

## Genetic monitoring of the Amazonian fish matrinhã (*Brycon cephalus*) using RAPD markers: insights into supportive breeding and conservation programmes

By A. P. Wasko<sup>1</sup>, C. Martins<sup>1</sup>, C. Oliveira<sup>1</sup>, J. A. Senhorini<sup>2</sup> and F. Foresti<sup>1</sup>

<sup>1</sup>Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, São Paulo; <sup>2</sup>Centro Nacional de Pesquisa de Peixes Tropicais/Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (CEPTA/IBAMA), Rodovia Prefeito Euberto Nemésio Pereira de Godoy, Pirassununga, São Paulo, Brazil

### Summary

The importance of genetic evaluations in aquaculture programmes has been increased significantly not only to improve effectiveness of hatchery production but also to maintain genetic diversity. In the present study, wild and captive populations of a commercially important neotropical freshwater fish, *Brycon cephalus* (Amazonian matrinhã), were analyzed in order to evaluate the levels of genetic diversity in a breeding programme at a Brazilian research institute of tropical fish. Random Amplified Polymorphic DNA fingerprinting was used to access the genetic variability of a wild stock from the Amazon River and of three captive stocks that correspond to consecutive generations from the fishery culture. Although farmed stocks showed considerably lower genetic variation than the wild population, a significantly higher level of polymorphism was detected in the third hatchery generation. The results seem to reflect a common breeding practice on several hatchery fish programmes that use a small number of parents as broodstocks, obtaining reproductive success with few non-identified mating couples. The obtained data were useful for discussing suitable strategies for the genetic management and biodiversity conservation of this species.

### Introduction

Freshwater fish farming has been one of the most expanding food producing sectors in Brazil in the last years, especially due to its high hydrographic potential, great number of native species and several favorable areas to this activity. Although one of the migratory fish groups with greater economic importance in the country is represented by the genus *Brycon* (Müller and Troschel 1844; Pereira Filho, 1994), several species of this genus have been considered endangered due to the growing impact of environmental disturbances (Ceccarelli and Senhorini, 1996). Among these species, the Amazonian matrinhã, *Brycon cephalus* (Günther 1869), stands for an extremely important fishery resource in the Amazonian region (Bittencourt and Cox-Fernandes, 1990) and is also considered as one of the main cultivated fish species in Brazil (Pereira Filho, 1994).

Aquaculture practices may inadvertently decrease the genetic variability present in farmed stocks by the selection and breeding of related individuals or by the use of a small number of parents as broodstock and, unless genetic records are maintained, there is a higher probability of increasing inbreeding. The current aquaculture industry management of the matrinhã is largely based on analyses of stocking

density, feeding, nutrition, physiology, behavior, and induced reproduction (Mendonça, 1994). However, information about its genetic structure is still scarce, in spite of its importance in designing appropriate strategies for the management and biodiversity conservation of this neotropical fish species. The synthesis of the data derived from genetic studies, together with those of other disciplines, can be applied directly to species management plans (O'Brien, 1994).

In recent years, several nuclear DNA techniques have been available in accessing genetic variability in fish species (Park and Moran, 1994; O'Reilly and Wright, 1995; Carvalho and Hauser, 1998) and have been employed in aquaculture studies (Harris et al., 1991; Mjølnerod et al., 1997; Coughlan et al., 1998; Norris et al., 1999). One of these approaches is the random amplification of polymorphic DNA [Random Amplified Polymorphic DNA (RAPD) or AP-PCR], which is based on amplification of several random segments of a genome using single short arbitrary primers that can detect polymorphism in the absence of specific DNA nucleotide sequence information (Welsh and McClelland, 1990; Williams et al., 1990). Although one of the drawbacks of using short oligonucleotides as random primers is the low stringency necessary for DNA amplification, which can lead to non-reproducible results, the possibility of working with anonymous polymorphic DNA and the low expense makes it a simple and rapid methodology in the assessment of genetic differences among several groups, including fish species (Bardacki and Skibinski, 1994; Bielawski and Pumo, 1997; Cagigas et al., 1999; Koh et al., 1999; Parker et al., 2002).

