

Determination of Electrophoretic Variations in the Isozyme System of Three Clariid Species

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Abstract

This study was conducted to determine the genetic characteristics and variations within and among the three employed clariid populations; the horizontal starch gel electrophoresis was used. Moreover, morphometric characteristics were measured to determine the proportions that can be accounted for by the genetic factors influence to the measured characters.

Preliminary test showed skeletal muscle exhibiting good resolution and maximum loci activity. Five proteins employed in the study resolved six loci that anodally migrated viz., *ADH-1*, *ME-1*, *SOD-1*, *IDDH-1,2* and *MDH-1**. Except for *MDH-1**, all other loci were monomorphic. Observed heterozygosity reflected high in *C. batrachus* ($H_L = 0.50$); low in *C. macrocephalus* ($H_L = 0.44$); and none of *C. gariepinus* ($H_L = 0.00$). Within-population genetic variations revealed *C. batrachus* ($\chi^2 = 0.22$ $p < 0.05$) conforming to the Hardy-Weinberg Law, while *C. macrocephalus* ($\chi^2 = 14.89$ $p > 0.01$) and *C. gariepinus* ($\chi^2 = 54.00$ $p > 0.01$) significantly deviated from the expected proportions. Intra- and interspecific variations observed were attributed to heterozygote deficit, restricted effective population size, founder effects, Wahlund effects and even to anthropogenic influence. Genetic factors influence to the measured morphometric characteristics reflected low results, ranging from 0.6% for BD to 37% for BG, suggesting that the environment influenced the large proportions.

The results are of value to conservation authorities, researchers, and aquaculturists concerned with commercial species production.

Knowledge of the genetic structure of natural populations is essential for effective management in conservation biology. Such information is important with regard to vanishing native fish species and those of introduced fish in a natural habitat. This paper provides text on the protein electrophoretic analysis of

genetic structure, characteristics, and variations of the vanishing native catfish, *Clarias macrocephalus*, the legally introduced *Clarias batrachus*, and the illegally introduced *Clarias gariepinus*. It is based on considerations that there are no population genetic studies (characterization, variations, and population structure) undertaken on the species to date in the country, which undertakings are deemed essential for their effective management, aquaculture works, and conservation measures. Moreover, this study measured some morphometric characteristics to determine the proportion of influence of genetic factors to the observed morphometric characters.

Population genetics studies on clariid species are limited (Jondeung and Na-Nakorn, 1986; 1992; Lawonyawut *et al.*, 1992; Changpong *et al.*, 1994; Chaiparinga, 1996; Na-Nakorn *et al.*, 1998) and, mostly, have been done outside the country. In the Philippines, clariid species studies have been conducted anent to culture and production, such as captive breeding and propagation (Carreon *et al.*, 1973; Hara and Aquino, 1975; Hara, 1977; Monje *et al.*, 1993), induced spawning (Pocsidio, 1978; Pacoli *et al.*, 1985; Fermin and Bolivar, 1991; Tan-Fermin, 1992; Tan-Fermin and Emata, 1993; Tan-Fermin *et al.*, 1994; Tambasen-Cheong *et al.*, 1995), and diet and nutrition (Cruz and Laudencia, 1976; Santiago and Gonzal, 1997).

At present, government and private hatcheries produce fry of this fish and those of African catfish, cognizant to the fish seed demand, without safeguard against the danger of hatchery fish escaping to the natural environment. The increase in nonadaptive 'hatchery gene' frequency, the breakdown of natural system of semi-isolated population, and the inter- and intraspecific hybridization are among the potentially harmful genetic effects posed when hatchery fish finds its way to natural water bodies.

A substantive knowledge of the genetic structure and variability of the population or the species in question, therefore, is needed as the inceptive step prior to putting into effect any conservation or aquaculture attempts. The natural genetic pattern within, between, and among populations must be maintained to prevent indigenous genetic resources deletion. Genetic preservation is essential in order to sustain

fishery production, forestry, and agriculture (Hilsdorf, 1995).

MATERIALS AND METHODS

The native *C. macrocephalus* and the Thai *C. batrachus* were obtained from Liguasan Marsh, Cotabato, while *C. gariiepinus* was obtained from Tanay, Rizal Province. Physical identification based on the occipital process and species identification were based on Froese and Pauly (2003). The collected native and Thai clariids were then transported alive to Iligan City, where tissue extraction was carried out. For *C. gariiepinus*, live specimens were brought to Binangonan, Rizal for tissue extraction. All extracted tissues were brought to SEAFDEC, Binangonan Freshwater Station, Binangonan, Rizal for biochemical analysis. A total of 150 tissues were used in this study.

