

# Recent approaches of fish breeding

## Abordagens recentes do melhoramento genético de peixes

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### Abstract

This review aims at addressing the main issues related to genetic improvement applied to fish species with zootecnical potential, such as selection, genetic parameters and use of biotechnology as well as address the importance of this area of study for the expansion of aquaculture and its development trend. Fish breeding is one of the most important areas of study for fish production development. Important techniques are applied in order to advance genetic progress in fish breeding (e.g., individual selection, genetic parameters estimation and the use of biotechnology). Traditional breeding in fish farming has been conducted mainly through biometric analysis, which helps in choosing selection criteria and in the assessment of genetic parameters such as heritability and correlations between interesting traits. The use of biotechnology in fish breeding has been successful; it is an important strategy that allows breeding results to be achieved faster, although at a greater cost than traditional breeding. Fish breeding in Brazil is currently in its initial stages; however, it is a field with great growth expectations, and there is still significant research to be conducted in this area.

**Key words:** biometry; biotechnology; carcass; genetic parameters; performance.

### Resumo

Esta revisão tem como objetivo a abordagem dos principais aspectos relacionados ao melhoramento genético aplicado em espécies de peixes com potencial zootécnico, tais como seleção, parâmetros genéticos e utilização da biotecnologia, bem como abordar a importância desta área de estudos para a ampliação da aquicultura e sua tendência de desenvolvimento. O melhoramento genético é uma das áreas

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prioritárias de estudo para o desenvolvimento da produção de peixes. Assim, importantes técnicas são aplicadas visando à obtenção de progresso genético no melhoramento de peixes, como por exemplo, a seleção individual, a estimativa de parâmetros genéticos e o uso da biotecnologia. O melhoramento genético tradicional na piscicultura tem sido realizado, principalmente, por intermédio de análises biométricas, que contribuem para a escolha dos critérios de seleção e para a estimativa de parâmetros genéticos, como a herdabilidade e as correlações. A utilização da biotecnologia no melhoramento de peixes tem sido notável para a piscicultura, a qual é caracterizada como uma importante estratégia que permite que sejam alcançados resultados mais rápidos no melhoramento, embora os seus custos sejam maiores quando comparados aos métodos tradicionais. O melhoramento genético de peixes no Brasil está em uma fase inicial e corresponde a um campo de grande aumento de expectativas, havendo ainda muitos estudos a serem realizados nesta área.

**Palavras-chave:** biometria; biotecnologia; carcaça; desempenho; parâmetros genéticos.

## Introduction

Total continental aquaculture production was about 31.6 million tons in 2006, and it is quickly expanding as the demand for food of aquatic origin has been increasing considerably (FAO, 2008). In aquaculture worldwide, fish represent 50.1% of total production (CAMARGO; POUHEY, 2005).

The farming of aquatic species is a quite recent phenomenon. In recent years, several companies have invested in intensive farming of these organisms. Studies in this field have been conducted mainly with the purpose of increasing environmental control of aquatic organisms' habitats and researching fundamental aspects of biology such as feeding and reproduction (GONZAGA; ZANIBONI FILHO, 2006).

However, domestication of the species used in aquaculture will not be complete until all biological aspects of these animals

are controlled; this includes genetics that, together with animal breeding, are among the priority areas of research and development in fish farming, especially for fish, freshwater prawns and marine shrimps (QUEIROZ et al., 2002).

The tools and study techniques used in fish breeding, such as selection and genetic parameters estimation, are similar to those used for other species, like mammals and poultry. Moreover, some techniques, such as chromosome manipulation and sexual reversion, are easily performed in several species of fish, which might contribute to their breeding (GALL, 1991).

Thus, this review aims at addressing the main issues related to genetic improvement applied to fish species with zootechnical potential, such as selection, genetic parameters and use of biotechnology as well as address the importance of this area of study for the expansion of aquaculture and its development trend.