The present study reports the result of a comparative analysis of one wild and three farmed populations of *B. cephalus*, in order to genetically evaluate an 11-year breeding programme of the species in a Brazilian research institute of tropical fish. The genetic variability within and among the different stocks was evaluated through the use of RAPD. The results were useful not only to characterize each fish stock, but also to highlight future tasks for a better hatchery management and for the biodiversity conservation of the species. Moreover, information about improper management strategies usually applied in hatchery fish programmes suggest that genetic monitoring is extremely necessary for fish culture. The expected outcome is to use molecular methodologies in order to maximize or at least maintain the level of genetic diversity on cultivated stocks of several fish species.

## Materials and methods

### Sample collection

A wild stock of *B. cephalus* from the Amazon River (municipality of Manaus, Amazonas State, Brazil), which had not been subjected to any supplemental stocking of fish, and three captive stocks of the species that correspond to consecutive generations from a national center of tropical fish research (CEPTA/IBAMA, Centro Nacional de Pesquisa de Peixes Tropicais/Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) (municipality of Pirassununga, São Paulo State, Brazil), were analyzed. The base population used to establish the breeding programme of *B. cephalus* at CEPTA/IBAMA was obtained from the Amazon River. The farming stocks, maintained in isolation in the fishery center research since 1985, had basically the same breeding, management, density stocking, and feeding regimes – the average stocking density was 250 g m<sup>-2</sup>, and the annual average temperature and dissolved oxygen was 26°C and 5.5 p.p.m., respectively. A total of 99 individuals were analyzed – 30 samples correspond to the wild stock (W), 11 samples correspond to the first cultivated generation (F<sub>1</sub>) that was obtained in 1991, 28 samples correspond to the second farmed stock (F<sub>2</sub>) obtained in 1995, and 30 samples correspond to the third farmed generation (F<sub>3</sub>) obtained in 2000. The F<sub>1</sub> generation hatchery stock was bred from five males and a single female (Mendonça, 1994), and the F<sub>3</sub> cultivated stock bred from a mixture of seven males and just one female of the F<sub>2</sub> hatchery generation (J. A. Senhorini, unpublished data). Unfortunately, there are no precise data about the number of animals of both sexes that were used to compose the F<sub>2</sub> farm stock.

### DNA extraction and amplification

DNA was extracted from fish caudal or anal fins using a TNES-urea-digestion buffer [10 mM Tris-HCl pH 8.0; 125 mM NaCl; 10 mM ethylenediaminetetraacetic acid (EDTA) pH 8.0; 0.5% sodium dodecyl sulfate; 4 M urea], as described in Wasko et al. (unpublished data). Further DNA purification was performed with phenol-chloroform (Sambrook and Russell, 2001). A set of six 10-mer RAPD oligonucleotides (RAPD Analysis Beads; Amersham Pharmacia Biotech, Stockholm, Sweden) (Table 1) were used as single primers to amplify the DNA samples, following the manufacturer's instructions. Each RAPD-PCR reaction mixture contained 50 ng of target DNA and 25 pmol of single primer in a total volume of 25 µl. Amplifications were carried out in a PTC-100™ (MJ

Research, Boston, MA, USA) thermocycler, with the following cycle programme: 45 cycles at 95°C for 1 min, 36°C for 1 min, and 72°C for 2 min. A negative control, without template DNA, was also included to monitor any possible contamination of the reactions with non-target template DNA. Furthermore, amplification of multiple template DNA concentrations of every sample was carried out for all primers surveyed as a means of identifying artifactual variation. DNA amplification fragments were electrophoretically separated on 2% agarose gel in TAE buffer (Tris-Acetic acid-EDTA). The gels were run for 4.5–6.5 h at 110V/150A. DNA bands were visualized using ethidium bromide staining (Sambrook and Russell, 2001) and UV illumination. Molecular weights were estimated using a standard DNA marker. The gel documentation system EDAS (Electrophoresis Documentation and Analysis System 120, Kodak Digital Science 1D, New Haven, CN, USA) was used in order to obtain digital images of the agarose gel and to identify each amplified fragment.

