Identification of Six Catfish Species Utilizing Isoelectrically Focused Muscle Tissue

Paul K. Whitehead,[1] *Texas Parks and Wildlife Department, P.O. Box 947, San Marcos, TX 78667*

William D. Harvey,[2] *Texas Parks and Wildlife Department, P.O. Box 947, San Marcos, TX 78667*

Abstract: Isoelectric focusing (IEF) and densitometric scanning were used to identify 6 species of catfish based on diagnostic bands and banding patterns produced by muscle proteins. Blue catfish (*Ictalurus furcatus*), white catfish (*Ictalurus catus*), and flathead catfish (*Pylodictis olivaris*) each have diagnostic bands in pH 4–5 gels that allow positive species identification. IEF of channel catfish (*Ictalurus punctatus*) musculature in this pH range exhibits a polymorphic system. One of the bands is diagnostic for this species when present, allowing identification of 58% of the channel catfish tested. Channel catfish without this band, yellow bullhead catfish (*Ictalurus natalis*), and black bullhead catfish (*Ictalurus melas*) are indistinguishable in the pH 4–5 range gels, but can be identified by banding patterns produced by IEF of proteins in pH 6–7 gels.

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Texas' freshwater fishing laws classify channel catfish, blue catfish, and flathead catfish as game species whose harvest, possession and sale are closely regulated. Black and yellow bullhead catfish are designated "rough fish" with no restrictions to harvest. As is the case in many states, illegal harvest and sale of catfish species in Texas is a substantial problem, and enforcement of fishing regulations applicable to these species is dependent upon correct species identification.

It is common practice for fishermen to remove the head and tail of a fish as soon as the catch is no longer transported by boat and to subsequently fillet the fish for storage or sale. Identification of harvested species from the remaining morphology and coloration of partially processed fish is a difficult task for law enforcement officials and fillet identification is virtually impossible. Clearly, development of a

[1]Present address: Department of Fisheries and Allied Aquacultures, Auburn University, AL 36849.
[2]Present address: Texas Parks and Wildlife Department, 4200 Smith School Road, Austin, TX 78744.

method through which law enforcement officials could identify species-of-origin in processed catfish samples would improve enforcement of regulations.

A variety of electrophoretic techniques has been applied to species identification of fish flesh. Connell (1953) first compared fish protein extracts using moving boundary electrophoresis. Other techniques developed subsequently for fish identification include starch gel zone electrophoresis (Thompson 1960), disc electrophoresis (Payne 1963, Thompson 1967), cellulose polyacetate strip electrophoresis (Lane et al. 1966), and isoelectric focusing or IEF (Lundstrom 1977).

The objective of this study was to develop IEF banding profiles that would enable identification of 6 catfish species. IEF can provide high resolution and reproducibility necessary for positive identification of fish species. Application of this technique to sarcoplasmic proteins of fish was successful in differentiating 9 species of *Lepomis* (Whitmore 1986), 4 species of *Morone* (Harvey and Fries 1987) and congeneric *Morone* hybrids (Fries and Harvey 1989).

Laboratory facilities and financial support for this project were provided by the Texas Parks and Wildlife Department (TPWD). Also, TPWD personnel throughout the state assisted in collection of specimens. The white catfish specimens were provided by Dr. R. Dunham and Auburn University. We relied heavily on the technical expertise of L. Fries during the development of these procedures. We wish to thank Dr. B. G. Whiteside, Dr. D. G. Huffman, L. T. Fries, Mike Ryan, and Dr. G. C. Matlock for their review of this manuscript.

Methods

Sample Collection and Treatment

Specimens used in this study consisted of 6 blue catfish, 11 black bullhead catfish, 26 flathead catfish, 37 yellow bullhead catfish, and 144 channel catfish collected from Texas fish hatcheries, streams, ponds, and reservoirs. One of the blue catfish and 2 of the channel catfish were collected in Louisiana. Although the white catfish does not occur in Texas, 9 individuals (collected in Alabama) were evaluated in this study because the white catfish is an important resource in nearby states.

Fishes were identified to species at the time of collection. Approximately 2 g of epaxial muscle was excised from either side of the dorsal spine and stored in 1.5 ml cryogenic vials. Several samples were collected from fish recovered on the third day of cove rotenone to assess muscle protein stability.

Samples were frozen as soon as possible and shipped to the A. E. Wood Fish Hatchery, Texas, for analysis. Samples were stored at the lab at either −70° C during testing or −40° C for semi-permanent storage.

Gel Preparation and Running Conditions

Polyacrylamide gels for IEF were prepared using the methods described by Fries and Harvey (1989). Gels were run on a horizontal electrophoresis unit cooled to 10.0° C. Power was supplied by a 2,500-W power supply with starting power set at 3.0 W.

