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# Evidence for genetic purity of captive and domestic striped bass broodstocks [1]

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**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 introgessive 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-

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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 (Avise 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 uL) 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 uM of each primer; 200 uM of each dNTP; 1.25 units of AmpliTaq DNA polymerase (Perkin Elmer)). Conditions for the amplification (50 ul) 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 |
| *Southern hybridizations* | | | | | |
| pSB5-12 | 120 | 1 | 1 | 1 | 0 |
| pSB5-33 | 139 | 1 | 2 | 1 | 0 |
| pSB10-59 | 122 | 3 | 1 | 3 | 0 |
| *PCR assays* | | | | | |
| 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 |

[a]For 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.

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