### Statistical RAPD analysis

Reproducible well-marked amplified fragments were scored and pairwise comparison of banding patterns was evaluated among samples amplified by each primer separately and for the combined data of primers, by calculating an index of genetic similarity using the coefficient method of Jaccard (1901). Mean bandsharing similarity indices between individuals within each stock and among stock samples were computed. For each genotype, the presence and absence of fragments were scored as 1 or 0, respectively, and each DNA fragment or RAPD marker was interpreted to represent a single locus. The percentage of polymorphic loci (*P*) was also calculated. Statistical analyses were performed using the NTSYS-PC version 1.70 (Numerical Taxonomy and Multivariate Analysis System) computer programme (Rohlf, 1993).

## Results

The six decamer oligonucleotides (Table 1), used as single primers to amplify DNA samples of *B. cephalus*, produced fragments ranging from approximately 150 to 2000 base pairs (bp) and each of them generated a unique pattern of amplification products. The total number of reliable amplified bands, generated by each primer separately and that were selected for further analyses, ranged from 11 (primer 1) to 22 (primer 3) with an average of 9.7 bands per individual and primer (Table 1). From a total of 104 fragments that were amplified by the six RAPD primers, 16 loci (15.4%) could be visualized in all the DNA samples of *B. cephalus*, while 88 loci (84.6%) were polymorphic (Table 1). Although some of the observed variations could be due to the RAPD technique itself and, therefore, would not be real polymorphisms, this seems not to be probable as control procedures were utilized in the PCR amplifications.

Primers were also evaluated based on intensity of bands, consistency within individuals, and presence of smearing. Repeated amplifications with the primers 1, 2, 4, 5, and 6 were reproducible, and generated good quality banding patterns, with intense stained fragments well separated from other bands, and of sufficient variability. Therefore, the bands produced by these five primers were selected for the genetic statistical analysis. From a total of 82 selected bands, 12 (14.6%) were conserved among all analyzed individuals, while 70 (85.4%) amplification products were polymorphic.

Table 1

Description of the decamer primers of random sequence (RAPD oligonucleotides) and summary of the characteristics of the amplification products. The total number of scorable loci and the number of polymorphic fragments are represented by *n<sub>t</sub>* and *n<sub>p</sub>*, respectively

Primer	Sequence (5'-3')	(G + C)%	Number of loci		Size range of fragments (bp)
			<i>n<sub>t</sub></i>	<i>n<sub>p</sub></i>	
1	GGTGC GGGAA	70	11	10	200–1650
2	GTTTCGCTCC	60	17	15	250–2000
3	GTAGACCCGT	60	22	18	200–1500
4	AAGAGCCCGT	60	20	18	150–1500
5	AACGCGCAAC	60	20	18	150–1500
6	CCCGTCAGCA	70	14	9	150–1500
Total			104	88	200–1500

Distinct levels of genetic variability were detected among the four analyzed stocks of *B. cephalus* (Tables 2 and 3). The three cultivated stocks of the species evidenced a lower genetic diversity than the wild stock, as a lower number of bands/alleles and a higher genetic similarity index were detected in the cultivated samples (Tables 2 and 3). While the percentage of polymorphic loci ( $P$ ) observed for the individuals of the wild stock was 85.36%, the cultivated stocks  $F_1$ ,  $F_2$ , and  $F_3$  evidenced a percentage of variable bands of 81.81, 72, and 79.74%, respectively (Table 2). Moreover, despite the fact that any of the tested primers evidenced a diagnostic band (present in all and exclusively the members of a determined stock), the frequency of several amplified fragments differed substantially among the analyzed stocks. As an example, a conspicuous band of approximately 650 bp, identified in *B. cephalus* using the primer 4, was detected in 53.33% of the animals from the wild stock, and in 81.81, 93.33, and 53.57% of the individuals from the cultivated generations  $F_1$ ,  $F_2$ , and  $F_3$ , respectively (Fig. 1).

