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Genetic Conservation of Brazilian Fishes - Present State and Perspectives

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Abstract

Wasko AP, Martins C, Oliveira C, Foresti F. Genetic Conservation of Brazilian Fishes - Present State and Perspectives. ARBS Ann Ver Biomed Sci 2004;6:79-90. Natural environments have been worldwide affected by the growing impact of anthropogenic actions that promote the reduction or the extinction of several vertebrate species. Aquatic ecosystems represent one of the most affected environments and many fish species and/or populations have been increasingly fragmented distributed due to habitat degradation, predatory fishing, introduction of exotic species, river sedimentation, deforestation, pollution, reduction of food resource, and construction of hydroelectric dams. Actually, more than 150 Brazilian fish species, including freshwater, estuary and coastal species, can be considered threatened. Information on the diversity, conservation biology and population analysis on threatened species or populations, with several DNA markers, can be extremely useful for the success of fish species-recovery and maintenance programs. Although DNA analysis in Neotropical fish species are just beginning, they tend to increase with the widespread attention to the use of molecular approaches to minimize problems related to the risk of extinction. The accumulation of information on biology and pattern of genetic variation of fish species, associated with ecological and demographic data, and also education and respect to the nature, constitutes a crucial task to develop efficient conservation strategies in order to preserve the genetic diversity in aquatic environments.
Key-words: conservation genetics, endangered species, fish, molecular markers

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Introduction

Natural environments have been worldwide affected by the growing impact of anthropogenic actions, leading to the destruction, fragmentation, and changes of natural environments, promoting the reduction or the extinction of several organisms (Wilson, 1988; Erlich, 1988; Avise, 1996). In recent years, a great number of vertebrate species have been designated at risk or are even extinct. Fishes comprehend a particularly threatened vertebrate taxon, with many species and/or populations becoming increasingly fragmented distributed.

About 77% of the Earth surface is covered by water. Considering the total water volume, 97.5% correspond to saltwater and only 2.5% correspond to freshwater. It is important to note that 68.9% of the freshwater represent the polar ice cap, glaciers and the permanent snow that cover the top of high mountains (Rebouças, 1999). Thus, the men have heavily exploited a very small amount of the freshwater. On the other hand, the aquatic ecosystems represent one of the most affected environments and the reduction/extinction of several fish species or populations have been related especially to habitat degradation, predatory fishing, introduction of exotic species, river sedimentation, deforestation, pollution, reduction of food resources, and construction of hydroelectric dams.

Several fish species contribute significantly to food production and the fishery industry represents at least 15% of the animal protein consumed in the world (FAO, 1998). However, despite the great economic importance of this animal group and the risk of extinction, little is known about the genetic diversity of natural populations, even though such information may be critical to their survival and conservation.

The careful selection of appropriate natural stocks, based on genetic criteria, can offer greater potential for success in species-recovery and maintenance programs (Quattro & Vrijenhoek, 1989). DNA-based studies on threatened animals have been one of the major interests in gathering information on the diversity, conservation biology and population analysis (O'Brien, 1994; Avise, 1996; Snow & Parker, 1998) in order to define priorities to the management of threatened species or populations (Moritz, 1994), to develop demographic models of small or fragmented populations (Lacy & Lindenmayer, 1995), and to analyze the fitness value of natural or captive populations (Nunney & Campbell, 1993; Lynch, 1996).

The purpose of this review is to present the current status of threatened Brazilian fish species, different conservation strategies, and the application of methodologies to access genetic variability of several species and populations, and also to present future perspectives to minimize the problems of the risk of extinction of these animals. Moreover, some examples of recently developed genetic markers that have been applied to conservation and population genetics management studies on Brazilian fish were also included.

Endangered Fish Species in Brazil

More than half of the vertebrates (51.1%) is represented by fish species, *i.e.*, around 24,618 species (Nelson, 1994). Although the number of fish species in Brazil is unknown, some authors have suggested that could exist 8,000 Neotropical freshwater species (Schaefer, 1998; Vari & Malabarba, 1998) and about 1,000 marine species. In the last two decades, about 700 new freshwater species were described (Vari & Malabarba, 1998), which represents about 40 new species by year!

