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DOI: 10.2225/vol14-issue6-fulltext-7 *SHORT COMMUNICATION*

Inbreeding evidence in a traditional channel catfish (*Ictalurus punctatus*) hatchery in Mexico

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Abstract

Background: Channel catfish are one of the most important aquaculture species raised for food purposes in Mexico. Two temporal samples were obtained from the largest channel catfish breeding hatchery in Mexico to identify changes in genetic diversity and inbreeding that are promoted by traditional hatchery management. **Results:** The genetic parameter analysis of 11 microsatellite loci showed no significant change in genetic diversity ($p > 0.05$). However, a significant heterozygosis deficiency was detected ($p < 0.001$), and genetic structure analysis indicated moderate differentiation between the temporally divided populations ($F_{ST} = 0.08$). A moderate level of inbreeding and a slight increase of the inbreeding coefficient from 0.23 to 0.27 were the result of traditional hatchery practices. To achieve an effective population size, the temporal approach resulted in a limited number of breeders to maintain genetic variability. **Conclusions:** Although no significant change in genetic diversity parameters was found, the heterozygote deficiency and low effective number of breeders suggest that there is a risk for increased inbreeding. Thus, we propose the need for controlled reproductive management and the establishment of genetic programs in hatcheries. Molecular tools can provide valuable information to facilitate the achievement of these goals.

Keywords: channel catfish, effective population size, genetic diversity, inbreeding

INTRODUCTION

Channel catfish (*Ictalurus punctatus*, Rafinesque 1918) is an endemic American freshwater fish species found in the main river and stream systems and reservoirs of Tamaulipas, Mexico (García de Leon et al. 2005). Due to its favourable weather conditions, Tamaulipas is the most important state in Mexico for the production of channel catfish, as it produces more than 70% of the fish consumed nationally and approximately 90% of the fry and fingerlings used on regional and national farms.

This species has been cultivated since 1970, and, in most farms, traditional breeding practices have been based on mass selection. For decades, catfish farmer's experiences have been the main resources for success in most farms. Signs of concern in local farms include sporadic mortalities and lower reproductive success and fry production. In some cases, these problems are related to the loss of genetic variability and inbreeding depression.

Molecular technologies have been proposed and partially applied as versatile tools for monitoring genetic variability in regional populations (Perales-Flores et al. 2007). Although producers face technical and basic issues that prevent the complete acceptance of these tools, some producers have invested time and money in monitoring genetic variability.

In this study, we used microsatellite data from samples obtained from a traditional channel catfish brood fish hatchery to identify changes in genetic diversity and inbreeding.

MATERIALS AND METHODS

Samples were collected randomly at two sampling times (in 2005 and 2009) from the largest channel catfish broodstock hatchery in Tamaulipas, Mexico (ACU). In total, 96 anal fin samples (n_{ACU05} = 50 and n_{ACU09} = 46) were collected. Broodfish were randomly selected from hatching ponds to ensure the equal representation of each population.

Traditional hatchery management consists exclusively of breeding and the production of fry and fingerlings for commercialization. Mating and spawning occur in rustic pond installations, and egg harvesting and incubation (March through May) occur in hatchery troughs. The annual fry production is typically 10 million fingerlings. Fifteen ha of rustic ponds are seeded annually. Traditional reproductive management is based on mass selection three months before the breeding season. This selection occurs when fish are 2 years old (with ages ranging from 1 to 3 years) and is based on size, conformation and other phenotypic traits. In general, the male:female ratio is 2:3. Traditionally, selected broodstock is from production farms of the same line.

DNA from fin samples was extracted using the GenElute Mammalian Genomic DNA Miniprep Kit (G1N350) (SIGMA-Aldrich®). All of the samples were analyzed at 11 microsatellite loci (Table 1), as previously described (Perales-Flores et al. 2007; Waldbieser and Wolters, 2007).

Table 1. Description of microsatellite loci and PCR conditions used to genotype channel catfish (*Ictalurus punctatus*) samples.

