific Hybridization

The hybrid between cachama (*Colossoma macropomum)* and morocoto (*Piaractus brachypoma)* is a sterile hybrid (cachamoto) that accounts for about 80% of aquaculture in Venezuela.

Cachama

Morocoto

Cachamoto

Photo credit: Miriam Requena (Venezuela)

Asian catfish

African catfish

The hybridization between Asian catfish (*Clarias macrocephalus – female)* X African catfish (*Clarias gariepinus* - male) produce a hybrid that is superior to both parents in regard to growth rate).

The hybrid is favored for Thai aquaculture.

Asian catfish is native to Southeast Asia, African catfish was introduced into Thailand

North American catfish

The hybrid results from the female of channel catfish (Ictalurus punctatus) x the male of blue catfish (Ictalurus furcatus) has commercial application

The hybrid exhibits the following traits:

- faster growth
- better feed conversion
- tolerance of low oxygen
- increased resistance to many diseases
- tolerance to crowded culture conditions
- uniformity in size and shape
- higher dress-out percentages
- increased harvestability by seining
- increased vulnerability to angling

Interspecific **Hybridization & reciprocal crossing**

The reciprocal cross between the male of channel catfish with the female of blue catfish, does not have the same superior production characteristics of the original hybrid

Indian major carps

Intergeneric Hybridization

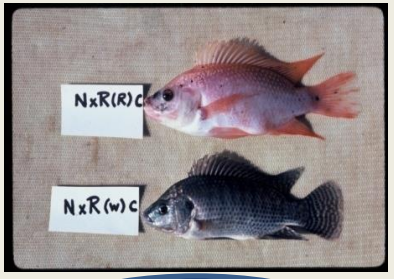
♀	♂	Hybrid
Labeo rohita	Catla catla	~ 60% hatchability – high mortality of hatchlings – fry growth was higher than rohu …..
Cirrhina mrigala	Labeo rohita	> 90% of eggs were fertilized. Most of the body characteristics were intermediate to those of the parents. Both the hybrids matured fully in two years
Labeo rohita	Cirrhina mrigala	
Labeo rohita	Cirrhina reba	Twenty percent of the fertilized eggs hatched out but all of them died on the third day

Source: H. Chaudhuri, Fish hybridization in Asia with special reference to India - FAO

Hatchery Broodstock

Hybrids which could be excellent for grow-out, **cannot be used as broodstock**

For species purity, if hybridization is carried out in a hatchery, hybrids should be kept in isolate



Case: Red tilapia

Ploidy induction in fish – why?

General

Sterility: (for environmental reasons): triploid (3n) fish are normally sterile (e.g. grass carp)

Higher growth rate: through saving the energy which could be spent in gonad development and spawning (triploidy)

Indirect means to produce triploidy (when tetraploidy (4n) mates with diploidy)

Species-specific (all-season oyster)

Oysters (e.g. Pacific oysters) are highly fecund.

Upon spawning –in summer- they turn watery, unpalatable & their weight is much reduced

Spawned-out oyster would be less marketable

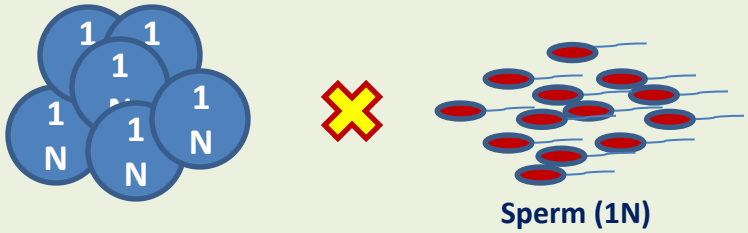
The adoption of the triploid oysters are characterized by their:

- Sterility
- Fast growth
- High meat quality (firm and more palatable)
- Could be marketed all-year round

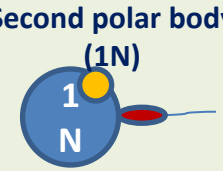
Triploid oyster was first developed in the early 1980s Afterwards, a tremendous growth in the production of triploid oyster took place; a large oyster hatchery produced about **11 billion** oyster larvae in 1994.

Triploidy induction in fish

Preparing for fertilization between haploid gametes (1N) each



Fertilization



Apply the shock soon after fertilization

Resulted cell has 3 chromosomes from: ova, 2nd polar body (**retained**) and from the sperm

3-N fish

Under normal situations, the second polar body is extruded and fallen and embryos with two chromosomes result
Shocking will prevent the second polar body from falling
The time and duration of shocking depends on species and water temperature

Types of shocking:
Heat shock: water bath
Cold shock: chiller, refrigerator
Pressure: pressure chamber
Chemicals: (e.g. cytochalasin B)

Triploidy (concerns and inspection protocol)

Issues of concern

Environmental concerns call for the use of triploidy fish based on their sterility

It is assumed that sterile fish (triploids) will not cause environmental damages compared to diploid fish

However, triploidy fish might out-compete their diploid counterparts in regard to food or the spawning grounds

Inspection (example of triploid grass carp) – No chance for any mistake

Before triploid grass carp leaves the facility, producers test their fish according to the protocol set by USFWS :

Individual fish must be blood tested by the producer using **coulter counter** to ensure it is triploid.