It is estimated that 4% of the known fish species (around 979 species) are in risk of extinction. On South America, the region that presents the richest freshwater fish fauna of the world, it is settled that 0.4% of the fish species are already endangered. By the end of the 20th century, at least 78 fish species have been considered endangered in Brazil, specially due to deforestation and pollution of rivers and oceans (Rosa & Menezes, 1996). However, these data seem to be underestimated.

National and international agencies are generally responsible for assigning risk or threat levels to potentially endangered taxa and for estimating the number of species under each category. These levels are: probably extinct, extinct in natural environments, critically in danger, in danger, vulnerable and presumable threatened. The last official list of endangered animals from IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) was presented in 2003. However, it did not include any fish species and, although several other non-governmental lists have been available in the last years, these were generally restricted to a specific region and were mostly incomplete. Anyway, these lists are useful to suggest how critical should be the total number of endangered fish in Brazil. Examples of these lists are given in Tables 1 and 2 that comprehend several threatened fish of the São Paulo state, including even aquarium species, as *Holacanthus ciliaris* (queen angelfish) and *Holacanthus tricolor* (rock beauty), and also species of great economic importance as *Pristis pectinata* (smalltooth sawfish), *Pristis perotteti* (largetooth sawfish) and *Brycon insignis* (Tietê tetra).

Actually, it is assumed that 152 Brazilian fish, including freshwater, estuary and coastal species, can be considered threatened, and that 4 species are already extinct (Ricardo Rosa - Universidade Federal da Paraíba, personal

Table 1: Threatened fish species of the São Paulo state (Brazil).

Species	Classification	Common name
Heteroconger longissimus	Osteichthyes/Anguilliformes/Congridae	enguia-de-jardim (Atlantic garden eel)
Cetorhinus maximus	Chondrichthyes/Lamniformes/Cethorhinidae	tubarão-filtrador (basking shark)
Carcharodon carcharias	Chondrichthyes/Lamniformes/Lamnidae	tubarão-branco (great white shark)
Manta birostris	Chondrichthyes/Myliobatiformes/Mobulidae	jamanta (manta ray)
Mobula hypostoma	Chondrichthyes/Myliobatiformes/Mobulidae	jamanta (devil ray/ lesser devil ray)
Ginglymostoma cirratum	Chondrichthyes/Orectolobiformes/Ginglymostomatidae	cação-lixa (nurse shark)
Rhincodon typus	Chondrichthyes/Orectolobiformes/Rhincodontidae	tubarão-baleia (whale shark)
Pristis pectinata	Chondrichthyes/Pristiformes/Pristidae	peixe-serra (smalltooth sawfish)
Pristis perotteti	Chondrichthyes/Pristiformes/Pristidae	peixe-serra (largetooth sawfish)
Spintherobolus papilliferus	Osteichthyes/Characiformes/Characidae/Cheirodontinae	
Glandulocauda melanogenys	Osteichthyes/Characiformes/Characidae/Glandulocaudinae	
Mimagoniates lateralis	Osteichthyes/Characiformes/Characidae/Glandulocaudinae	(croaking tetra)
Coptobrycon bilineatus	Osteichthyes/Characiformes/Characidae/Tetragonopterinae	
Hyphesobrycon duragenys	Osteichthyes/Characiformes/Characidae/Tetragonopterinae	
Hyphesobrycon melanopleurus	Osteichthyes/Characiformes/Characidae/Tetragonopterinae	
Phallotorhynus fasciolatus	Osteichthyes/Cyprinodontiformes/Poeciliidae	guaru/barrigudinho
Phallotorhynus jucundus	Osteichthyes/Cyprinodontiformes/Poeciliidae	guaru/barrigudinho
Campellolebias dorsimaculatus	Osteichthyes/Cyprinodontiformes/Rivulidae	
Leptolebias aureoguttatus	Osteichthyes/Cyprinodontiformes/Rivulidae	
Chaetodon sedentarius	Osteichthyes/Perciformes/Chaetodontidae	peixe-borboleta (reef butterfly fish)
Elacatinus figaro	Osteichthyes/Perciformes/Gobiidae	neon (golden goby)
Ptereleotris helenae	Osteichthyes/Perciformes/Microdesmidae	(hovering goby)
Gramma sp.	Osteichthyes/Perciformes/Grammatidae	grama (gramma)
Centropyge aurantonotus	Osteichthyes/Perciformes/Pomacanthidae	peixe-anjo anão (flameback angel)
Holacanthus ciliaris	Osteichthyes/Perciformes/Pomacanthidae	ciliaris (queen angelfish)
Holacanthus tricolor	Osteichthyes/Perciformes/Pomacanthidae	tricolor (rock beauty)
Epinephelus itajara	Osteichthyes/Perciformes/Serranidae	mero (jewfish)
Heptaterus multiradiatus	Osteichthyes/Siluriformes/Pimelodidae	
Pimelodella kronei	Osteichthyes/Siluriformes/Pimelodidae	bagre cego (blind catfish)
Pimelodella sp.	Osteichthyes/Siluriformes/Pimelodidae	bagre cego blind catfish)
Trichogenes longipinnis	Osteichthyes/Siluriformes/Trichomycteridae	
Trichomycterus paolencis	Osteichthyes/Siluriformes/Trichomycteridae	
Hippocampus erectus	Osteichthyes/Syngnathiformes/Syngnathidae	cavalo marinho (lined seahorse)