The values of mean similarity indices ( $J$ ) within and among the four stocks, obtained using data of the primers 1, 2, 4, 5, and 6 separately and of a combination of these primers, are given in Table 3. A mean genetic similarity index of 0.58 was observed for the wild stock of *B. cephalus*. The cultivated stocks  $F_1$ ,  $F_2$ , and  $F_3$  of the species showed mean genetic values of 0.62, 0.71, and 0.63, respectively. Although each individual primer had differed in the overall level of genetic variability detected, it was possible to verify that the animals of the cultivated stocks presented a higher genetic similarity index and, therefore, lower variability, when compared with the wild stock samples from the Amazon River.

Table 2  
Percent of polymorphic loci ( $P$ ) detected in each stock of *Brycon cephalus*. Values were obtained for each RAPD primer separately (1, 2, 4, 5, and 6) and for combined data of the primers (c.d.)

Primers	Percent of polymorphic loci (analysed stocks)			
	Wild (W)	Cultivated ( $F_1$ )	Cultivated ( $F_2$ )	Cultivated ( $F_3$ )
1	90.90	81.81	72.72	90.90
2	88.23	82.35	82.35	82.35
4	90.00	88.23	82.35	88.88
5	90.00	88.88	68.75	78.94
6	64.28	64.28	50.00	57.14
c.d.	85.36	81.81	72.00	79.74

Table 3  
Values of mean pairwise band genetic similarity index among individuals of each stock of *Brycon cephalus*, obtained for each RAPD primer separately (1, 2, 4, 5, and 6) and for combined data of the primers (c.d.)

Primers	Mean genetic similarity index (analysed stocks)			
	Wild (W)	Cultivated ( $F_1$ )	Cultivated ( $F_2$ )	Cultivated ( $F_3$ )
1	0.58	0.69	0.77	0.60
2	0.54	0.58	0.68	0.62
4	0.53	0.58	0.63	0.55
5	0.51	0.56	0.76	0.64
6	0.72	0.74	0.78	0.75
c.d.	0.58	0.62	0.71	0.63

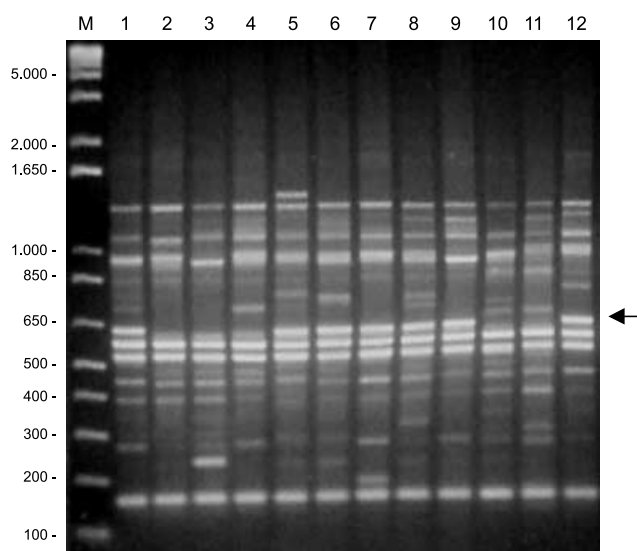


Fig. 1. RAPD patterns of individuals of *Brycon cephalus* (1–12) using primer 4. Arrow denotes a band of approximately 650 bp