## Selection and fish breeding

According to Resende et al. (2008), fish breeding might increase productive gain to every generation, which is already considered high compared to other animals. The main benefit, according to these authors, is the increase in the fish growth, which directly influences production cost decrease and fish farmers income improvement.

Thus, one of the tools for increasing production in order to meet the increasing demand for aquatic species is the development of improved fish lineages. One breeding method for doing so is the use of selection. Every improved generation of fish is the basis for the next generation; therefore, the seventh generation of selected fish is estimated to achieve double the growth rate of the original population. Improved lineages are distributed to producers who are then able to produce high quality fry and juveniles to be sold for fattening. In this way, animal breeding is spread to future generations, which results in gains for the productive system (RESENDE et al., 2008). According to Hinrichs et al. (2006), the genetic gain of fish populations might be maximized if genetic evaluation is used, thus obtaining a value that can be used to represent the true genetic merit of individuals; animals are then selected on the basis of this merit.

The genetic selection for native fish such as tilapia (*Tilapia rendalli*) resulted in the rapid spread of improved lines that replaced non-improved lines in Brazil until the end of 1990s (KUBITZA et al., 2007). On the other hand, zootechnical gains in fish are still in their initial phases, and there is much yet to be studied in this area.

Another study using Nile tilapia conducted by Leonhardt et al. (2006) found

that the existence of genetic variability in a population is an initial requirement for applying for selection. Once this genetic variability is confirmed, the families and desired traits of the target population are identified. According to Resende et al. (2008), identification of genetic variability makes it possible to select reproducers by choosing the ones that present the highest growth rate, then using them for consecutive breeding.

Tilapias are disease-resistant individuals that exhibit good adaptability in captivity and reproduce easily. All tilapia producers searching for good reproducers should consider growth speed, resistance to disease and body shape in addition to filet yield due to market demands when aiming for selection (GONÇALVES et al., 2003).

As for carnivorous fish that present a strong tendency for cannibalism, such as pintado (*Pseudoplatystoma corruscans*) and dourado (*Salminus maxillosus*), selection of individuals conditioned on feed intake in some generations might result in lines with less cannibalistic behavior and better feed acceptance. This has been demonstrated in other species of carnivorous fish around the world (KUBITZA et al., 2007). According to the same authors, some producers of juvenile pintado and dourado in Brazil have already noticed differences, such as a decrease in cannibalism among juveniles produced from flocks of domesticated matrix in comparison to flocks of wild matrix.

In Houston et al. (2008) study, progress in easily measurable traits, such as growth of sexual maturity in salmon (*Salmo salar*), was rapid and significant. Studies related to molecular genetics currently aim to increase resistance to diseases like infectious pancreatic necrosis, which is the main disease in this species.

With regard to catfish (*Ictalurus punctatus*), Liu et al. (2003) noted that the existence of numerous families for these fish leads to a great variability of the specie, making it possible to apply phenotypic selection. However, according to the same authors, genotypic selection with molecular markers would be very efficient for identifying those animals with the best traits in terms of performance.

### **Selection for carcass yield in fish**

Evaluation methods for animal productivity that are effective and allow for fast evolution in breeding programs have been researched in an effort to achieve greater efficiency in farm fishing as a zootechnical activity (CREPALDI et al., 2008).

Carcass yield has been one of the main goals of research on increasing efficiency in animal production systems, especially for fishes. Selection programs have made it possible to improve traits of economic interest, not only by searching for animals with better carcass yield, but also by increasing yield in noble cuts, which are more valued in the consumer market. Therefore, several studies have been conducted with the aim of improving this trait (CREPALDI et al., 2008).

Studies related to selection for carcass yield increase in tilapia, according to Santos et al. (2007), can lead to greater interest in breeding these species or lines and serve as a reference for other possible studies on fish breeding. In addition, information obtained from this kind of research might be of importance in determining the ideal weight for slaughtering and the final characterization of the product as well as establishing fish breeding programs.