Source: Programa Estadual para a Conservação da Biodiversidade - PROBIO/SP (1998) - Secretaria do Meio Ambiente - Governo do Estado de São Paulo, Brazil.

Table 2: Probable threatened fish species of the São Paulo state (Brazil).

Species	Classification	Common name
Plectrypops retrospinnis	Osteichthyes/Beryciformes/Holocentridae	cardinal (cardinal soldierfish)
Carcharhinus limbatus	Chondrichthyes/Carcharhiniformes/Carcharhinidae	tubarão galha-preta (blacktip shark)
Carcharhinus maou	Chondrichthyes/Carcharhiniformes/Carcharhinidae	tubarão galha-branca (whitetip shark)
Carcharhinus obscurus	Chondrichthyes/Carcharhiniformes/Carcharhinidae	tubarão fidalgo (dusky shark)
Carcharhinus plumbeus	Chondrichthyes/Carcharhiniformes/Carcharhinidae	tubarão galhudo (sandbar shark)
Carcharhinus signatus	Chondrichthyes/Carcharhiniformes/Carcharhinidae	cação-baía, tubarão-tuninha (night shark)
Prionace glauca	Chondrichthyes/Carcharhiniformes/Carcharhinidae	tubarão azul (blue shark)
Carcharias taurus	Chondrichthyes/Lamniformes/Odontaspididae	mangona (grey nurse shark)
Rhinobatos horkelii	Chondrichthyes/Rhinobatiformes/Rhinobatidae	raia-viola (guitarfish)
Squatina guggenheim	Chondrichthyes/Squatiniformes/Squatinidae	cação-anjo (angular angel shark)
Oligobrycon microstomus	Osteichthyes/Characiformes/Characidae	
Rachoviscus crassiceps	Osteichthyes/Characiformes/Rachoviscus	tetra (golden tetra)
Catabasis acuminatus (*Brycon insignis*)	Osteichthyes/Characiformes/Characidae	piabanha (Tietê tetra)
Pseudocorinopoma heterandria	Osteichthyes/Characiformes/Characidae/Glandulocaudinae	
Sardinella brasiliensis	Osteichthyes/Clupeiformes/Clupeidae	sardinha verdadeira (Brazilian sardine)
Lutjanus analis	Osteichthyes/Perciformes/Lutjanidae	caranho-vermelho (mutton snapper)
Rachycentron canadum	Osteichthyes/Perciformes/Rachycentridae	beijupirá (cobia)
Cephalopholis fulva	Osteichthyes/Perciformes/Serranidae	catuá (coney)
Epinephelus nigritus	Osteichthyes/Perciformes/Serranidae	garoupa (warsaw grouper)
Epinephelus niveatus	Osteichthyes/Perciformes/Serranidae	cherne/garoupa (snowy grouper)
Mycteroperca microlepis	Osteichthyes/Perciformes/Serranidae	badejo-da-areia (gag grouper)
Neoplecostomus paranaensis	Osteichthyes/Siluriformes/Loricariidae	cascudo
Neoplecostomus ribeirensis	Osteichthyes/Siluriformes/Loricariidae	cascudo
Pseudotocinclus tietensis	Osteichthyes/Siluriformes/Loricariidae	cascudo
Rineloricaria pentamaculata	Osteichthyes/Siluriformes/Loricariidae	cascudo (whiptail catfish)
Pimelodella meeki	Osteichthyes/Siluriformes/Pimelodidae	bagre
Steidachneridion parahybae	Osteichthyes/Siluriformes/Pimelodidae	surubim
Balistes vetula	Osteichthyes/Tetraodontiformes/Balistidae	peixe-porco/cangulo-rei (queen triggerfish)

