



ELSEVIER

Aquaculture 137 (1995) 41–44

Aquaculture

Evidence for genetic purity of captive and domestic striped bass broodstocks¹

L. Curry Woods *, Bert Ely, Gilles Leclerc, Reginal M. Harrell

*University of Maryland, Department of Animal Sciences, Crane Aquaculture Facility, P.O. Box 1475,
Baltimore, MD 21203, USA*

University of South Carolina, Department of Biological Sciences, Columbia, SC 29208, USA

University of Maryland, Horn Point Environmental Laboratory, Cambridge, MD 21613, USA

Abstract

The culture of striped bass or its hybrids is currently one of the fastest growing segments of aquaculture in the United States. Although this industry is still in the early stages of development, it is already estimated that cultured striped bass and hybrids exceed that of the wild harvest. One major problem limiting the growth of the industry is the dependency on wild brood stock for seed supply. The Crane Aquaculture Facility (CAF) maintains the largest Chesapeake Bay (Maryland, USA) population of captive (F_1) and domestic (F_2 or greater) striped bass. These striped bass originated from wild populations of Chesapeake Bay where hybrids of *Morone* exist sympatrically, and where evidence of introgressive hybridization among *Morone* has occurred. Given this evidence, we felt it was imperative to screen all of the CAF stock for genetic purity before selective breeding efforts were initiated. We utilized genomic DNA techniques to validate genetic purity because of the ease of sampling and the high level of sensitivity to introgressive hybridization. No white bass alleles were found among the samples tested. Thus, white bass alleles if present at all are extremely rare in the CAF striped bass stocks.

Keywords: *Morone saxatilis*; Introgressive hybridization; Genomic DNA; Genetic markers

1. Introduction

The Crane Aquaculture Facility (CAF) maintains a large population of captive (F_1) and domestic (F_2 and F_3) striped bass which originated from wild populations of the Chesapeake Bay (Maryland, USA) and since 1983 has been involved in domestication efforts (Woods and Sullivan, 1993; Woods et al., 1992). One concern associated with domesticated brood-

* Corresponding author.

¹ University of Maryland Agricultural Experiment Station Contribution Number 9107.

stock development is whether the breeder is starting with genetically pure stocks. The presence of congeneric genes sequestered within the founder stock could have far reaching implications in future progeny-based selection efforts. It is known that: (1) hybrid *Morone* were intentionally stocked into the natural populations of Chesapeake Bay, from which our founder stocks were derived; (2) *Morone* hybrids are reproductively viable (Harrell, 1984); (3) that outcrossing of natural populations of striped bass with hybrids has been reported numerous times (Avisé and Van Den Avyle, 1984; Crawford et al., 1987; Forshage et al., 1988; Fries and Harvey, 1989). Even more alarming, Harrell et al. (1993) reported that 3% of field identified hybrid *Morone* taken from the Chesapeake Bay in 1991 exhibited evidence of backcrossing. Thus, genetically, there is a reasonable chance that the CAF captive and domestic stocks may not be pure striped bass.

Given the evidence of introgressive hybridization among *Morone*, and the value of the broodstock domestication efforts, we felt that it was imperative to screen all individuals of the captive and domestic CAF stocks for genetic purity before selective breeding efforts were initiated. We had previously demonstrated that a number of striped bass and white bass (*M. chrysops*) alleles could be discriminated on the basis of restriction fragment length polymorphisms using Southern blots (Harrell et al., 1993). In addition, we have developed several PCR-based assays which discriminate between the two species (Leclerc and Ely, unpublished data). Therefore, it was relatively straight forward to use these techniques to validate the genetic purity of the CAF stock of striped bass.

2. Materials and Methods

Whole blood samples (ca 1000 μ L) were taken from individual CAF broodstock, mixed with a 13.2% EDTA solution to prevent clotting, and stored frozen (-80°C) until used for DNA isolation. Nuclear DNA isolations and Southern hybridizations were performed as described in Harrell et al. (1993).

The construction of plasmid DNA pSB5-12, and pSB5-33 is described elsewhere (Harrell et al., 1993). These plasmids have been shown to be useful for the detection of the introgression of conspecific alleles in wild striped bass. A third plasmid, pSB10-59, contained a 371 bp HindIII-EcoRI striped bass nDNA fragment inserted into a pUC19 vector. These three plasmids were used to hybridize to Southern (1975) blots of genomic DNA cut with HindIII, HaeIII, and PstI, respectively. In each case, species-specific restriction fragment length polymorphisms were observed.

Four PCR-based assays developed from three anonymous striped bass loci (SB1-10, SB5-1, and SB5-21) and the striped bass growth hormone gene (SB-GH) were used to further validate genetic purity. The PCR-primers for the growth hormone gene (T. Chen, personal communication) and the anonymous loci were designed from DNA sequences obtained from both ends of cloned striped bass nDNA fragments (Leclerc et al., 1995). Approximately 20 ng of uncut genomic DNA were added to the PCR mixture (60 mM Tris-HCl, pH 9.0; 2 mM MgCl_2 ; 0.5 μ M of each primer; 200 μ M of each dNTP; 1.25 units of AmpliTaq DNA polymerase (Perkin Elmer)). Conditions for the amplification (50 μ l) were: initial denaturation for 6 min at 94°C followed by 34 cycles in a thermal cycler (denaturation: 1 min at 94°C ; annealing: 1 min at 59°C ; extension: 3 min at 72°C), and a

final incubation for 10 min at 72°C to ensure maximum full-length product formation. Approximately 15 ul of amplified DNA was used for subsequent analyses.