Different methods of assessing carcass yield are used in animal production, including assessment by weighting, biometry of specific areas such as the loin eye, and techniques that keep the animals alive, like carcass evaluation by ultrasonography (CREPALDI et al., 2008). Moreover, if these techniques are used in the initial selection of a reproducer's flock, it can advance animal breeding programs, according to the same authors, and carcass evaluation of traits is a reliable technique that makes it possible to keep fish with superior zootechnical traits inside the flock. Over many years of breeding systems, catfish have undergone several selection processes in the search for greater carcass yield; ultrasonography has also been used with this specie (BOSWORTH et al., 2001).

According to Reidel et al. (2004), analysis of carcass traits and morphometric features in fish species allow for comparisons to differentiate individual capacity of production and, consequently, the selection process. According to Bosworth et al. (2001), catfish with smaller head, deeper body and thick in the visceral cavity have greater yield based on correlation estimates. The selection for this body shape could, therefore, increase filet yield.

Carcass yield does not depend on the length or height of Nile tilapias at the time of slaughter (GONÇALVES et al., 2003). Only weight and size of filet would be related to carcass yield. According to the same authors, meat production is related more to fish height than to fish length; therefore, taller fish would tend to present a better slaughter weight as well as a higher filet weight.

During a study conducted by Reidel et al. (2004), male curimatás (*Prochilodus lineatus*) were noted to be smaller in body size than females and to present a lower

filet yield. Head length in comparison to body length was bigger for male curimatás than for females, indicating that males have a relatively larger head, which resulted in the reduced yield. Among piavuços (*Leporinus macrocephalus*), another native species analyzed by Reidel et al. (2004), the males were also found to be smaller than females; however, the filet yield for males was higher than that of females. Thus, female curimatás and male piavuços tend to be more likely to obtain better carcass yield when breeding these species.

Although the selection process for fish species is efficient, its productive performance might be affected by environmental factors. Therefore, a favorable fish breeding environment is essential so that animals can express their real genetic potential (MAREGONI et al., 2008). Working with juvenile Nile tilapia, Margoni et al. (2008) showed that increased stock density causes alterations in feed consumption and, consequently, production costs.

Type of feeding can also significantly influence fish production, and performance is frequently affected by the food supplied to animals in production system. Piedras and Pouey (2004) reported that the growth and survival of peixe-rei fry (*Odontesthes bonariensis*) tended to be affected by the kind of feed they were given. Temperature is another important environmental factor in fish development. In a study conducted by Piedras et al. (2004), the performance traits of jundiá fry (*Rhamdia quelen*) were influenced by oscillations in water temperature. Therefore, the environmental conditions to which fish are subjected must be controlled so that the selection process of reproducers and, consequently, their breeding, become more efficient.

## Genetic parameters

Heritability corresponds to the portion of total variation in a trait that is of additive genetic nature, is considered an important genetic parameter for defining more adequate breeding methods (PEREIRA, 1999). The response to selection of individuals is a linear function of the heritability of the trait to be improved (FUNK et al., 2005).

Traits with low heritability, such as survival and fertility in fish, have little additive genetic action, and are therefore more influenced by the environment in which animals are raised (VEHVILÄINEN et al., 2008). These same authors subjected rainbow trout (*Oncorhynchus mykiss*) to two different environments in an attempt to estimate the heritability of survival traits; in both cases, heritability was low, suggesting that selection response to these traits tend to be unfavorable.

Silva (2007), working with Nile tilapias, observed high heritability for performance and carcass traits such as weight and length at 60 and 150 days old, daily weight gain, daily length gain, eviscerated carcass weight and carcass yield. According to this study, these traits tend to respond to selection.

Genetic correlation measures the probability of two different traits being affected by the same genes, i.e., the correlation between the genetic values of two traits (PEREIRA, 1999). Genetic correlations often make it possible for two traits to be improved at the same time (FUNK et al., 2005), most often when they are evaluated in fish of different ages (ROCHA et al., 2002). Therefore, it is essential to understand correlations between traits of interest in a population when studying animal breeding;

it is also important to establish zootechnical rates that can support other studies on selection and breeding (ROMAGOSA et al., 2003).