## Discussion

The actual hatchery programme of *B. cephalus* at CEPTA/IBAMA can be considered well established pertaining to stocking density, feeding, nutrition, physiology, behavior, and induced reproduction (Mendonça, 1994; Senhorini et al., 1998). However, there is still a lack of genetic data of the potential breeders, and only a low number of couples have been used in the induced reproductions that occur annually. Molecular markers are currently used in order to identify the levels of genetic variation within and between stocks of *B. cephalus* and also for individual genetic characterization. Therefore, the present study intended to contribute to a practical and theoretical framework to the fishery management and genetic conservation of the species.

Even with the limited survey of primers and animal samples used in the analyses, it was possible to identify a considerable amount of genetic variability within the wild and the three cultivated stocks of *B. cephalus* and to detect significantly distinct band sharing-based similarity values and different polymorphic loci percent among the stocks. Although each stock could be characterized by a different level of genetic diversity, an overall variability decrease was observed in the successive cultivated stocks (generations  $F_1$ ,  $F_2$ , and  $F_3$ ) of the species, which seems to reflect a common practice in hatchery fish programmes that generally use a low number of genetically non-identified breeders in induced spawning, which can lead to the occurrence of possible consanguineous mating. Moreover, polymorphism seemed to be lost in the hatchery generations. A reduction in the number of polymorphic loci can also be correlated to the limited number of wild specimens and/or populations used to settle hatchery stocks (Winkler et al., 1999), which occurred in the fishery establishment of *B. cephalus* at CEPTA/IBAMA as only one wild population from the Amazon River represented the first breeding stock of the species. A genetic variability decrease was also observed in hatchery stocks of another species of the genus *Brycon* – *B. cf. reinhardti* (Galhardo and Toledo Filho, 1987).

Numerous studies using genetic markers correlated the domestication process in fish species with a decrease in genetic variability (Vespoor, 1988; Ferguson et al., 1991; Youngson et al., 1991; Taniguchi et al., 1993; Mjolnerod et al., 1997;

Koh et al., 1999; Reilly et al., 1999; Winkler et al., 1999). This fact can be associated with several events, such as the use of few animals to establish hatchery stocks (Allendorf and Ryman, 1987), employment of inappropriate reproductive strategies (Gile and Ferguson, 1990; Whitler and Beacham, 1994), and even genetic drift and inbreeding (Tave, 1986; Ferguson, 1995). A gradual loss of genetic variability caused by inbreeding seems to be the most common feature in cultivated fish stocks after some generations (Bielawski and Pumo, 1997; Bouza et al., 1997; Koh et al., 1999; Hansen et al., 2000). Moreover, an endogamy depression can lead to a continuous loss of genetic variability, which can even predispose to future survival, growth and reproductive performance reduction (Kincaid, 1983), as already observed in fish species such as *Oncorhynchus mykiss* (Gjerde et al., 1983; Su et al., 1996), *Brachidanio rerio* (Vonhertell et al., 1990), *Poeciliopsis occidentalis occidentalis* (Quattro and Vrijenhoek, 1989), and *Scophthalmus maximus* (Imsland et al., 2001).

The identification of a significant increase in genetic diversity in the third hatchery stock (generation F<sub>3</sub>) of *B. cephalus* can be correlated to the casual choice of genetically non-related breeders or even associated to the employment of different artificial reproductive strategies. Although it is not known how many fish were used for generating F<sub>2</sub>, it is also possible that only a very small number of animals were used for producing this stock. This could also explain the drop in diversity of the F<sub>2</sub> fish and the higher genetic variability values in the F<sub>3</sub> generation. Proper and uniform management and breeding programmes have to be employed not only to prevent such genetic variability oscillations but also to prevent high levels of inbreeding depression that could be the probable outcome of the present management model used in the farming programme of the species.