Horizontal starch gel electrophoresis and histochemical staining procedures were carried out following the modified protocols of Aebersold *et al.* (1987) and Macaranas (1991). With the gel slices in the appropriate enzyme staining mixtures, the gels were incubated at 35°C until distinct banding patterns become visible. Band scoring was carried out immediately.

The information from the yielded zymogram was analyzed and the basis of genetic variability assessment within and between the employed clariid populations. Enzyme Commission (EC) numbers and abbreviations followed the recommendations of Shaklee *et al.* (1990). Enzyme loci were designated by the enzyme abbreviation followed by a number. For enzyme systems encoded by more than one loci, the most anodal locus was designated as 1. In loci with more than one allele, the most common allele was arbitrarily designated as 100 and the other loci were scored relative to the mobility of the most common allele.

Following the recommended equation of Ferguson (1980), allele frequencies p and q were determined from the number of identifiable enzyme phenotypes obtained from each individual fish.

Heterozygosity was another means of measuring genetic variations among the employed catfish species and was measured employing the formula:

$$H_L = 1 - \sum x_i^2 \quad \text{where: } H_L = \text{heterozygosity per locus}$$

X_i = frequency of the *i*th allele
at a locus

Based on the Hardy-Weinberg principle (Hartl and Clark, 1984), genotypes *F/F*, *F/S* and *S/S* have the frequencies of p^2 , $2pq$, and q^2 , respectively.

A test for departure from the Hardy-Weinberg expectations was determined using the chi-square test (χ^2). The null hypothesis (H_0) states that the tested catfish populations conform to the Hardy-Weinberg Equilibrium. The greater the departure from the Hardy-Weinberg proportions, the poorer the fit. In this study, the significance level is set at 0.05. The expected genotype frequencies were calculated based on the hypothesized ratio of Hardy-Weinberg proportions, which is 1:2:1 (1*F/F*; 2*F/S*; 1*S/S*). The degree of freedom (*df*) was calculated as $c - 1$ where *c* is the total number of data categories. The influences of genetic factors to the observed morphometric characters were analyzed using the Systat Version 10.2.

RESULTS AND DISCUSSIONS

Isozyme Variation

Initially, preliminary test was undertaken on all extracted tissues (i.e., eye, liver and muscle) to determine which of the tissue samples showed good resolution by exhibiting maximum loci activity. As shown in Table 1, the skeletal muscle tissue exhibited a wide range of enzyme activity and maximum elucidation of bands compared to the eye and liver tissues. Thus, it was appropriate to employ skeletal muscle in this undertaking.

Table 1. Elucidation and enzyme activity of various tissue samples

Enzyme	Abbreviations	E.C. number	Tissues		
			Eye	Liver	Muscle
Superoxide dismutase	SOD	1.15.1.1	*	*	√
Malic enzyme	ME	1.1.1.40	*	*	√
L-Iditol dehydrogenase	IDDH	1.1.1.14	√	*	√
Alcohol dehydrogenase	ADH	1.1.1.1	∅	√	√
Malate dehydrogenase	MDH	1.1.1.37	∅	√	√

Legend:

- √ - good resolution and high enzyme activity; tissue best used to score loci
- * - not good resolution, minimal enzyme activity; tissue not taken to score loci
- ∅ - no activity; no elucidation of loci

Several studies (Romana, 1985; Degani and Velth, 1990) have shown that enzymes are tissue-specific and that loci activity is expressed better by specific tissue. Although loci activity are better elucidated and expressed in muscle, a generalization on tissue specificity could not be formulated in this regard because of the limitation of enzymes employed in the study.

Using skeletal muscle tissue, the study resolved six loci in five enzymes, which are reflected in Table 2, *viz.*, *SOD-1*, *IDDH-1*, *2*, *ADH-1*, *MDH-1**, and *ME-1*. **Except for *MDH-1****, all other loci were monomorphic, which means that no variations in the elucidated loci were observed. In the zymogram, the resolved protein non-allelic variations elucidations exhibited the single band that anodally migrated for *SOD-1*, *ADH-1*, and *ME-1* and two bands for *IDDH* (i.e. *IDDH-1* and *IDDH-2*) in all sampled individuals in the three populations, revealing that only one allele was elucidated by these enzymes in the samples. Since the mentioned enzymes were monomorphic, it was expected that their allelic frequency was 1.