Source: Programa Estadual para a Conservação da Biodiversidade - PROBIO/SP (1998) - Secretaria do Meio Ambiente - Governo do Estado de São Paulo, Brazil.

communication). The inclusion of fish species at a new official list of endangered animals from the Brazilian fauna would offer more subsidies to conservation programs as this information is fundamental_to give support to the biodiversity maintenance and recovery efforts of the species.

Choosing Stocks and Biological Materials for Conservation Purposes

Conservation biology is mainly based on population studies that aim for the definition of unities and priorities to be used on strategies of natural or captive stocks management (Lacy & Lindenmayer, 1995). These data can contribute to the definition of priority regions for the establishment of areas of high diversity or of endemism, field management of endangered populations, and planning strategies of maintenance of captive breeding (Seal, 1988; O'Brien & Mayr, 1991; Rojas, 1995). Moreover, data on species recovery priorities can be used for the identification and/or creation of distinct gene banks - *in situ*, *ex situ* and *in vitro* - as proposed by Toledo Filho *et al.* (1992) (Table 3).

While *in situ* gene banks regards to populations that did not suffer great impacts and are maintained in natural environments, *ex situ* and *in vivo* gene banks should be created in order to minimize problems generated by disturbed environments. Great or irreversible environmental alterations lead to a perspective of hatchery management of the affected fish species. In this case, it is necessary to select individuals that represent the genetic diversity present in natural populations in order to perform a future establishment of gene flow between captive stocks and natural populations (Ryder, 1986; Seal, 1988). As so, the correct selection of the

Table 3: Characterization of distinct gene banks.

Gene bank	Purpose	Material	Local
wild (*in situ*)	preservation of the genetic diversity of wild populations and native species on natural (wild) environments	wild populations	protected natural areas (parks or refuges)
cultivated (*ex situ*)	preservation of the genetic diversity of wild populations and native species in hatchery conditions	wild breeders (P) and descendents (F1, F2 and F3 generations)	governmental stations and/or particular fishery farms
preserved (*in vitro*)	preservation of the genetic diversity of wild or captive populations using several biomaterials	DNA, gametes, embryos, tissue culture, museum collections	universities, museums, governmental research stations, private fishery laboratories

According to Toledo Filho *et al.* (1992).

stocks that would be used for this purpose is of capital importance.

Toledo Filho *et al.* (1992) proposed a description and characterization of each of the stocks generally used on conservation programs that aim for future reintroduction on natural environments. Their proposal is given below.