Estimates of genetic correlations between weight, body length, body height and head length ranged from moderate to high for most associations at different developmental stages of common carp (*Cyprinus carpio*). Therefore, when selecting for one of these traits, the others will also benefit, according to Rocha et al. (2002). Genetic correlations in Nile tilapia regarding the live weight and length of fish of different ages as well as carcass weight were also high, according to Silva (2007), making it clear that indirect selection for performance and carcass traits in these species is possible.

## **Biotechnologies used in fish breeding**

Research in the field of biotechnology in fish farming is of great importance, according to Igarashi and Magalhães Neto (2001). Progress in fish breeding has been the subject of many discussions and reviews throughout the last decade (LEONHARDT et al., 2006). The main goal of this research has been to increase the number of new lines through the development of modern technologies.

With advances in molecular genetics and genetically modified organism techniques, it has become possible to obtain genetically modified individuals, i.e., organisms that have had genes from another organism inserted in their genome and then transferred to their descendents (ARAGÃO, 2003). Genetically modified organisms often have advantages over other non-improved individuals. For example, it is possible to increase the survival

time in salmon through genetic modification. However, it is necessary to take great care to avoid releasing these genetically modified fish into the environment, as it could cause an ecological unbalance in the local populations (HOWARD et al., 2004).

According to Rexroad et al. (2008), rainbow trout have achieved great importance in aquaculture as well as in fishing sports. Genome mapping in this specie has made it possible to identify important genes that affect traits of economic interest.

Quinn et al. (2008) report that sequencing the salmon genome makes it possible to better understand the genetics of this fish, which is a species of great economic interest that has an important role in aquatic ecology when in its native habitat. Resistance to disease has also been studied in fish breeding. Several species, like salmon, have their production damaged because they are vulnerable to some disease. Therefore, alternatives have been researched through genetic mapping to identify individuals that might present this trait (MOEN et al., 2004).

Moreira et al. (2007) conducted a study that evaluated the genetic variability of two varieties of Nile tilapia (*Oreochromis niloticus*), Chitralada and Red Stirling, and their progeny, which were subjected to breeding on intensive raising systems using microsatellite markers. This study used DNA extraction and amplification by PCR (Polimerase Chain Reaction) to determine that the variability between these two Nile tilapia strains was high and that heterosis was able to improve zootechnical performance of progenies. Knowledge about DNA markers has evolved beyond the experimental phase and been incorporated into aquaculture in a practical and efficient way, according to Leonhardt et al. (2006).

Silva et al. (2011) used F1 generations of silver catfish of the genus *Rhamdia*, for a study to identify individuals with triploid cytogenetic tools, showing that in the analyzed species occurred a high number of triploid individuals, 46.8%. Taking into account that the triploid species have partial or total sterility, this study highlights the importance of cytogenetic monitoring in repopulating programs aimed at genetic conservation.

Catfish production is subject to a constant breeding process for trait selection as much as for molecular genetics techniques (QUINIOU et al., 2007) and is an excellent candidate for genomic studies (LIU et al., 2003). According to Cereali et al. (2008), the biggest families of catfish are of great interest in cytogenetic studies due to significant variations in chromosome number among the species in these families, as well as the rearrangement of chromosomes, which suggests an intense karyotypic evolution. The catfish genome has regions that control important production traits (QUINIOU et al., 2007). Among these are growth rate, food conversion, resistance to disease, body shape and filet yield, and tolerance for low oxygen and low quality water (LIU et al., 2003). Thus, with complete construction of the genome, it will be possible to locate these regions and search for new ways to improve this specie.

Thus, Odegard et al. (2011) reported that breeding, using tools of genomic analysis, is essential for achieving genetic progress in fish species. In order to improve these individuals productivity is necessary to understand their genomic structures, so that it becomes possible to extend the methods of genetic selection and identification of molecular markers associated with production traits (KANG et al., 2008).