As indicated, *MDH* showed polymorphism in *C. macrocephalus* and *C. batrachus* in the zymogram and electrophoretogram (Figures 1 and 2), while *C. gariepinus* was monomorphic. The allelic frequencies of the catfish populations employed in this study are reflected in Table 3. It should be noted that allelic or gene frequency was an indicator of genetic variability since it showed different electrophoretic mobilities of enzymes in a single locus. The allelic frequency variation observed in this undertaking reflected the genetic plasticity of the species.

Table 2. Loci abbreviations and Enzyme Commission (EC) numbers for each protein analyzed in three catfish populations.

Protein	Locus	E.C. numbers
Superoxide dismutase	<i>SOD-1</i>	1.15.1.1
L-Iditol dehydrogenase	<i>IDDH-1,2</i>	1.1.1.14
Alcohol dehydrogenase	<i>ADH-1</i>	1.1.1.1
Malic Enzyme	<i>ME-1</i>	1.1.1.40
Malate dehydrogenase	<i>MHD-1*</i>	1.1.1.37

*showed polymorphism

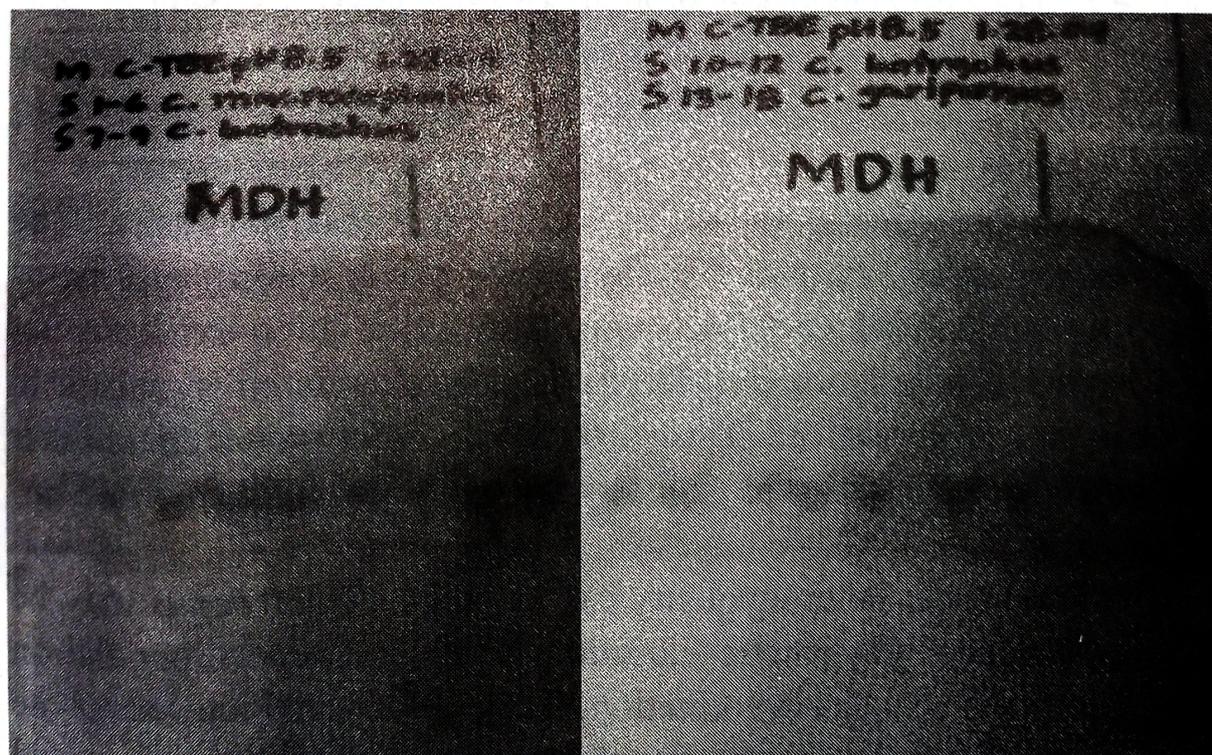


Figure 1. Zymogram showing isozyme variation in malate dehydrogenase (*MDH*). Samples 1-6 - *C. macrocephalus*, samples 7-12 - *C. batrachus*, samples 13-18 - *C. gariepinus*.

