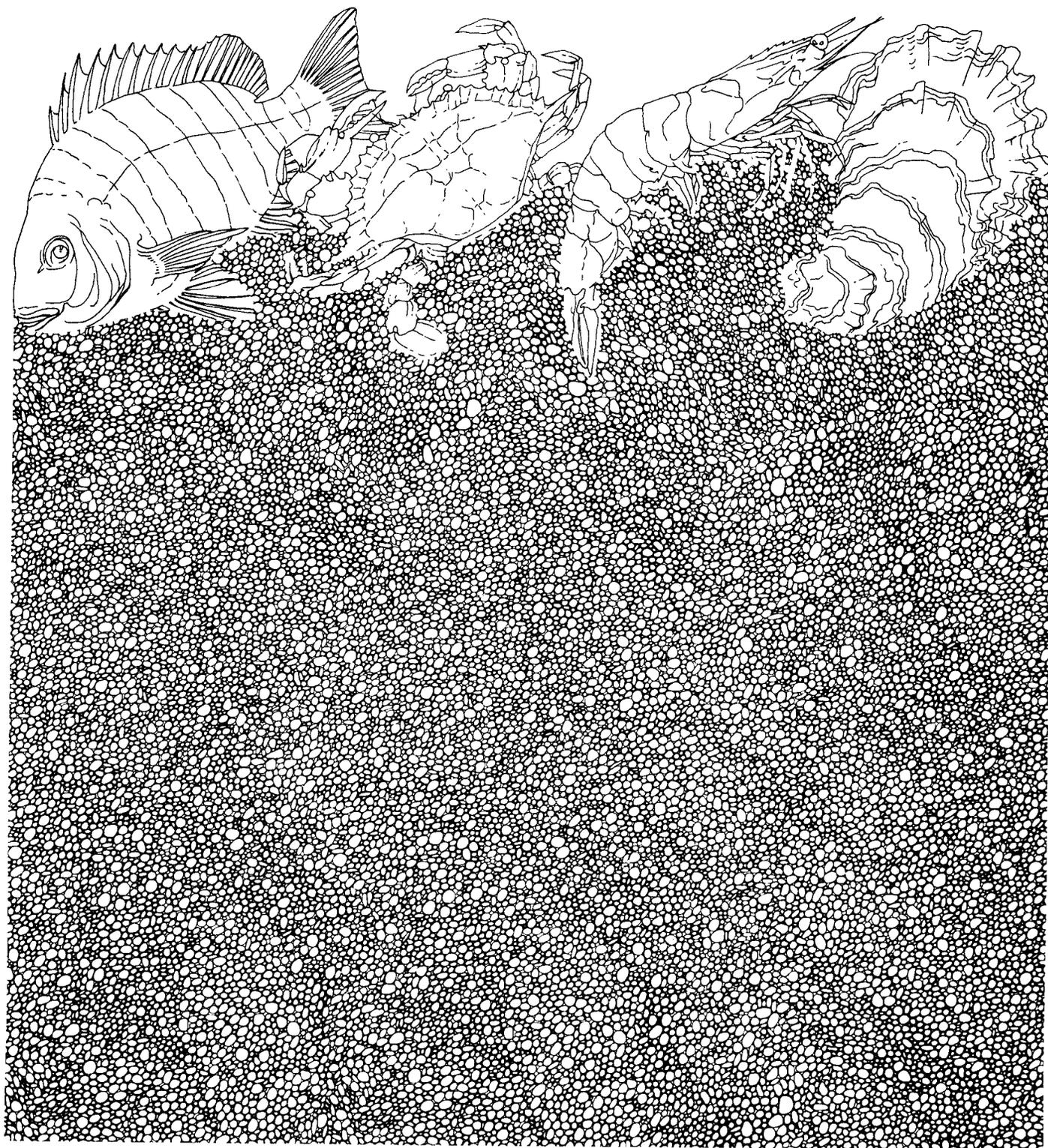


# A Comparison Of Snook and Fat Snook Muscle Protein By Isoelectric Focusing

by Timothy L. King, Anne Henderson-Arzapalo and Anthony F. Maciorowski

Management Data Series Number 141  
1988

Texas Parks and Wildlife Department  
Coastal Fisheries Branch



**ERRATA**

for

Management Data Series Number 141

entitled

A Comparison Of Snook and Fat Snook  
Muscle Protein By Isoelectric Focusing

Page 2, 1st paragraph, last sentence should read:

1200 V, instead of 200 V

A COMPARISON OF SNOOK AND FAT SNOOK MUSCLE  
PROTEIN BY ISOELECTRIC FOCUSING

by

Timothy L. King, Anne Henderson-Arzapalo  
and Anthony F. Maciorowski

MANAGEMENT DATA SERIES  
No. 141  
1988

Texas Parks and Wildlife Department  
Coastal Fisheries Branch  
4200 Smith School Road  
Austin, Texas 78744

## ACKNOWLEDGMENTS

Partial financial support for this project was provided by the Federal Aid in Sport Fish Restoration Act under Project F-36-R. The Act is popularly known as the Dingell-Johnson Act after its congressional sponsors and is administered by the U. S. Fish and Wildlife Service for the conservation and management of sport fisheries.