According to Hemmer-Hansen et al. (2011), the new sequencing technologies have revolutionized the discovery of genes and polymorphisms throughout the genome of the species. Quinn et al. (2008) report that by sequencing the genome of fish is possible to obtain better results in the genetic understanding of these species.

An important tool for genomic studies is the use of candidate genes, which consist of genes from known biological function and are involved in the development or physiology a particular characteristic (PEIXOTO et al., 2009). Permits the link between genotype and biological function, extending the observation of genetic variation (HEMMER-HANSEN et al., 2011). A major effort has been applied on the isolation and analysis of genes of interest in several species of fish, including salmon, trout, halibut (KANG et al., 2002) and tilapia (BLANCK et al., 2009).

As a result of the increase of technology in the biological area, especially regarding to genomic sequencing, a large individual genetic variation can be seen established. The variations that occur with greater frequency in the genome are highlighted as single nucleotide polymorphisms or SNPs, which consist of mutations point created in different alleles (KONGCHUM et al., 2010). For its part, these regions throughout these individual's genome are abundant molecular markers and have been receiving great attention in recent studies (KITAOKA et al., 2010).

Many of these polymorphisms are associated with productive traits of economic importance. According to Blanck et al. (2009) study of polymorphisms in candidate genes has contributed information that can be applied as selection criteria in breeding programs of species, especially aquaculture.

This practice aims to identify molecular markers that can be used in selection programs for improvement of traits difficult to measure (NINOV et al., 2007). Similarly, studies based on the identification of markers can be applied to the detection of variability between strains and association with the phenotype (MARKLIUND; CARLBORG, 2010), and such studies potentiate more genomic research (GROENEN et al. 2008).

The identification of polymorphisms can serve as a starting point for marker assisted selection (MAS), a very important tool in today's scientific scene (KITAOKA et al., 2010). In modern animal breeding programs it is used the association of a marker gene or a set of genes, so the selection for this marker is more efficient than for the very characteristic (JANGARELLI et al., 2011).

Several studies have shown the importance of identifying polymorphisms in genes of fishes. Blanck et al. (2009), by working with Nile tilapia, have identified a significant correlation between growth hormones' gene polymorphisms and body characteristics. Likewise, Kocour and Kohlmann (2011), by studying *Tinca tinca* L., have identified polymorphisms in genes of this species. Kang et al. (2002) pointed out that there are variations in genes and their association with body weight in halibuts. In this same segment, further investigations have been conducted with some genes that affect growth characteristics, such as the genes of prolactin, growth factor similar to insulin and myostatin.

Other studies aimed at broadening the understanding of the function of some important aquatic organisms' genes have used the post-transcriptional silencing of genes, known as RNA interference or RNAi, which

consists of a dsRNA that links to a target sequence promoting their degradation and blocking the expression (ESTRADA et al. 2007). Thus, some researchers have shown the use of RNAi as an important tool for studies. Estrada et al. (2007) evaluated the biological function of the myostatin gene in aquatic organisms, noting that the gene silencing, promoted by RNAi, results in an increase in muscle, since myostatin is involved in muscle deposition. Lee et al. (2009) also used this methodology to study the suppression of the myostatin gene in transgenic zebrafish, where the fish had a double muscling phenotype. These results may contribute to a breakthrough for aquaculture if they achieve commercially important species.

Triploidization can also be used to improve fish production. This technique consists of applying a shock (heat, pressure or chemical) to a fertilized fish egg before the second polar body extrusion, blocking effectively meiosis II (JOHNSON et al., 2007). As a consequence, the fertilized egg has three haploid nuclei: one from the egg, one from the sperm and another from the second polar body and the three haploid nuclei fuse to form a triploid zygotic nucleus. The techniques for obtaining triploid fish have two main goals: growth improvement and sterility. Triploid fish have been raised in several species such as tilapia, catfish, trout and salmon (MOREIRA, 2001).

Obtaining triploid fish allows for stabilization in fish production (JOHNSON et al., 2007). According to the same authors, triploidization increases phenotypical variation in growth and resistance traits in salmon.