The species level differences for genetic markers SB1-10, SB5-21, and SB-GH were detected by revealing an interspecific polymorphic restriction site using the restriction endonucleases DraI, HaeIII, and PvuII, respectively, followed by agarose gel electrophoresis. The amplified DNA generated with primers for the marker SB5-1 was analyzed directly in 1.2% agarose gel to detect species-specific size differences. Control DNA samples from known striped bass and white bass were compared on each gel.

3. Results and Discussion

The molecular markers used in this study revealed, through agarose gel electrophoresis, different banding patterns for striped bass and white bass. Depending on the marker, one to five alleles were diagnostic for striped bass while white bass controls displayed one or two alleles (Table 1).

Armed with the knowledge that it is highly probable that the CAF founder stocks are genetically pure striped bass, we can now go forward with traditional progeny-based selection efforts. In addition, the genetic molecular efforts we have undertaken pave the way for the development of markers for the identification of specific genes responsible for desirable quantitative traits. The availability of genetic markers for specific genes or combinations of genes responsible for a desirable quantitative trait affords culturists the ability to circumvent several generations of progeny-based selection efforts by initiating a marker based selection approach. The simple screening of broodstock for the presence of a gene responsible for a desirable trait would allow for first generation detection of that trait, thereby eliminating the problem caused by undetected, undesirable alleles.

Table 1

Genetic purity analysis of domesticated striped bass. Values indicate the number of diagnostic marker alleles present

Marker	Control ^a		Domestic Fish		
	N	Striped Bass	White Bass	Striped Bass	White Bass
<i>Southern hybridizations</i>					
pSB5-12	120	1	1	1	0
pSB5-33	139	1	2	1	0
pSB10-59	122	3	1	3	0
<i>PCR assays</i>					
SB1-10	205	2	2	2	0
SB5-1	205	1	2	1	0
SB5-21	205	2	1	1	0
Growth hormone	205	2	2	2	0

^aFor the initial experiments, 14 individuals of each species were obtained from two geographically distinct areas and tested to establish species-specific differences. Subsequently, several hundred South Carolina (USA) striped bass were tested and no white bass alleles were observed.

Acknowledgements

Funding for this project was provided in part by the University of Maryland Agricultural Experiment Station, the United States Department of Agriculture Northeastern Regional Aquaculture Center, the University of Maryland Center for Environmental and Estuarine Studies (CEES), and the University of South Carolina Cooperative Institute for Fisheries Molecular Biology. This research is listed as CEES contribution number 2706.

References

- Avise, J.C. and Van Den Avyle, M.J., 1984. Genetic analysis of reproduction of hybrid white bass × striped bass in the Savannah River. *Trans. Am. Fish. Soc.*, 113: 563–570.
- Crawford, T., Freeze, M., Fourt, R., Henderson, S., O'Bryan, G. and Phillip, D., 1987. Suspected natural hybridization of striped bass and white bass in two Arkansas reservoirs. *Proc. Ann. Conf. Southeastern Assoc. Fish and Wildlife Agencies*, 38: 455–469.
- Forshage, A.A., Harvey, W.D., Kulzer, K.E. and Fries, L.T., 1988. Natural reproduction of white bass × striped bass in a Texas Reservoir. *Proc. Ann. Conf. Southeastern Assoc. Fish and Wildlife Agencies*, 40: 9–14.
- Fries, L.T. and Harvey, W.D., 1989. Natural hybridization of white bass with yellow bass in Texas. *Trans. Am. Fish. Soc.*, 118: 87–89.
- Harrell, R.M., 1984. Tank spawning of first generation striped bass × white bass hybrids. *The Progressive Fish-Culturist*, 46: 75–78.
- Harrell, R.M., Xu, X.L. and Ely, B., 1993. Evidence of introgressive hybridization in Chesapeake Bay *Morone*. *Mol. Mar. Biol. Biotechnol.*, 2:291–299.
- Leclerc, G.M., Ely, B., Xu, X.L. and Harrell, R.M., 1995. Gynogen production in hybrid striped bass. *J. World Aquacult. Soc.*, in press.
- Southern, E.M., 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.*, 98: 503–517.
- Woods III, L.C. and Sullivan, C.V., 1993. Reproduction of striped bass (*Morone saxatilis*) broodstock: monitoring maturation and hormonal induction of spawning. *Aquacult. Fish. Manage.*, 24: 213–224.
- Woods, III, L.C., Bennett, R.O. and Sullivan, C.V., 1992. Reproduction of a domestic striped bass brood stock. *The Progressive Fish-Culturist*, 54: 184–188.