We appreciate the assistance of Ms. L. Fries and Dr. W. Harvey with the electrophoresis, and also the Florida Department of Natural Resources for supplying Florida snook fry for pond culture.

## ABSTRACT

Sarcoplasmic protein extracts of snook (Centropomus undecimalis) and fat snook (C. parallelus) were compared by thin-layer polyacrylamide gel isoelectric focusing. A pH gradient from 3.0 (anodally) to 10.0 (cathodally) was used to distinguish protein phenotypes of the two species. The selected gradient conditions failed to differentiate between pond cultured C. undecimalis derived from Florida broodstock and wild-caught C. undecimalis from Texas.

## INTRODUCTION

Electrophoretic separation of proteins has enhanced understanding of systematic and evolutionary relationships, ontogenesis, and population structure of numerous organisms (Lundstrom 1977, Whitmore 1986). Modifications of conventional electrophoresis such as mitochondrial DNA analysis, two-dimensional electrophoresis, and isoelectric focusing have increased effectiveness in inter- and intraspecific identification (Mosher et al. 1985, Whitmore 1986).

Thin-layer polyacrylamide gel isoelectric focusing (IEF) is ideally suited for the examination of gross protein profiles of fishes due to the experimental flexibility and rapidity of analysis (Lundstrom 1979, Coulson 1981, Mosher et al. 1985). The inherent high resolution of IEF allows the production of species specific protein profiles not normally attained by conventional electrophoretic techniques. The present study provides a preliminary evaluation of IEF-produced sarcoplasmic protein profiles for differentiating snook (Centropomus undecimalis) from fat snook (C. parallelus), and compares protein profiles of pond-cultured snook derived from Florida broodstock to wild caught snook from Texas.

## MATERIALS AND METHODS

Three snook and three fat snook were collected by rod-and-reel, bag seine, or gill net from the lower Laguna Madre and Brownsville ship channel, Brownsville, Texas between October 1986 and March 1987. Florida snook fingerlings were spawned and pond cultured as described by Maciorowski et al. (1986) and Henderson-Arzapalo et al. (1987). Fish were frozen prior to analysis.

Muscle tissue was excised from the lateral musculature immediately below the dorsal fin of thawed specimens. Tissues were homogenized in equal volumes of deionized water and centrifuged at 1,400 G for 15 minutes. The resultant supernatant was retained for analysis.

Isoelectric focusing was performed on 0.25-mm polyacrylamide gels. The gel solution consisted of 2 ml of 29.1% (wt/vol) acrylamide and 0.9% N,N'-methylenebisacrylamide, 0.6 ml of pH 3-10 (Serva Fine Biochemicals, Westbury, NY) ampholytes, and 10  $\mu$ l of TEMED. The gel solution was placed into a flask and degassed for 5 minutes. Polymerization was initiated by the addition of 56  $\mu$ l of 10% ammonium persulfate to the gel solution. The acrylamide solution was poured onto a GELBOND PAG film (FMC Bioproducts, Rockland, MN) placed on the lower section of a gel mold. The gel mold consisted of two 3-mm thick glass plates separated by 0.25-mm spacer tape. The upper glass plate was lowered onto the gel and the plates were clamped. The gel was allowed to polymerize for 1 h before the mold was disassembled and the GELBOND PAG film, with gel attached, was applied to the IEF apparatus.

IEF was performed on an LKB 2117 Multiphor II electrofocusing apparatus powered by a LKB 2197 power supply and thermoelectrically cooled by an LKB 2219 Multitemp II thermostatic circulator (LKB Instruments, Gaithersburg, MD). The gel was placed on the cooling platform over a thin layer of light paraffin

oil to ensure appropriate thermal conductance. Electrode strips were soaked in an anolyte (0.05 M aspartic acid and 0.05 M glutamic acid) and a catholyte (0.05 M arginine and 0.05 M lysine) placed parallel to each other at opposite edges of the gel corresponding to the electrodes on the apparatus. The gel was prefocused for 1 h to ensure the establishment of a consistent pH gradient. The protein extracts were loaded onto a 15-sample application mask (5  $\mu$ l capacity), and focused for an additional 2 h. Focusing was performed at a constant 10 C, 200 V and 4 W.

Immediately following completion of electrofocusing, gels were fixed for 5 minutes in a solution of 4% sulfosalicylic acid and 12.5% trichloroacetic acid. Following fixation, gels were placed in 300 ml of wash solution consisting of 40% methanol and 10% glacial acetic acid for 3 minutes. Protein phenotypes were stained with a 5% Coomassie Blue R-250, 40% methanol, and 10% glacial acetic acid solution for 10 minutes. The gel was destained in wash solution until the background cleared and was allowed to air dry. Gels were examined visually.