For better understanding of the cell events related to fish production, cytogenetics should be applied to fish breeding. According to Cruz et al. (2001), cytogenetics is a

specialized area of genetics related to chromosomes and their association with genetic and phenotypic events.

Many studies on the hybridization of species and sexual reversion have been performed in fish, contributing to a better understanding of the mechanisms of sexual determination, especially in Nile and other species of tilapia (LEONHARDT et al., 2006).

According to Boscolo et al. (2001), interest in tilapia farming has grown over the last eight years due to the use of sexual reversion technology, which consists of developing single sex populations to prevent fish reproduction in captivity. This technique is carried out by using feed with masculinizing hormones in new-born progenies until three to four weeks of age. The advantages of this technique include preventing energetic waste with reproductive activity, animal uniformity and reduction of sexual maturity effects in meat quality, among others (CESAR et al., 2009).

Hybridization consists of crossing species using pure lines (RIBEIRO, 2001). Hybrid formation is potentially interesting in the evolution and reproduction of fish, as it has some advantages, such as rapid growth and greater resistance to diseases (LIU et al., 2007).

Characterization of genetic variability in flocks is important for establishing a breeding program. When conducting breeding in a population through traditional or molecular techniques, great initial genetic variability assures the existence of enough variability to achieve the desired improvements in successive generations (RESENDE et al., 2008).

With regard to the genetic characterization of tilapia, there have been

losses in the heterozygosity of flocks according to Melo et al. (2006). This was supported by analyses conducted by these authors on six flocks of tilapia carried out in southeast Brazil; the Israel and Nile flocks were the most genetically similar, while the Chitralada and Taiwan flocks had the fewest genes in common. As Red tilapias (*Oreochromis* sp) are hybrid, they were the most genetically distinct of the six flocks.

Genetic variability reduction might occur in a single generation and therefore resistances to diseases as well as the capacity to adapt to new environments were also reduced because of reproductive management of fish, according to Lopes et al. (2008).

According to Carneiro (2007), problems in aquaculture, such as inbreeding, might occur as a result of successive crossing that selects genes of specific interest through inbreeding, thus reducing genetic diversity. Building up a genetic data bank with frozen fish semen from several males can be used to fertilize eggs from females, significantly decreasing the risks of inbreeding without the need to maintain a large flock of reproducers. Carneiro (2007) further notes that this genetic material could also be used to recover fish population.

Genetic studies conducted with preserved material from a frozen sperm bank might help decrease the fishing stock limitations of different species by allowing them to adjust of fishing in a region (RESENDE et al., 2005).

Techniques for inducing reproduction in fish using hormones have become essential in animal breeding for several species, according to Zaniboni Filho and Weingartner (2007). Hypophysation may be used for artificial procreation of fish, as it places special emphasis on efficiency,

practicability and economy. This process consists of using collected fresh or preserved pituitary glands from donor fish that are then used to provoke final maturity of gametes in reproducers (FURUYA; FURUYA, 2001).

Application of this process requires selection of reproducers, which is considered the most important phase of the egg-laying process. The criteria for this process are based on subjective traits, such as females with expanded soft abdomens and swollen and reddish genital papilla (ZANIBONI FILHO; WEINGARTNER, 2007).

## Conclusion

Traditional breeding in fish farming has been conducted mainly through biometric analysis, which helps in choosing selection criteria, and assessment of genetic parameter

such as heritability and correlations. Practices for fish selection are often performed by the producers themselves and do not always achieve the desired results. Tilapia is the most farmed and studied specie in Brazil due to its adaptability to captivity.

The use of biotechnology in fish breeding has been successful; it is an important strategy that allows breeding results to be achieved faster, although at a greater cost than traditional breeding.

Fish breeding is still in its early stages, but it has become a field with strong growth expectations. There is still significant research to be conducted in this area beyond animal breeding and the different kinds of technologies that have been used in production. These studies have become essential for continue to advance the field and search for genetic progress in species.

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