Preliminary IEF in broad range gels (pH 3–10, pH 3–5, and pH 5–8), and

subsequent IEF in narrow range gels covering one pH unit from pH 3 through pH 9 indicated that species specific proteins could be discriminated in gels containing single pH unit ampholytes with advertised (label) ranges of pH 4–5 and pH 6–7. Gels with pH 4–5 ampholytes were run using techniques described by Fries and Harvey (1989). Gels with pH 6–7 ampholytes were run with a 0.5-M acetic acid (pH 2.5) anolyte solution and a 1.0-M sodium hydroxide (pH 12.7) catholyte solution. Initial voltage for these gels was 200 V and 4–6 hours of run time was required for complete focusing (final voltage = 2000 V).

About 30 minutes to 1 hour before application to the gel, samples were removed from the freezer and allowed to thaw at room temperature. Freezing and thawing the samples in this manner ruptured the cell membrane, producing an exudate containing the soluble sarcoplasmic proteins. Approximately 1 μl of exudate was collected in a disposable pipette and placed directly on the gel surface using a variety of application masks. The sample volume needed varied from 0.5 μl to 1.5 μl, depending on the mask used.

Mechanical damage to the gel caused by the mask was avoided in both pH ranges by placing the application mask 2 cm from the cathode. All gels were prefocused for 1 hour before application of samples, and masks were removed after 1 hour of run time.

An Ingold combination surface electrode was used at the conclusion of each run to measure gel pH at 1 cm increments from the anode to the cathode. Gels were maintained at separation temperature during measurement of pH gradients. After these measurements were taken, current was re-applied to the gel to sharpen the focus of any bands that had started to diffuse. Gels were then fixed and stained as described by Harvey and Fries (1987).

Determination of Isoelectric Point

Quantitative evaluation of band migration was performed with an LKB 2222–010 UltroScan XL laser densitometer (LKB Produkter). Each lane was scanned, the scanned signal was integrated, and distances from the cathode were reported to 0.10 mm. The pH readings taken at the conclusion of the run along the 1 cm increments of the gel were plotted against respective distances from the cathode.

Distance from the cathode of each diagnostic band was determined from densitometric scanning. Scanning of each lane was begun at the same point on each gel. These distances were used to interpolate isoelectric points from the pH gradient. All pI values were identified to the nearest 0.01 pH unit to aid in differentiation of protein phenotypes. In narrow range gels such as these, small differences in isoelectric points correspond to large differences in migration distance.

Results

Gel surface pH

Broad range (pH 3–10) ampholytes comprised 20% of the total ampholytes in pH 4–5 gels and 30% in pH 6–7 gels. Under conditions used in these analyses,

broad-range ampholytes used in formulation of pH 4–5 and 6–7 ampholytes resulted in gel pH range that was consistently linear through a wider pH range than indicated by the manufacturer. Linear pH range of the pH "4–5" gels was approximately pH 4.1–5.4 and linear range of "6–7" gels was approximately pH 5.2–7.5. Despite the differences in apparent pH range and label range, we refer to these pH ranges as "4–5" and "6–7" for consistency. In those non-linear areas of the gel, bands which appear to be similar in migration may have been very different in pI. Therefore, we could not reliably assign a pI to bands outside linear ranges and such bands were not included in these analyses.

pH 4–5 Gels

Proteins with isoelectric points in the range of pH 4–5 proved to be extremely stable over time for the 6 catfishes studied. Whitmore (1986) suggests that these fish proteins are parvalbumins characterized by low isoelectric points, low molecular weights, calcium binding properties, and water solubility. Two additional parvalbumin properties are resistance to thermal and acid denaturization, both common to most animal intracellular enzymes (Scopes 1982). Samples collected on the third day post-rotenone application displayed considerable decomposition. Exudate could not be recovered from these samples, but a portion of the decomposing tissue of paste-like consistency was applied to gels and run with interpretable results in pH 4–5 gels.

Blue catfish can be consistently identified by diagnostic bands with mean isoelectric points of 4.26 and 4.74 (Fig. 1). All 5 of the other catfish species can be distinguished from blue catfish by possession of a faint band with a mean pI of 4.77. Flathead catfish have a single diagnostic band in this range with a mean pI of 4.19, while white catfish have a single diagnostic band at pI 4.70. Bands found to be diagnostic for the blue catfish, flathead catfish, and white catfish occurred in all individuals of each of these 3 species.