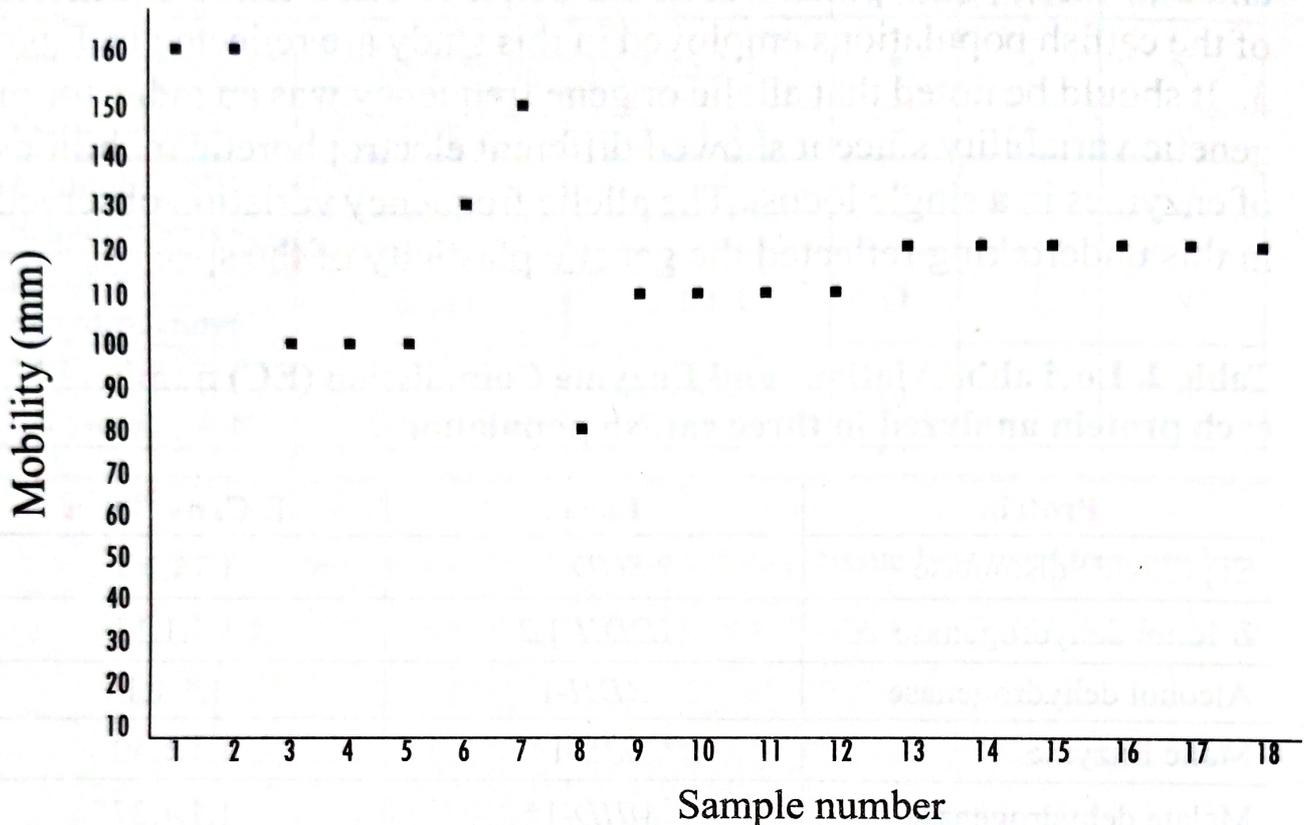


Figure 2. Electrophoretogram of *MDH* Isozyme patterns of *Clarias spp.* Samples 1-6 - *C. macrocephalus*, samples 7-12 - *C. batrachus*, samples 13-18 - *C. gariepinus*

Another genetic variability indicator is heterozygosity. The genetic variations amount, as measured using heterozygosity, is shown in Table 3, which reflects considerable variation existence not only in within-population but also among closely related organisms in the polymorphism extent. It was noted that *C. batrachus* had higher heterozygosity compared to *C. macrocephalus* and *C. gariepinus*.

The higher heterozygosity exhibited by *C. batrachus* may be ascribed to the high genetic variation level the founder population had during the species introduction in the 1970's, which led to the species' successful adaptive capability. Such capability increased species reproductive fitness that enabled it to establish itself very well in almost all freshwater environs in the country. This finding supports the claim and observation of Juliano *et al.* (1989) that *C. batrachus*

successfully established itself in almost all inland waters after its introduction in the country.