Loci	Repeated motif	Primers (sense/antisense) → 5'3'	PCR conditions	
			T_mºC[1]	$MgCl_2$[2]
Ip265	$(CA)_{32}$	AGAGGTTGAAATAAAACACAGCC/ AAGACCCCACTTCCATCATC	55	2.00
Ip077	$(GT)_{15}$	GAAACACAATGTACAGTAAGCTG/ GCTGCTTCTTATGGAATCTC	55	1.75
Ip591	$(GT)_7(GA)_{20}$	CTGCTTTAGGTCCACCCACTGC/ AGGCACTTGACATTTAGCCTGC	55	1.75
Ip607	$(GA)_{24}$	TCAGGCACAAATCTTGTGATGG/ TTGTAGTTCTGCCTCTAACCGC	50	2.00
Ip427	$(CA)_{24}$	CATTTTGCTAGGTGCGCGCACG/ GGTGCCTTTATATGTATATAC	50	2.00
Ip070	ATAG	ATCATTTTCTGCTTCTTATACATAGGCT/ CCTTTAGATGAACTCACCTGCC	55	3.00
Ip128	AAAG	GATCCACTGAGAAATAAGAGCACA/ GGAGTATAGCACAGAAACACGAA	55	3.00
Ip038	ATAG	GTGTGCCTGATTTACTAATGATAAG/ TGTATTGGTATAGAACACATTAGCC	50	3.00
Ip189	AAT	GATCCTGTGCTAAAGAAACCAAG/ GTGCCGCAGTGTGTTGTAAA	55	3.00
Ip195	AAT	AACTGTCATTTACACACATTCATCTA/ GCAGGTCTGTCGTCATCTA	50	3.00
Ip273	AAG	CGTTTTACTTCCTCATACAGCAC/ GCACCAAGAGACCTGTGACA	50	3.00

[1]Annealing temperature during PCR.
[2]$MgCl_2$ concentration (mM).

PCR reactions were performed in a final volume of 10 µL composed of 50 ng DNA, 1.25 U Taq DNA polymerase (Promega Co, Madison, WI, USA) and 0.5 mM dNTPs. The MgCl2 concentrations and temperature profiles varied, as shown in Table 1. PCR products were denatured at 95ºC for 5 min and were electrophoresed on a 6.5% polyacrylamide-bisacrylamide gel (as shown in Figure 1) for 2 hrs on a LI-COR 42001G sequencer. The allele sizes were determined by SAGAGTTM™ software using the IRDye® 800 Sizing Standard ranging from 50-350 bp.

Fig. 1 Image illustrating the sequencing gel pattern of an Ip273 trinucleotide microsatellite motif.

Previously, an analysis was performed by MICRO-CHECKER (Van Oosterhout et al. 2004) to identify genotyping errors in the microsatellite panel used in this study. The Hardy-Weinberg equilibrium (HWE) was tested, and population-diversity parameters were examined. Expected (H_E) and observed (H_O) heterozygosities were estimated using GENEPOP version 4.0 software (Rousset, 2008).

Using microsatellite allelic frequencies, the average number of alleles (A) and the effective number of alleles were estimated following the proposed formula $Ae = 1/\Sigma x_i^2$, where x_i = frequency of the allele at each locus (Li et al. 2004). Complementarily, genetic diversity (G), allelic richness (Ar) and inbreeding coefficients (F_{IS}) were estimated by FSTAT version 2.9.3 (Goudet, 2001).

The differences in parameters according to loci and sampling time were analyzed through a paired *t* test and Bonferroni comparison of means using InfoStat/Professional version 2.0 software.

Genetic structure was assessed by analysis of F_{ST} comparison, as computed by GENEPOP version 4.0 software (Rousset, 2008). Finally, N_e, the effective population size, was estimated using the NeESTIMATOR program (Ovenden et al. 2007) using a temporally based N_e estimator (N_T).

RESULTS AND DISCUSSION

The preliminary analysis revealed that microsatellite loci showed no dropout and no stuttering bias. However, a homozygote excess was estimated in some loci, and null allele frequencies were identified in four and five loci from the 2005 and 2009 populations, respectively. As temporal samples included in this study were from a domestic farm population, homozygosis was an inherent assumption that was revealed by further analysis.

The average genetic parameters estimated by temporal sampling are shown in Table 2. All diversity parameters in the present study are comparable to those determined in previous reports by Waldbieser and Bosworth (1997) for channel catfish wild populations, DeWoody and Avise (2000) for freshwater fishes and Perales-Flores et al. (2007) for farm channel catfish in Tamaulipas, Mexico. The *t* test analysis that was performed to detect possible changes between genetic parameters in temporal samples showed no significant difference between populations, as determined by loci comparison ($p > 0.05$).

Table 2. Average values of genetic variability parameters in two temporal samples in a traditional channel catfish farm.