1. Donor stock that corresponds to wild native or non-native populations, hatchery populations, or hybrid populations originated by wild x hatchery populations. At this level, particular attention should be given to several characteristics, as spawning season, genetic, morphological and ethological features, isolation level, ecological specialization, and local adaptation in order to detect population subdivision.
2. Founder stock that corresponds to a sample obtained from the donor stock that will be used as broodstocks. Particular attention should be given to the minimum number of the sample (it is usually suggested at least 25 males and 25 females) in order to avoid lower levels of heterozigosity and loss of rare alleles.
3. Reproductive stock that corresponds to a sample of the founder stock effectively used as breeders. Particular attention should be given to the effective number of breeding animals and to monitor crossings between genetically non-related individuals in order to avoid higher levels of consanguinity and genetic drift and to detect the levels of surviving, growth, and fertility.
4. Resettlement stock that corresponds to the individuals from the F_1 generation produced from the reproductive stock and that will be used for reintroduction.
5. Receiver stock that corresponds to the resident population that will receive the cultivated animals. Particular attention should be given to the monitoring of the natural and reintroduced stocks in order to analyze the adaptation of these animals to the environment and to detect competition, predation, introduction of parasites and possible hybridization.

Although particular attention should be given to all these parameters, few studies using genetic markers have been developed in order to detect several problems that occur on fishery programs that aim for future reintroduction on natural environments (Arias *et al.*, 1995; Meffe, 1995; Cross, 2000; Hansen *et al.*, 2001).

Molecular Markers and Genetic Conservation

One of the main purposes of managing and conservation programs of endangered species is the maintenance of the better quantity of genetic variation. As so, the original genetic variation present in natural populations has to be identified and used in conservation programs. Additionally, to evaluate the success of a breeding program and to improve its efficiency, it is necessary to estimate the level of genetic variation of captive stocks and compare it to the genetic variability found in natural populations.

Morphogenetic markers, generally associated to phenotypic characters, and biochemical or enzymatic markers, associated to multiple molecular forms of proteins, represent the initial approaches that were used to analyze characteristics that reflect the genetic composition of fish stocks. The simplicity and the low cost of the electrophoretic analysis made them one of the tools that were greatly used for the genetic characterization of fish species and/or populations (May & Krueger, 1990). Several studies using these genetic markers were performed in Brazilian fish (Galhardo & Toledo Filho, 1987; Galhardo, 1989; Calcagnotto, 1993; Revaldaves *et al.*, 1997; Beheregaray & Levy, 2000; Calcagnotto & Toledo, 2000). Cytogenetic markers also can be successfully used on population genetics and fishery management (Ryman & Utter, 1987) and have been applied in Brazilian fish conservation studies, especially to identify species and hybrids and to determine population subdivision (Almeida-Toledo *et al.*, 1987; Ramirez-Gil *et al.*, 1998).

More recently, molecular markers of the nuclear genome (nDNA), together with mitochondrial DNA (mtDNA) patterns, have been considered potentially important tools in studies related to fish population structure, as they can better provide the detection of polymorphism and secure information about the variability and similarity levels among distinct populations (Ferris & Berg, 1987; Avise, 1994; Carvalho & Pitcher, 1995). The detection of nuclear genome variability began with the use of restriction enzymes, through analysis of restriction DNA length fragment polymorphism (RFLP) (Grodzicker *et al.*, 1974). Later, with the advent of the PCR (Polymerase Chain Reaction) (Mullis & Faloona, 1987; Saiki *et al.*, 1988), several other molecular markers were available, including distinct repetitive DNA sequences as the minisatellites (Jeffreys *et al.*, 1985) and the microsatellites or SSR (Simple Sequence Repeats) (Litt & Luty, 1989), and random amplified polymorphic DNA (RAPD) (Welsh & McClelland, 1990; Williams *et al.*, 1990). Microsatellites and RAPD markers have been routinely used on distinct fish species as they have favorable characteristics to conservation and genetic diversity studies - they are abundant and present high levels of polymorphism.

These molecular technologies have opened a new chapter in fish species conservation efforts, and genetic markers have been used in several studies involving the identification and characterization of species, populations or hybrids, identification of parentage, identification of founders on new populations, estimation of gene flow, determination of population structure, determination of the effective population size, lineage identification, determination of genetic variability on wild and captive populations, identification of homing fidelity, evaluation of the genetic impact of the introduction of cultivated fish on natural populations, determination of reproductive strategies to the cultivation and restocking, and identification of genetic markers associated to economic characteristics (Carvalho & Hauser, 1998).