Table 3. Genetic variability of the three catfish populations for *MDH**

Species	Allele frequencies		Heterozygosity	Chi-square values (χ^2)
	<i>F</i>	<i>S</i>		
<i>C. macrocephalus</i>	0.67	0.33	0.44	14.89**
<i>C. batrachus</i>	0.50	0.50	0.50	0.22 ^{ns}
<i>C. gariepinus</i>	1.0	0.00	0.00	54.00**

**significant at 1% level

^{ns} not significant

The lower heterozygosity observed in *C. macrocephalus* may be ascribed to species population size reduction from a large one and/or fluctuation sometime in the past which could be inferred to be due to the species local niche degradation, the fish's commercial exploitation, and the species adaptive incapability to nature's new introduced conditions. Fish population reduction formed from a large one resulted in allele frequencies divergence, thus allele was lost, and consequently there was a genetic variability reduction. Corollary accounts were those of Na-Nakorn *et al.* (1998) who noted that the low heterozygosity results obtained in their experiment on *C. macrocephalus* were due to effective population size reduction in addition to population bottleneck. Quilang (2003) also cited similar explanations on the genetic variability low level obtained in his study of the silver perch, *Leiopotherapon plumbeus* Kner, (1864) isozyme variations conducted in Laguna de Bay. He stressed that population size reduction resulting from unrecorded effective population size restriction in the past accounted for the low genetic variability results.

Although there have been no published reports on the significant drop in fish catch for this species, personal interview conducted by this researcher with fishermen residing around Liguasan Marsh indicated significant fish catch decline in the area. In the past, according to them, catfish catch came in a large volume. Chikli *et al.*, (1998) (cited in Quilang, 2003) gave the following as potential low gene diversity

explanation in *S. aurita*: restriction on effective population size owing to variance in reproductive success, demographic instability, historical bottlenecks in population size, selection, and artifacts and statistical biases. The lower heterozygosity in *C. macrocephalus* obtained in this study may be ascribed to this explanation.

The lack of heterozygosity within population such as that shown by *C. gariepinus* is attributed to the species' limited quantities and low genetic variability in the founder population during its illegal introduction by private individuals resulting in severe population bottleneck which often occurs when a small group of emigrants is introduced. Inasmuch as *C. gariepinus* was illegally introduced, no one knows the sex ratio between the males and females in the founder population, which nevertheless resulted in a peculiar bottleneck. Half of the alleles in any generation come from each sex, and any sex ratio departure from equality enhances the opportunity for random genetic drift. It should be noted that the African *hito* population assessed in this study was considered pure hatchery stock from a hatchery in Tanay, Rizal Province, where hatchery production used small parental numbers, which can cause genetic variation loss in the population. Hatchery operators based their parental selection on the desired trait wanted to be observed in the progenies, in which the alternate high level allele may be of greater importance to the population's well being in the shorter term.

In within-population genetic variation, the examination by comparing observed genotypic distribution with expected Hardy-Weinberg proportions revealed that *C. batrachus* ($\chi^2=0.22, p>0.01$) conformed to the expected proportions, while *C. macrocephalus* ($\chi^2=14.89, p<0.01$) and *C. gariepinus* ($\chi^2=54.00, p<0.01$) significantly deviated from the expected values (Table 3).

The observed genotypic frequencies deviation from the expected Hardy-Weinberg proportions at *MDH* coding loci in *C. macrocephalus* and *C. gariepinus* populations are ascribed to heterozygote deficit obtained in the samples.

Heterozygote deficit shows either that selection is taking place or

that the sample was a mixture of two or more populations characterized by different allelic frequencies. Also, periodic population bottlenecks and/or founder events resulting from adverse climatic conditions, i.e., drought or *El Niño*, may increase mating probability between relatives, which, in turn, increases the frequency of homozygous individuals and decreases the frequency of heterozygous individuals relative to that which may be expected from random mating, thus leading to genetic variability reduction. It is, therefore, inconceivable that in breeding, which usually results in heterozygote deficit, contributes to the Hardy-Weinberg discrepancies in the *C. macrocephalus* and *C. gariepinus* populations obtained in the present study.

Moreover, Engelbrecht and Mulder, (1999) disclose that Hardy-Weinberg disequilibrium in a population may be attributed to the following factors: natural selection, small effective population sizes, sampling error, self-sorting crossing, founder effects, the Wahlund effect, and even anthropogenic influences.

Genetic variability reduction places constraint on the population's or species' future adaptive potentials and hence survival. As disclosed by Leberg (1990), sufficient genetic variation amount is essential for long-term population and species survival and adaptability, since it enables these species to respond to adverse environmental and biotic occurrence.