Afterward, a USFWS inspector visits the facility and randomly retests 120 fish from each prospective shipment.

If the 120 fish are triploid, a certificate is issued verifying that every fish in the prospective shipment is triploid.

If **even one** diploid is found during the inspection, no certificate is issued and every fish in the shipment must be individually retested by the producer.

Another 120 randomly selected fish must pass another USFWS inspection. If the inspection passes, and a certificate is issued, triploid grass carp may be shipped from the facility

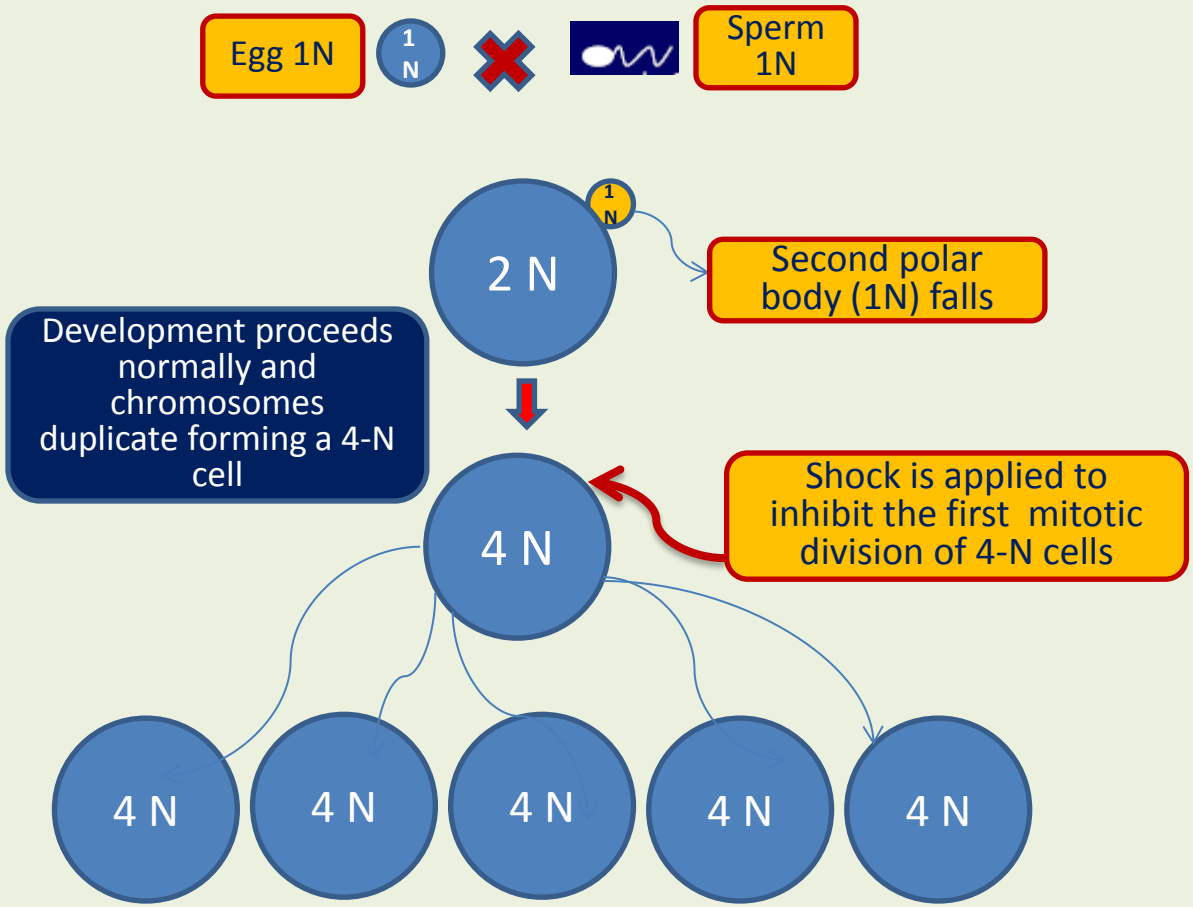
Tetraploidy induction in fish

Summary of the process:
No shock is applied after fertilization and so 2nd polar body is extruded and fallen (as normal) - embryos with 2 chromosomes result

Enough time should be allowed till cell chromosomes duplicate forming 4N

Shock is applied to inhibit the first mitotic division resulting in a 4N cells that divides normally afterwards producing tetraploid fish

Shock could be heat shock, cold shock, pressure or use of specific chemicals; timings, levels and durations of treatments vary



Hormonal masculinization and feminization

Male or female genotype is established upon fertilization; however, the phenotypic sex shows-up later

The un-differentiated sex period varies according to species (e.g. about 21 days in tilapia)

During the sex determination period, male hormones (androgens) or female hormones (oestrogens) could be used to alter the phenotypic sex of treated fry to either all-male or all-female populations.