Parameter	ACU_{05}	ACU_{09}
H_O	0.669	0.630
H_E	0.860	0.855
HWE	$p < 0.0001$	$p < 0.0001$
F_{IS}	0.23	0.27
G	0.864	0.858
A	12.3	13.5
Ar	10.60	10.84
Ae	6.80	7.80
N_T	-	21.0

These results could be a consequence of empirically mixing selection broodstock from other hatcheries, which is a common practice on traditional farms. As previously reported by Perales-Flores et al. (2007), this practice maintains genetic diversity in the genetic pool as a whole.

Conversely, the analysis of heterozygosity showed a slight reduction in average H_O for the ACU_{09} population. Considerably lower values for H_E were found in the respective samples, in keeping with the low estimates found by Perales-Flores et al. (2007), which were reflected in HWE deviations and thus indicated non-random mating patterns for both samples. Further analyses indicated significant heterozygote deficiencies in both samples ($p < 0.001$).

Previous reports in different fish species have confirmed that intensive domestic management stimulates the significant reduction of genetic diversity (Kohlmann et al. 2007; Thai et al. 2007, Ha et al. 2009; Na-Nakorn and Moiekun, 2009). Bottleneck events may occur, due to population isolation, reduction of effective number of breeders and/or genetic drift caused by a small number of founders (Harlt and Clark, 2007). In agreement with these assertions, a study of domestic channel catfish in the United States (Mickett et al. 2003) reported lower allelic informative parameters. However, it is commonly recognized that the industry of channel catfish in that country has become notably intensive, which probably has caused a loss of genetic variation (Waldbieser and Bosworth, 1997).

Genetic structure analysis by fixation index F_{ST} comparison showed a moderate genetic variation ($p < 0.001$) between temporal samples (Hartl and Clark, 2007). As F_{ST} is directly related to the variance in allele frequency between populations and, conversely, to the resemblance among individuals within populations (Holsinger and Weir, 2009), it was interesting to identify moderate differentiation within the hatchery despite the time that had elapsed between sampling. However, this difference is caused by the selection criterion of broodstock replacement based on the selection of breeders from regional farms that buy fingerlings from this hatchery.

Interestingly, the estimated degree of F_{IS} (Table 2) increased over a four-year period from 0.23 to 0.27. It is known that repeated selection within the same strains carries a risk of decreasing diversity when a small number of families and high mass selection intensities are used (Thai et al. 2007). This reduced diversity could increase the inbreeding coefficient, resulting in an excess frequency of mating between relatives (Charlesworth, 2009), which may be induced by unintentional, intensive use of related breeders. As the nature of inbreeding indicates, higher levels of this parameter suggest an increase of genes from a common ancestor and, eventually, a risk of inbreeding depression.

The consequences of inbreeding channel catfish are slight. Bondari and Dunhan (1987) studied two generations of inbred populations of channel catfish and estimated inbreeding of approximately 0.35% and 19% inbreeding depression in growth rate. However, they indicated that survival rates were not affected by inbreeding. Kinkaid (1983) found a large quantity of susceptible traits for inbreeding depression in fish populations, such as growth, survival, egg production and feed efficiency. Periodical monitoring of these parameters could allow improved planning of breeding strategies.

The effective population size, which is closely related to increased inbreeding, was estimated using the temporal N_e method. This estimation showed that the effective number of breeders available (N_T) was

21. The only disadvantage of the temporal method is that the timescale sampling requires at least two generations (Leberg, 2005), while the advantage of the analysis is that it considers changes in allele frequencies that occur between samples taken from a small population.

The effective number of broodstock determines the rate of change in the composition of a population caused by genetic drift (Charlesworth, 2009). Although breeding management is partially monitored by considering age and size as the main selection criteria for each reproductive season, hatcheries do not keep records of the selected broodstock, which means that, at each breeding season, broodstock may include inbred male and female breeders.

Further monitoring at periodic time intervals may be necessary to promote consistent estimates and to eliminate any source of bias. Finally, increasing the sample size could be a useful strategy in future studies.

The understanding of diversity parameters, the effective number of breeders and the inbreeding risks for broodstock hatcheries can facilitate the development of better breeding strategies that consider the genetic characteristics of local populations. Such strategies would promote genetic improvement, possibly through the use of heterosis and/or the reintroduction of foreign strains.

CONCLUDING REMARKS

Microsatellite data analysis did not show significant evidence of a loss of genetic diversity associated with management practices ($p > 0.05$). However, as a consequence of traditional hatchery practices, a significant deficiency of heterozygosity and a slight increase in the inbreeding coefficient were identified. In addition, the effective number of breeders was low. There is a need for the implementation of organized reproductive management and the establishment of genetic analysis programs. Molecular approaches may yield useful data for monitoring and achieving these goals.

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