The agreement of observed and expected allelic frequencies, such as that exhibited by *C. batrachus* ($\chi^2 = 0.22, p > 0.01$) (Table 3), is the fundamental nature of the Hardy-Weinberg Law and is applied only to equilibrium population, which means that gene pool frequencies are inherently stable. The law states that in a panmictic population (a population is panmictic if individuals can mate at random within it), there is a genotypic equilibrium determined solely by the frequencies of alleles. The simplest derivation of the law is that alleles segregate independently in the formation of gametes; random union occurs between spermatozoa and ovum during fertilization, which occurs during exchange of genetic materials, i.e., DNA, and parents mate at random in the population. Aside from the condition of panmixia, for the Hardy-Weinberg Equilibrium to be observed, there should be no

operation of natural selection, genetic drift, mutation, migration, and the population should be very large (Ferguson, 1980; Purdom' 1993; Carvalho and Pitcher, 1995).

Although populations in equilibrium are rare in nature (perhaps they do not exist), the Hardy-Weinberg Law indicates what happens at a given locus in large random-mating population in the absence of evolutionary change. Conformance to Hardy-Weinberg proportions is important, but is not an absolute criterion for inferring the genetic nature of electrophoretic banding pattern (Storer *et al.*, 1979; Altukhov, 1981).

Genetic Influence on Morphometric Characteristics

The discussion on this portion of the paper focuses on the proportions of genetic influence to the morphometric characteristics expression, which includes total length (TL), standard length (SL), body weight (BW), body girth (BG), body depth (BD), and head length (HL).

The proportion of variation of genetic factor influence to some morphological species characters employed in this study is reflected in Table 4. Accordingly, in determining the influence, the independent/predictor variable represents the species genotypes, which accounts for the variation in the dependent/predicted variable, which was the measured morphometric trait. As shown in the table, the proportion of influence of genetic factors on the measured traits or characters was low and ranged from 0.6% for BD to 37.0% for BG. The remaining large proportion was contributed by a non-genetic factor, the environment.

Table 4. The proportion of genetic factors on some morphometric characters of the species employed in the study

Morphological characters	N	R² (%)
Total Length (cm)	90	8.5%
Standard Length (cm)	90	2.4%
Body Weight (g)	90	11.7%
Body Girth (cm)	90	37.0%
Body Depth (cm)	90	0.6%
Head Length (cm)	90	29.2%

According to Ferguson (1980), the largest source of intra-specific variation is the effect of environmental factors, which can cause temporary, cyclic, or permanent changes in the individual. Many of the morphological features of organism can change during the lifetime of an individual or be determined during development in response to environmental conditions. This assertion was upheld by Allendorf *et al.* (1987) who pointed out that phenotypic variations of organism are largely contributed by environmental factors and that the least part of the variation observed in a population is determined genetically. The greater susceptibility to environmental factors is attributed to the unique physiological characteristics of the fish. First, the indeterminate growth capacity of most fishes permit greater phenotypic adjustment to environmental factors, i.e., food availability and crowding. Second, fish is more sensitive to variations in temperature directly affecting metabolic processes. Third, age and size at sexual maturity are interrelated in fish in a way that permits great flexibility without loss of reproductive success.

Fishes are the product of their genes, the environment, and the interaction between the two (Kapuscinski and Jacobson, 1978). The interaction between the genotypes and environment was distinctly reflected in the study conducted by Monkonsawad (1996) employing XY and YY tilapia (*O. niloticus*) genotypes cultured in ponds (67.67%), cages (48.80%), and tanks (32.80%). The study revealed that the higher growth gain obtained among fishes cultured in pond is ascribed to the type of environment where the fish was cultured.

As indicated by Viola and Arieli (1989), the environment is one of the major factors influencing growth performance. The capacity for growth is genetically determined, but environmental factors determine how much of this potential is actually reached.

In conclusion, the theoretical perspective, which states that genetic variations exist within or even among closely related organisms, is supported by the findings of this study. This is evident in the yielded genetic characterization results, variations in the extent of polymorphism, and within-population genetic variation.

The proportion of variations of the influence of genetic and non-genetic factors on morphometric characteristics showed that non-genetic factors contributed largely on the expression of trait. In conclusion, the perspective that phenotypic variations of organisms is largely contributed by non-genetic (i.e., environmental) factors and that the least part of the variation observed in a population is determined genetically, is supported by the findings of this study.

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