Conservation Genetics of Brazilian Fish

The declining and extinction of several fish species have prompted governmental agencies and scientists to restore degraded environments, conducting researches in ecology and developing propagation programs for species perpetuation and reintroduction. However, to date, almost all the conservation studies using molecular markers are restricted to the North American and European fish fauna (Ferguson *et al.*, 1995; Vrijenhoek, 1998). Although these analyses in Neotropical fish species are just beginning, they tend to increase with the widespread attention to the application of molecular approaches to minimize problems related to the risk of extinction. The gain of information on biology and pattern of genetic variation of fish species, together with ecological and demographic data, can be strongly important to develop efficient conservation strategies.

Some examples of how molecular markers have been used in population and conservation studies with distinct Brazilian fish species are given below.

The São Francisco River Example

The construction of dams is one of the major factors that contribute to the decline of fish populations since it promotes drastic changes in the aquatic ecosystems (Gup, 1994). Stocks of *Brycon lundii* and *Prochilodus marggravii*, two of the most important commercial migratory fish species that occurs in the São Francisco River basin, have been declining and are in risk of extinction as a consequence of the intensive deforestation and construction of hydroelectric dams. It has been observed that several specimens of these two species collected downstream the Três Marias dam (Minas Gerais State) in the Medium São Francisco River system, have smaller size and immature gonads during the spawning season, which can be related to less favorable environmental conditions to their reproduction. A distinct condition has been observed 30 Km downstream the dam in a less disturbed environment, where these animals generally have normal size and developed gonads (Sato *et al.*, 1995).

Molecular analysis using RAPD markers suggested the occurrence of population structuring in *Brycon lundii* and in *Prochilodus marggravii* - individuals of each species collected closest to the hydroelectric dam seem to represent an unique stock and the animals from the second area could comprehend at least two co-occurring populations that have genetic differences and a co-migrating behavior during the spawning season. Moreover, the correlation between ecological and genetic data seems to indicate that the proposed population structuring scenery of each species could be related to a competition for resources defense or to homing behavior and reproductive site fidelity (Hatanaka, 2001; Wasko & Galetti, 2002). These data were extremely useful to define different unities of *B. lundii* and *P. marggravii* of the São Francisco River system to be used in reproductive and restocking programs, which gives greater support to the biodiversity maintenance and recovery efforts of these Neotropical fish species.

The Paraná-Paraguai River Basin Example

The decline or extinction of several fish species in the Upper Paraná River system has been associated to the interruption in their migratory routes caused by several hydroelectric dams that have been built along the basin during the last decades (Gomes, 1999) since most of them represent an effective obstacle for migratory movements. The decrease in the number of individuals of the migratory fish *Prochilodus lineatus*, observed in the last decade (Agostinho *et al.*, 1999), indicate the urgent necessity of a more effective biological monitoring, including data on the genetic structure of the species in order to maintain its genetic variability.

Mitochondrial DNA sequencing and RAPD analysis evidenced that distinct stocks of *Prochilodus lineatus* from the Paraná River basin do not present population subdivisions, since the restriction in the movement of this migratory fish due to the hydroelectric dams did not seem to have substantially changed its DNA composition. However, a genetic heterogeneity was detected among the analyzed wild populations, which has to be considered in hatchery programs - the Miranda River stock had a greater sequence variation in fragments of mitochondrial DNA genes and also a distinct frequency of some RAPD fragments, which suggests the preference choice for individuals of this wild stock to compose founder stocks in fisheries and conservation projects in the surroundings areas (Revaldaves, 2001).

The Pantanal and Amazon River Basin Examples

The genetic diversity within and among native populations of two commercially important fish species - pacu (*Piaractus mesopotamicus*) from the Pantanal region and tambaqui (*Colossoma macropomum*) from the Amazon region - was identified and characterized, using restriction fragment analysis of mitochondrial DNA. A low genetic variability level was detected among native stocks of each species. The hypothesis to explain this scenario is based mainly upon the Pantanal and the Amazon geological history and it was suggested that the genetic uniformity found in *P. mesopotamicus* and *C. macropomum* fall into the phylogenetic category IV proposed by Avise (1989), in which populations present intensive gene exchange and do not present subdivisions caused by historic or recent geographic barriers (Calcagnotto, 1998).