The genetic diversity of wild populations seems not to be fully exploited in the present culture of *B. cephalus*; a genetic variability reduction could even be magnified in future hatchery generations if new management strategies are not implemented. To maintain long-term hatchery generations of *B. cephalus* without a significant decrease in genetic variability, some strategies can be proposed – for instance, genotyping of potential breeders before setting up the next cultivated stocks, or backcrossing of farmed stocks to wild fish populations. It would also be important for continuing the genetic studies in coming years, in order to verify if there is gene flow between different wild populations, and how much this contributes to the genetic variability compared with the genetic diversity within each population.

Although one major drawback of the RAPD methodology is the impossibility to determine if an individual is homozygous or heterozygous due to its dominant character, the results showed that the technique stands for a simple and cost-efficient approach to gain information about general genetic variability within and between wild and farmed populations of *B. cephalus*. Moreover, although the present analyses represent primary genetic data on *B. cephalus*, the observed results are useful for guiding future fish breeding policies.

## Conclusion

The careful selection of natural stocks and potential breeders, based on genetic criteria, can determine the success and sustainability of hatchery and conservation programmes (Quattro and Vrijenhoek, 1989; Crozier, 1994). The suggested approaches for *B. cephalus* fishery management could ensure a

minimum increase in inbreeding rate in each cultivated generation. Moreover, it would be important for continuing the genetic characterization and monitoring of their natural and captive stocks in the coming years. To pursue this issue we are currently continuing the genetic diversity analyses using RAPD and microsatellite markers. Appropriate results will be of great value not only for fish farmers concerned with commercial production of the species but also to meet the needs of the conservation programme, i.e. the development of an integrated strategy that conserves as much genetic diversity within a species as possible and ensures the presence of utilizable fish resources.

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## References

- Allendorf, F. W.; Ryman, N., 1987: Genetic management of hatchery stocks. In: Population genetics and fishery management. N. Ryman, F. Utter (Eds). Washington Sea Grant Program. University of Washington Press, Seattle, pp. 141–159.
- Bardacki, F.; Skibinski, D. O. F., 1994: Applications of the RAPD technique in tilapia fish: species and subspecies identification. *Heredity* **73**, 117–123.
- Bielawski, J. P.; Pumo, D. E., 1997: Randomly amplified polymorphic DNA (RAPD) analysis of Atlantic Coast striped bass. *Heredity* **78**, 32–40.
- Bittencourt, M. M.; Cox-Fernandes, C., 1990: Peixes migradores sustentam pesca comercial. *Ciência Hoje* **11**, 20–24.
- Bouza, C.; Sanchez, L.; Martinez, P., 1997: Gene diversity analysis in natural populations and cultured stocks of turbot (*Scophthalmus maximus* L.). *Anim. Genet.* **28**, 28–36.
- Cagigas, M. E.; Vasquez, E.; Blanco, G.; Sánchez, J. A., 1999: Combined assessment of genetic variability in populations of brown trout (*Salmo trutta* L.) based on allozymes, microsatellites, and RAPD markers. *Mol. Biotech.* **1**, 286–296.
- Carvalho, G. R.; Hauser, L., 1998: Advances in the molecular analysis of fish population structure. *Ital. J. Zool.* **65**, 21–33.
- Ceccarelli, O. S.; Senhorini, J. A., 1996: *Brycon*. Viabilização da produção de alevinos. *Panorama da Aqüicultura Maio/Junho*, 10–11.
- Coughlan, J. P.; Imsland, A. K.; Galvin, P. T.; Fitzgerald, R. D.; Nævdal, G.; Cross, T. F., 1998: Microsatellite DNA variation in wild populations and farmed strains of turbot from Ireland and Norway: a preliminary study. *J. Fish Biol.* **52**, 916–922.
- Crozier, W. W., 1994: Maintenance of genetic variation in hatchery stocks of Atlantic salmon, *Salmo salar* L.: experience from the River Bush, Northern Ireland. *Aquacult. Fish. Manage.* **25**, 383–392.
- Ferguson, M. M., 1995: The role of molecular genetic markers in the management of cultured fishes. In: Molecular genetics in fisheries. G. R. Carvalho, T. J. Pitcher (Eds). Chapman & Hall, London, pp. 81–103.
- Ferguson, M. M.; Ihsen, P. I.; Hynes, J. D., 1991: Are cultured stocks of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) genetically similar to their source population? *Can. J. Fish Aquat. Sci.* **48**, 118–123.
- Galhardo, E.; Toledo Filho, S. A., 1987: Estudo genético-bioquímico de pirapitinga do sul, *Brycon* sp., da Estação de Paraibuna, CESP. *Ciência e Cultura* **39**, 722.
- Gile, S. R.; Ferguson, M. M., 1990: Crossing methodology and genotypic diversity in a hatchery strain of rainbow trout (*Oncorhynchus mykiss*). *Can. J. Fish Aquat. Sci.* **47**, 719–724.
- Gjerde, B.; Gunnes, K.; Gjedrem, T., 1983: Effect of inbreeding on survival and growth in rainbow trout. *Aquaculture* **34**, 327–332.
- Hansen, M. M.; Nielsen, E. E.; Ruzzante, D. E.; Bouza, C.; Mensberg, K. L. D., 2000: Genetic monitoring of supportive breeding in

