Identification of *Morone* Species and Congeneric Hybrids using Isoelectric Focusing

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Abstract: The 4 North American species of the genus *Morone* were evaluated using isoelectric focusing for determination of species specific protein phenotypes. Each species could be characterized by a pair of protein bands that had isoelectric points in the 3.0 to 5.0 pH range. These diagnostic protein bands were then used to successfully identify 3 congeneric hybrids. The technique of isoelectric focusing yields results that are accurate in determination of species within this genus and serves as a powerful complement to other electrophoretic techniques in analyses of *Morone* populations.

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The last few years have seen a dramatic increase in the use of electrophoretic techniques in fisheries management. This increase reflects a growing awareness of the need for a more accurate genetic characterization of both fish and wildlife populations than that which can be provided through use of morphological or meristic indices. In addition, the technology necessary for these types of genetic characterizations has become much more readily available over the last few years. One of the more recent electrophoretic techniques, isoelectric focusing (IEF), was shown by Lundstrom (1981) to be a powerful tool in identification of fish species. Through development of a library of protein profiles, Lundstrom was able to identify unknown individuals with a very high success rate. More recently, Whitmore (1986) used IEF to characterize members of the *Lepomis* complex.

As part of the Texas Parks and Wildlife Department's (TPWD) broodstock certification program, we evaluated the utility of IEF in identification of the species and available congeneric hybrids within the *Morone* complex. Four members of the genus *Morone* are found in North America—the striped bass (*Morone saxatilis*), the white bass (*M. chrysops*), the white perch (*M. americana*), and the yellow bass (*M. mississippiensis*). While the individual species can be identified using starch-

gel electrophoresis (Otto 1975), TPWD's program of screening populations of striped bass and white bass for brood stock collection and certification requires a technique that is both less expensive and time-consuming. In this study we applied IEF to muscle protein extracts from each of the *Morone* species and from 3 congeneric hybrids. Our objectives were to characterize these species and to determine the utility of IEF as an alternative to starch-gel electrophoresis for identification of the species and hybrids of this genus.

Fish used in this study were provided by several individuals: Clay Young, United States Fish and Wildlife Service; Allen Forshage, Timothy Broadbent, Paul Seidensticker, Ken Kurzawski, Barry Lyons, and Charles Inman, TPWD; Howard Kerby, North Carolina State University; Tim Mulligan, Chesapeake Biological Lab, University of Maryland; and Dick Snyder, Pennsylvania Fish Commission. Initial electrophoretic evaluations were conducted by Kathryn Kulzer, TPWD. Much of the transportation and processing of samples was done by Vernon Staats and Tony Owens, TPWD.

Methods

Sample preparation

Striped bass, white bass, striped bass × white bass hybrids, yellow bass, striped bass × yellow bass hybrids, and yellow bass × white bass hybrids were collected and identified by personnel of the TPWD. White perch were collected from the Chesapeake Bay, Maryland and from the Susquehanna River, Maryland (Table 1). Of the hybrids evaluated in this study, only the yellow bass × white bass hybrids were the result of natural hybridization. Other hybrids were artificially produced in hatcheries and subsequently stocked. Taxonomic status of each individual was confirmed using starch-gel electrophoresis and the specific criteria of Otto (1975). The results of these analyses were not made known prior to our IEF analysis.

All fish samples were frozen as soon as possible after collection and transported to TPWD lab facilities. Approximately 1 g of epaxial muscle was excised from each fish. Samples were frozen at 0° C or below until thawed for analysis by IEF.

Gel Preparation and Running Conditions

Gels were prepared according to LKB Application Note 2217 (LKB-Produkter AB, Gaithersburg, Maryland) using acrylamide and N,N'-methylene-bisacrylamide so that a final composition of 7.5% T and 3.0% C was achieved. The gels also contained 3.0% W/V of narrow range (pH 3.0–5.0) carrier ampholytes. Resultant gels were 0.25 mm thick and were formed using the "flap" technique suggested by Radola (1980).