Channel catfish show 3 banding patterns in the 4–5 pH range, produced by the presence or absence of 2 bands with pI's of 4.43 and 4.62 (Fig. 2). The pI 4.43 band occurred alone in 42% of the fish tested, the pI 4.62 band occurred alone in 24% of the fish tested, and the 2 bands occurred together in 33% of the fish tested. Occurrence of the pI 4.62 band was diagnostic for channel catfish, but occurrence of the pI 4.43 band alone in a channel catfish produces the same pattern in this range as both bullhead catfish species. Therefore, 42% of channel catfish and both bullhead species displayed similar banding patterns in pH 4–5 gels.

pH 6–7 Gels

Proteins with isoelectric points in the pH range of 6–7 denatured easily and banding patterns were not as straightforward as those found in pH 4.0–5.0 gels. As a result, banding patterns of fish proteins focused in pH 6–7 gels were less reproducible than the patterns produced by focusing of parvalbumins in pH 4–5 gels. Deliberate attempts to denature exudates by 5 minute immersion in an 80° C water bath caused loss of all protein activity, suggesting that diagnostic bands in this range are

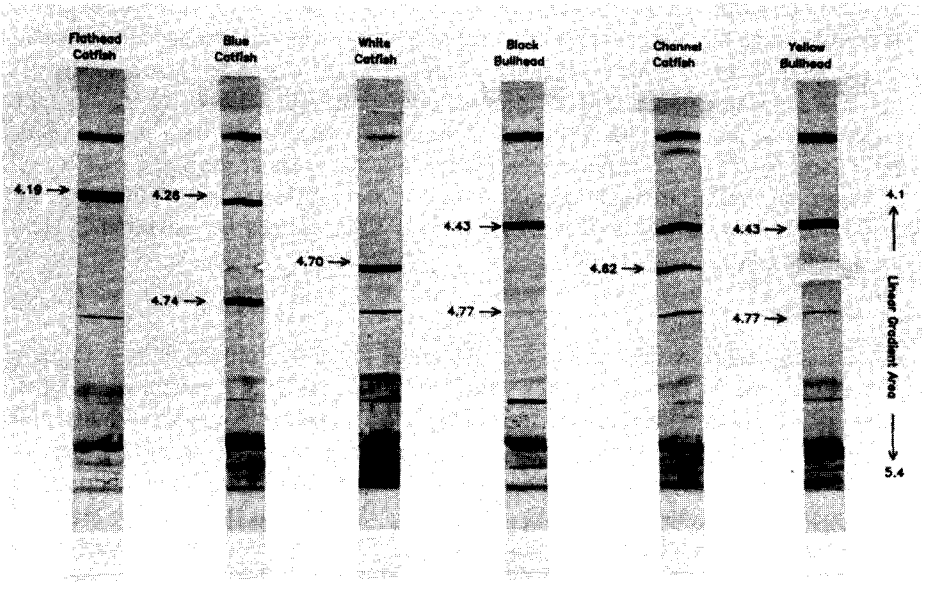

Figure 1. Banding patterns of 6 species of catfish produced by isoelectric focusing of muscle proteins in pH 4-5 polyacrylamide gels. Channel, black bullhead, and yellow bullhead catfish are indistinguishable from each other in this pH range in the absence of the pI 4.62 band.

not thermostabile parvalbumins. Overall banding patterns, in addition to individual diagnostic bands, had to be considered in determination of catfish species identification in this pH 6–7 gels.

Yellow bullhead catfish consistently demonstrated 7 bands in pH 6.0–7.0 gels. These bands had mean pI's of 5.52, 5.58, 5.62, 5.67, 5.70, 5.77, and 5.82 (Fig. 3). These bands were found in 65%, 88%, 76%, 59%, 100%, 65%, and 88%, respectively, of all yellow bullheads. The pI 5.70 band (100% occurrence) appears to be diagnostic for this species.

Black bullhead catfish consistently demonstrated 6 bands in pH 6–7 gels with mean isoelectric points of 5.75, 5.77, 5.80, 5.81, 5.85, and 5.90. Bands with pI 5.75 and 5.77 occurred in only 50% of the fish examined whereas the other 4 bands occurred in all black bullheads. Densitometry recognized bands with pI's of 5.75 and 5.77 as a single band 50% of the time. Single banding was also evident for the pI 5.80 and 5.81 bands at a similar frequency. Bands with pI 5.85 and pI 5.90 were considered diagnostic in distinguishing black bullheads, as both were cathodal to all 7 bands of the yellow bullhead. The pI 5.90 band also distinguished this species from channel catfish.

Channel catfish samples focused in pH range 6.0–7.0 gels displayed 7 bands with mean isoelectric points of 5.67, 5.73, 5.75, 5.77, 5.80, 5.85, and 5.95.