- brown trout (*Salmo trutta* L.) using microsatellite DNA markers. *Can. J. Fish Aquat. Sci.* **57**, 2130–2139.
- Harris, A. S.; Bieger, S.; Doyle, R. W.; Wright, J. M., 1991: DNA fingerprinting of tilapia, *Oreochromis niloticus*, and its application to aquaculture genetics. *Aquaculture* **92**, 157–163.
- Imsland, A. K.; Foss, A.; Naevdal, G.; Stefansson, S. O., 2001: Selection or adaptation: differences in growth performance of juvenile turbot (*Scophthalmus maximus* Rafinesque) from two close-by localities off Norway. *Sarsia* **86**, 43–51.
- Jaccard, P., 1901: Étude comparative de la distribution florale dans une portion des Alpes et des Jura. *Bull. Soc. Vaudoise Sci. Nat.* **37**, 547–579.
- Kincaid, H. L., 1983: Inbreeding in fish populations used for aquaculture. *Aquaculture* **33**, 215–227.
- Koh, T. L.; Khoo, G.; Fan, L. Q.; Phang, V. P. E., 1999: Genetic diversity among wild forms and cultivated varieties of discus (*Symphysodon* ssp.) as revealed by random amplified polymorphic DNA (RAPD) fingerprinting. *Aquaculture* **173**, 485–497.
- Mendonça, J. O. J., 1994: Criação de espécies do gênero *Brycon* no CEPTA/IBAMA. In: I Seminário Sobre Criação de Espécies do Gênero *Brycon*, Centro de Pesquisa e Treinamento em Aquicultura. Pirassununga, Brazil, pp. 31–48.
- Mjølnerod, I. B.; Refseth, U. H.; Karlsen, E.; Balstad, T.; Jakobsen, K. S.; Hindar, K., 1997: Genetic differences between two wild and one farmed population of Atlantic salmon (*Salmo salar*) revealed by three classes of genetic markers. *Hereditas* **127**, 239–248.
- Norris, A. T.; Bradley, D. G.; Cunningham, E. P., 1999: Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations. *Aquaculture* **180**, 247–264.
- O'Brien, S. J., 1994: A role for molecular genetics in biological conservation. *Proc. Nat. Acad. Sci. USA* **91**, 5748–5755.
- O'Reilly, P.; Wright, J. M., 1995: The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. *J. Fish Biol.* **47**, 29–55.
- Park, L. K.; Moran, P., 1994: Developments in molecular-genetic techniques in fisheries. *Rev. Fish Biol.* **4**, 272–299.
- Parker, R. W.; Paige, K. N.; De Vries A. L., 2002: Genetic variation among populations of the Antarctic toothfish: evolutionary insights and implications for conservation. *Polar Biol.* **25**, 256–261.
- Pereira Filho, M., 1994: Estudos desenvolvidos no INPA (Manaus-Amazonas) com o matrinhã, *Brycon cephalus* (Gunther, 1869). In: I Seminário Sobre Criação de Espécies do Gênero *Brycon*, Centro de Pesquisa e Treinamento em Aquicultura. Pirassununga, Brazil, pp. 25–30.
- Quattro, J. M.; Vrijenhoek, R. C., 1989: Fitness differences among remnant populations of endangered Sonoran topminnow. *Science* **245**, 976–978.