From the conservation genetic point of view, it is advisable that, in order to compose *ex situ* gene banks, the stocks of *P. mesopotamicus* and *C. macropomum* from different rivers of Pantanal and Amazon, respectively, should be stocked in different tanks to preserve its historical and biological adaptations to the different habitats (Calcagnotto, 1998).

The Paraná River Basin – Guaíra Falls Example

The Guaíra Falls were considered a geographic barrier that separated two distinct areas in the Paraná River - the Upper Paraná and the Parano-Platense provinces (Bonetto, 1986) - until 1982, when the hydroelectric dam of Itaipu was built. However, it is possible that these falls did not represent an absolute barrier for the dispersion of migratory fish since some agile species, as *Prochilodus lineatus*, *Salminus maxillosus* and *Leporinus elongatus*, could be capable of overcome this barrier during periods of exceptional floods (Agostinho *et al.*, 1997). On the other hand, even in these occasions, it seems difficult that Siluriformes species, such as *Pseudoplatystoma corruscans*, could climb the barrier of Guaíra Falls (Godinho *et al.*, 1991). Population mixture on this species could be related to unidirectional migration and descent of adult individuals, eggs and larvae at the Guaíra Falls.

Results of RAPD analysis on samples of *Pseudoplatystoma corruscans* from three distinct regions that were influenced by Guaíra Falls - Upper Paraná River floodplain (200 Km upstream the Guaíra Falls, at the Upper Paraná province), Itaipu reservoir, and downstream the Yacyretá reservoir (at the Parano-Platense province) - indicated the existence of genetic differentiation among them, suggesting that the Guaíra Falls isolated reproductively the populations of *P. corruscans*. Moreover, it was also observed a greater genetic similarity between the populations from the reservoir of Yacyretá and Itaipu and a greater genetic distance between the Upper Paraná River floodplain and Itaipu reservoir, suggesting the existence of different spawning areas, which partially avoid genetic homogenization of these two last populations (Sekine, personal communication).

The Paraíba do Sul River Basin Example

The Paraíba do Sul River basin represents a greatly affected environment and several economically important fish species from this region have been suffering an extremely high decline due to agricultural activities, aquatic pollution, mining, construction of hydroelectric dams and deforestation.

RFLP analysis of mitochondrial DNA was carried out on wild, reintroduced and cultivated populations of a migratory fish, *Brycon opalinus*, found in this hydrographic basin, in order to investigate their genetic structure. The results suggested that wild stocks management should take in count the actual population structure of *B. opalinus*. Additionally, the hatchery stocks of the species presented haplotype lineages that were not detected on the wild populations, which could be used as a marker in reintroduced fish to evaluate the reproductive and competitive success of reintroduced individuals in contact with wild populations (Hilsdorf, 1999).

Conclusion

Many Brazilian fish species are under threat due to overexploitation, habitat degradation, introduction of exotic species, river sedimentation, deforestation, aquatic pollution, reduction of food resources, and construction of hydroelectric dams. Unfortunately, little is done to maintain these species or enhance their populations. Primary actions consist on establishing protected areas and trying to minimize the results of environmental impacts by restocking and imposing fiscal measures against polluters, predatory fishing and construction of hydroelectric dams. Secondary actions should include ecological, ethological and genetic studies in order to analyze the distribution of the species/populations and to identify the factors that influence their distribution. The improvement and the application of molecular technologies and their use in a greater number of endangered Brazilian fish species will be crucial for the characterization of stocks and maintenance of the genetic diversity in aquatic environments.

Finally, biological conservation also depends on the behavior of human societies and, once value-based policies limit conservation programs success more than does biological knowledge (Meffe & Viederman, 1995; Wagner, 1996), education and respect for nature could represent some of the most crucial tasks to the conservation of biotic diversity.

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