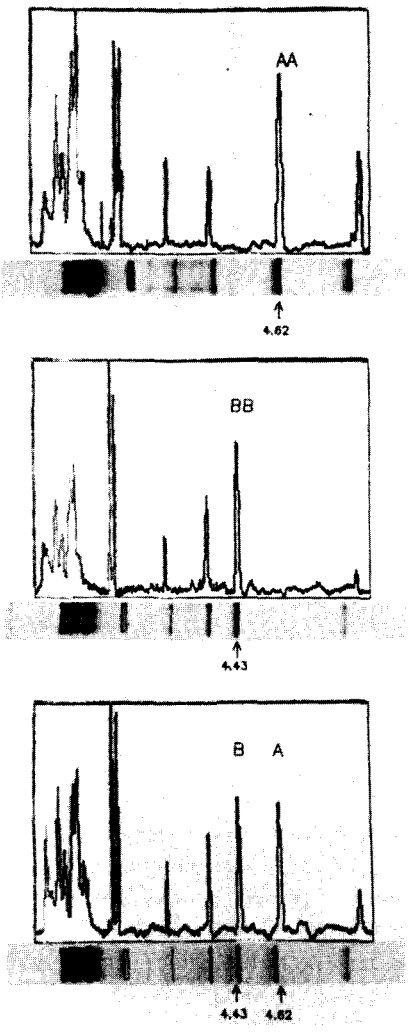

Figure 2. Channel catfish banding patterns and muscle protein densitometric scan of pH 4-5 polyacrylamide gels. The pI 4.62 band is unique to channel catfish and distinguishes this species from bullhead catfish in this pH range.

However, only bands at pI 5.85 and pI 5.95 were present in all 144 channel catfish tested. Bands at pI's 5.75 and 5.77 appears to be artifacts of a single protein and were again treated as such by scanning software for 50% of samples. The pI 5.95 band was intensely stained, present at 100% frequency and unique to channel catfish in this pH range. The pI 5.85 band shares the first 2 characteristics, but was also present in black bullhead catfish, and is not diagnostic for channel catfish.

In general, yellow bullhead catfish had the most anodal banding pattern, followed by black bullhead catfish and then channel catfish. Blue catfish, white catfish, and flathead catfish can also be distinguished in the 6.0–7.0 range; however, identification of these species in pH 4–5 gels is enhanced by the thermostability of the

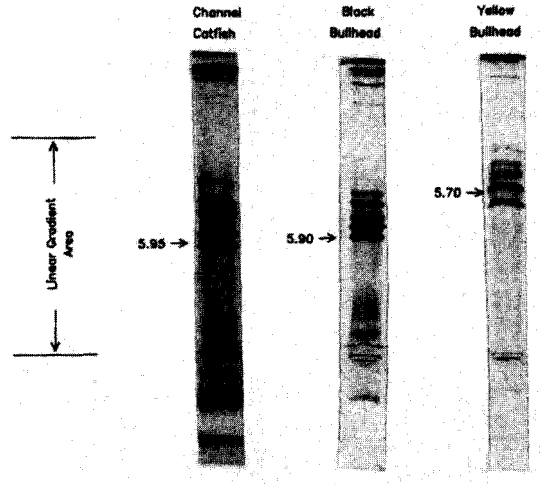

Figure 3. Banding patterns of channel, black bullhead, and yellow bullhead catfish is produced by isoelectric focusing of muscle proteins in pH 6-7 polyacrylamide gels. These three species can be discriminated in this range by the presence of unique bands occurring at 100% frequency.

parvalbumins evaluated and the fact that the 4 principal game species of catfish can be individually identified in this pH range.

Discussion

The 6 species of catfish evaluated in this study can be consistently identified using IEF in pH 4–5 and pH 6–7 polyacrylamide gels. The presence of unique discriminatory bands in each of the species allows a straightforward interpretation of the gels and the technique is relatively simple and quite repeatable.

The technique that we have described does have some limitations. The instability of the discriminatory proteins in pH 6–7 requires that samples be handled with substantial care. Texas state game wardens often have to transport confiscated evidenciary samples over long distances with much accompanying freezing and thawing. As a result, the utility of IEF (as we have described it) for discrimination of the channel catfish and the bullhead species relies on use of samples which can be shown to have undergone minimal denaturization. This, at least in our opinion, may limit the utility of this technique in law enforcement situations involving confiscation of frozen fish or fillets which may have not been maintained in a controlled environment.

The Texas Parks and Wildlife Department Law Enforcement Division has used results obtained through IEF in successful prosecution of fishing regulation violations involving several catfish species. The thermostability of the purported parvalbumins found in pH 4–5 gels makes them very useful as evidence in prosecution of cases

involving identification of blue catfish, yellow catfish, white catfish, and (in some cases) channel catfish.

Another, unforeseen, use of IEF of catfish musculature is in genetic marking of hatchery stocks. TPWD has begun breeding programs for the fixation of the pI 4.43 and 4.62 bands of hatchery channel catfish for use as genetic markers. Preliminary evaluation of channel catfish populations in reservoirs across the state found many with high frequencies of 1 band or the other. Stocking of these genetically marked catfish will be used to evaluate the stocking strategies used in establishing or enhancing channel catfish fisheries.

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