Gels were run on a flatbed IEF apparatus (LKB) and were cooled at 10° C. For these narrow pH range gels, initial power was 3.0 watts with a limitation of 1,500

Table 1. Sources and number of individuals of *Morone* used in the evaluation of isoelectric focusing as a fishery management technique.

Species	*N*	Collection site
M. saxatilis	70	Lake Livingston, Texas
	10	Inks Lake, Texas
	50	Lake Texoma, Texas
M. chrysops	50	Lake Buchanan, Texas
	20	Lake Palestine, Texas
	10	Lake Somerville, Texas
	27	Lake Pat Mayse, Texas
M. mississippiensis	5	Toledo Bend, Texas
	10	Lake Jackson, Texas
M. americana	4	Cheasapeake Bay, Md.
	6	Susquehanna River, Md.
M. saxatilis × *M. chrysops*	10	North Carolina State Univ., Raleigh, N.C.
	4	Lake Somerville, Texas
	4	Lake Palestine, Texas
	20	Lake Bridgeport, Texas
	49	Lake Sam Rayburn, Texas
M. saxatilis × *M. mississippiensis*	3	Toledo Bend, Texas
M. chrysops × *M. mississippiensis*	1	Toledo Bend, Texas
	1	Lake of the Pines, Texas
	1	Lake Ray Hubbard, Texas

volts. Optimum protein separation was achieved with a 1.0-M phosphoric acid anolyte solution (pH 1.6) and a catholyte solution of 2.0-M ethylenediamine, 0.025-M arginine, and 0.025-M lysine (pH 11.5). Current was adjusted so that starting voltage was 150 volts. Current at this voltage was 9 to 15 mAmps.

Gels were prefocused for 2 hours prior to sample application. Samples were applied to the surface using an application mask with 1.0 mm × 10.0 mm wells (FMC Corporation, Rockland, Maine). The application mask was placed 2.0 cm from the cathode. While samples are often homogenized before application, we have had excellent results using a direct tissue application method (Saravis et al. 1979). Sample application mask was removed after 30 minutes of run-time. In these narrow range gels, the current tended to stabilize before the proteins were sharply focused. Therefore, these gels were focused until the 1,500 volt limitation was reached.

Determination of the pH gradient of the gel was made using a surface pH electrode (LKB) at the termination of the run. Gel pH gradient was measured at 1.0 cm intervals along a straight line in the middle of the gel and was measured from the anode to the cathode.

Gels were fixed for 5 minutes in 20% trichloroacetic acid and then washed for 5 minutes in a 35% ethanol–10% acetic acid destaining solution. Gels were stained for 5 minutes with 0.5% Coomassie Brilliant Blue R-250 in destaining solution. Gels were destained with several changes of destaining solution then allowed to air dry.

Results

Species Identification

Each of the 4 *Morone* species could be identified through a pair of protein bands, presumably parvalbumins (Whitmore 1986), with isoelectric points in the pH 3.0 to pH 5.0 range of the gel (Fig. 1). The yellow bass was characterized by bands at pl = 3.85 and pl = 4.20; the striped bass at pl = 3.90 and 7 pl = 4.95; the white bass at pl = 3.95 and pl = 4.45; and the white perch at pl = 3.85 and pl = 4.43. Comparisons of samples of the same species collected from different locations indicated that while some variation in non-diagnostic proteins was evident, no variation was found in diagnostic proteins.

Of considerable interest was the fact that the Susquehanna River population of white perch was distinguishable from the Cheasapeake Bay samples. This variation was consistent between these populations; however, these 2 populations showed no variation in the protein bands that were used to differentiate this species from the other three evaluated.

Hybrid Identification

Using the band enumeration established as characteristic for each of the 4 *Morone* species, hybrid recognition and classification was straightforward. Each of the 3 different hybrids evaluated displayed the characteristic bands of both parental species (Fig. 1). For instance, the striped bass × white bass hybrid demonstrates both characteristic bands of the striped bass plus the 2 characteristic bands of the white bass.