## RESULTS AND DISCUSSION

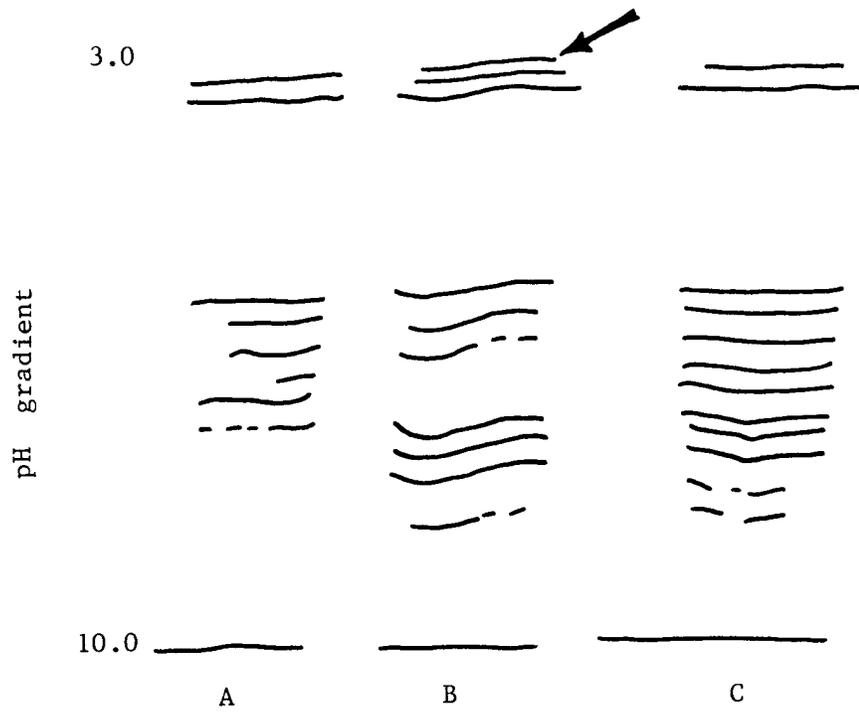
Isoelectric focusing of sarcoplasmic protein extracts indicated muscle protein phenotypes of snook and fat snook differed. The muscle protein electrographs for the two species (Figure 1) suggest clear protein differences in the lower pH range. Fat snook apparently possess a set of proteins which form a slightly more acidic band of proteins than snook. The highly acidic bands in fat snook extracts are probably parvalbumin proteins since they share the unique characteristics of low isoelectric points and low molecular weight (Whitmore 1986). There appears to be additional protein differences in the mid-pH range. However, the pattern is indistinct at the pH gradient employed.

Intraspecific variation between Texas and Florida snook was not detected. But, variation may occur and was masked in this study. Many of the protein bands resolved in the pH 3-10 gradient appear to have the same isoelectric point (Figure 1). A narrower pH gradient may increase resolution of snook muscle proteins, thereby allowing identification of Texas and Florida subpopulations.

## LITERATURE CITED

- Coulson, S. E. 1981. Enhancement of complex patterns in isoelectric focusing with improved isogel performance. *Journal of Electrophoresis*. 2:229-236.
- Henderson-Arzapalo, A., R. L. Colura, and A. F. Maciorowski. 1987. Snook fingerling production in saltwater ponds. Federal Aid Project Report F-36-R Segment II, Objective 2. Texas Parks and Wildlife Department, Coastal Fisheries Branch. Austin, Texas.
- Lundstrom, R. C. 1977. Identification of fish species by thin-layer polyacrylamide gel isoelectric focusing. *Fishery Bulletin*. 75:571-576.
- Lundstrom, R. C. 1979. Fish species identification by thin layer isoelectric focusing. *Journal of the Association of Official Analytical Chemists*. 62:624-629.
- Maciorowski, A. F., A. Henderson-Arzapalo, D. E. Roberts, Jr., R. L. Colura, and C. E. McCarty. In Press. Fingerling production of snook in saltwater ponds. Proceedings of the Thiry-eighth Annual Meeting of the Gulf and Caribbean Fisheries Institute.
- Mosher, R. A., W. Thorman, A. Graham, and M. Bier. 1985. The formation of stable pH gradients with weak monovalent buffers for isoelectric focusing in free solution. *Journal of Electrophoresis*. 6:545-551.
- Whitmore, D. H. 1986. Identification of sunfish species by muscle protein isoelectric focusing. *Comparative Biochemistry and Physiology*. 84:177-180.

Figure 1. Enlarged sarcoplasmic protein electrographs for snook and fat snook focused on a pH 3.0-pH 10.0 gradient. (A) snook (Texas), (B) fat snook, (C) snook (Florida). Arrow indicates additional proteins present in fat snook muscle tissue.



Dispersal of this publication conforms with Texas State Documents Depository Law, and it is available at Texas State Publications Clearinghouse and Texas Depository Libraries.

PWD-RP-3400-294-5/89