- Reilly, A.; Elliott, N. G.; Grewe, P. M.; Clabby, C.; Powell, R.; Ward, R. D., 1999: Genetic differentiation between Tasmanian cultured Atlantic salmon (*Salmo salar* L.) and their ancestral Canadian population: comparison of microsatellite DNA and allozyme and mitochondrial DNA variation. *Aquaculture* **173**, 459–469.
- Rohlf, F. J., 1993: NTSYS-PC: Numerical taxonomy and multivariate analysis system, Ver. 1.80. Applied Biostatistics, Steauket, NY.
- Sambrook, J.; Russell, D. W., 2001: Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Senhorini, J. A.; Mantelatto, F. L. M.; Casanova, S. M. C., 1998: Growth and survival of larvae of the Amazon species “matrinxã”, *Brycon cephalus* (Pisces, Characidae), in larviculture ponds. *Boletim Técnico do CEPTA* **11**, 13–28.
- Su, G-S.; Liljedahl, L-E.; Gall, G. A. E., 1996: Effects of inbreeding on growth and reproductive traits in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **142**, 139–148.
- Taniguchi, N.; Sumantadinata, K.; Lyman, S., 1993: Genetic change in the first and second generations of hatchery stock of black seabream. *Aquaculture* **35**, 309–320.
- Tave, D., 1986: Genetics for fish hatchery managers. AVI Publishing, Westport, WA.
- Vespoor, E., 1988: Reduced genetic-variability in 1st-generation hatchery populations of Atlantic salmon (*Salmo salar*). *Can. J. Fish Aquat. Sci.* **45**, 1686–1690.
- Vonhertell, U.; Horstgenschwark, G.; Langholz, H. J.; Jung, B., 1990: Family studies on genetic-variability in growth and reproductive-performance between and within test fish populations of the zebrafish, *Brachydanio rerio*. *Aquaculture* **85**, 307–315.
- Welsh, J.; McClelland, M., 1990: Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* **18**, 7213–7218.
- Whitler, R. E.; Beacham, T. D., 1994: Genetic consequences of the simultaneous or sequential addition of semen from multiple males during hatchery spawning of chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* **126**, 11–23.
- Williams, J. G. K.; Kubelik, A. R.; Livak, K. J.; Rafalski, J. A.; Tingey, S. V., 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18**, 6531–6535.
- Winkler, F. M.; Bartley, D.; Díaz, N. F., 1999: Genetic differences among year classes in a hatchery population of coho salmon (*Oncorhynchus kisutch* (Walbaum, 1792)) in Chile. *Aquaculture* **173**, 425–433.
- Youngson, A. F.; Martin, S. A. M.; Jordan, W. C.; Vespoor, E., 1991: Genetic protein variation in Atlantic salmon in Scotland: comparison of wild and farmed fish. *Aquaculture* **98**, 224–231.

**Author's address:** Adriane P. Wasko, Departamento de Morfologia, Instituto de Biociências, Universidade Estadual Paulista, 18618-000, Botucatu, São Paulo, Brazil.  
E-mail: awasko@ibb.unesp.br