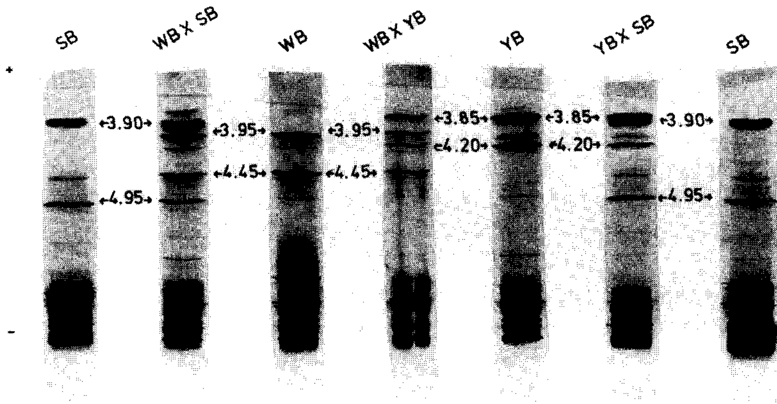

Figure 1. Characteristic banding patterns and isoelectric points of species specific proteins in striped bass (SB), yellow bass (YB), white perch (WP) and white bass (WB), and congeneric hybrids. Note that the discriminatory bands of both parentals are evident in F_1 hybrids.

Discussion

The 4 species in the genus *Morone* were originally described based upon morphological or meristic characters, and the species can usually be identified with little problem after reaching adult stages. However, the hybrids of these species, particularly hybrids of striped bass × white bass and yellow bass × white bass, can be very difficult to identify using meristic or morphometric criteria. Moreover, use of morpho-meristic characters is of little help in identification of non-F_1 hybrids (Forshage et al. 1986). In this evaluation, through isoelectric focusing of muscle proteins, we were able to rapidly and accurately identify the *Morone* species and 3 different hybrid types.

While we do not know the exact mechanism of inheritance for these protein bands, we have demonstrated IEF banding patterns which were suggestive of non-F_1 hybrids (or backcrosses) of striped bass and white bass in evaluations done prior to this investigation (Forshage et al. 1986). This non-F_1 hybrid status was verified using starch-gel electrophoresis of known discriminatory enzyme loci (Avise and Van Den Avyle 1984) which suggests that IEF will function well as a screening procedure for hybrid reproduction in suspect populations.

The technique associated with IEF is, in our opinion, more delicate than that of starch-gel electrophoresis. While a wealth of technical information concerning IEF can be easily obtained, the quality of results is largely a matter of trial and error. However, results can be characterized by exceptional resolution when the technique is mastered and the equipment is properly configured.

There are several advantages of IEF when compared to starch-gel electrophoresis. Whitmore (1986) points out that in addition to large numbers of proteins that can be evaluated, the minute amounts of tissue necessary for sufficient analysis through IEF can be taken using non-invasive techniques—which do not require sacrifice of the animal. Isoelectric focusing is an endpoint technique and as such does not require the critical timing that is inherent to other procedures. In addition, we would add the advantage of significantly reduced run-time compared with other techniques we have employed. We can consistently process samples, make acrylamide gels, and complete most analyses in less than 7 hours as compared to the 12 to 24 hours necessary for starch-gel electrophoresis. With this comes the ability to process large numbers of samples in a short period of time. This is a requisite for evaluating striped bass and white bass broodfish when results must be known prior to stocking of fry from these broodfish. The protein stains used in IEF do not require the expensive reagents (e.g. NADP) that are often required in histochemical staining and the proteins themselves are more resistant than are isozymes to degradation through improper handling.

The utility of IEF in many aspects fisheries management makes this technique a very useful complement to other electrophoretic techniques. Its use in identification of fish species and hybrids has been demonstrated herein and in other similar studies (Forshage et al. 1986, Whitmore 1986). In addition, we have used this technique to verify genetic status of potential record fish, screening reservoir pop-

ulations to assess natural reproduction of hybrids, and in prosecution of game-law violators.

It is not our suggestion that IEF can replace more conventional electrophoretic procedures; it certainly will not. It is but one of several biochemical techniques that is now readily available and may have distinct advantages over others in certain types of evaluations. The choice of technique, then, is largely dependent upon both the task at hand and of the nature of the systematic question being asked.

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