All-male **tilapia** is targeted because of their higher growth rate and to eliminate the unwanted reproduction. **Trout** females are desirable because of their late sexual maturity, faster growth and superior flesh quality compared to males.

Most effective male hormone is 17-methyltestosterone while 3-oestradiol is among the most effective feminization compounds.

Feminization is applied as an intermediate step towards the production of YY super male of tilapia

All-female trout eggs

Credit: Troutlodge (USA)

Hormonal sex-reversal in fish is widely adopted

However, such practice is not permitted in some countries for reasons related to the safety of operators as well as for environmental reasons

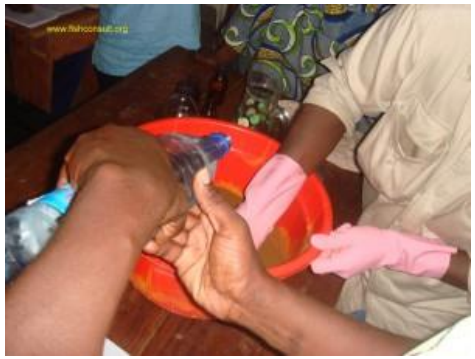
Hormonal masculinization and feminization

Nile tilapia swim-ups are sex-reversed to all-males by feeding 30–60 mg 17α-methyltestosterone/kg feed for 21–28 days. Expected male proportion **should exceed** 95% of treated fry

Silastic implants of 17 α - methyltestosterone has been used in the sex reversal of grass carp to males. Implants are placed in the fish at 85mm and the hormone is released until the fish reach about 200 mm

Coho salmon have been sex-reversed to females by bathing the embryos in 25 µg/l of 17β-oestradiol, followed by oral administration of 10 mg/kg 17β-oestradiol to fry

In general, several factors influence the effectiveness of sex reversal including species, genetics, type of hormone, dosage of hormone, duration and timing of treatment



Hormone-treated feed preparation in Benin

Credit: Ismael Radwan (Egypt)

Advanced genetic technologies

Gynogensis:
Used for the production of off-springs having its genetic make-up only from mother. This is achieved through the use of irradiated sperm using UV which destroy its DNA but still activate the ova development.

Androgensis:
Used for the production of off-springs having its genetic make-up only from father upon the fertilization of UV-irradiated ova by normal sperm (UV destroys the ova DNA)

Both approaches are used to produce highly inbred lines as required by some breeding programs

Advanced genetic technologies
Genetic engineering (gene transfer)

Fish are ideal organisms for genetic engineering programs because of:

- High fecundity
- Short generation (utilized for other purposes including human health)
- External fertilization
- Large size ova

Genetic Engineering

Gene transfer

Phases of application

- Successful insertion of the gene
- Expressing the transferred gene
- Heritability of traits related to transferred genes

Concerns:

- Will remain at experimental stage for some time
- Significant opposition
- Biosafety is a must during all phases of the experimentation

Genetically modified organism (s) (GMOs) – Genetic engineering organism

A living organism that inherited traits from another organism through the insertion of a gene using genetic engineering techniques

Resulting organisms are always declared as GMOs according to biosafety regulations

This technology has been used in several areas including plants, vaccines, foods, and medicine

In relation to fish, genetic engineering research has commenced in several research institutes

Fish with short generations could serve as an experimental animal whereas the outcomes may explain a phenomenon or could be applied to the target organisms with longer generations or could be extended to human (zebra fish and human muscular dystrophy)

Genetics in:
Stock Enhancement Programs

Facts:

There are genetic differences between hatchery-produced stock and the wild population of the same species

Predator avoidance, aggressiveness and homing are traits of importance to wild stocks while not that important at all in aquaculture

Noticeable problems may result from the mating of hatchery-produced stocks with their wild counterparts

Stock enhancement

Mitigation of possible risks

For a species with a long spawning seasons, broodstock planned for stock enhancement should be collected over the whole spawning season to avoid selecting for particular spawning period

In order to reduce the possibility of domestication, seed produced in hatcheries should not be grown for the use as broodstock in stock enhancement programs. **Instead**, broodstock should be taken from the wild –whenever possible- to maintain genetic diversity

Ideal enhancement programs are the ones that include breeding with no genetic changes; in order to preserve the inherent, non-domesticated genotypes

Some international stock enhancement programs designate special hatcheries for stock enhancement (managed differently from those serving aquaculture)

Conclusion

The possibility of enhancing fish production through husbandry practices has delayed the utilization of genetics due to cost and time required as well as the **difficulty impression**

As aquaculture develops and challenges continue, it became obvious that husbandry practices **have limits** especially with issues of genetic nature (e.g. cold tolerance)

Artificial propagation in hatcheries represents a main step towards the application of genetics in aquaculture

Fish genetics programs are the responsibility of research institutes, while mass production takes place in hatcheries or multiplying stations

The application of genetics that has been seen fancy in the past or even in the present would be in practice in the near future