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# Differentiation of Postlarvae of *Penaeus aztecus* and Penaeus duorarum from the Gulf of Mexico Using Isoelectric Focusing

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with annual temperature maxima and the coral mass spawning. I believe the occurrence of *M. tarapacana* with the annual mass spawning of corals at these banks to be potentially important. Mass spawning by corals at the Flower Garden Banks is a significant environmental event in the region and may be in part responsible for the seasonal habitat association of several planktivorous elasmobranchs occurring there during this period.

*Mobula tarapacana in the western Atlantic.*—Notarbartolo-di-Sciara and Hillyer (1989) were first to observe *M. tarapacana* in the western North Atlantic. Their identification of mobulids from aerial observations made between Puerto la Cruz and Isla Margarita, Venezuela, was based on morphological characters. They observed *M. tarapacana* primarily over deep waters (>200 m), between April and November, with observations being most numerous in July. Notarbartolo-di-Sciara and Hillyer (1989) thought that this biologically productive area served as an important feeding habitat for mobulid rays. The only mobula species known to occur in the western North Atlantic prior to the first observation of *M. tarapacana* were *Mobula hypostoma* and, possibly, *Mobula mobular* (Bigelow and Schroeder, 1953; Notarbartolo-di-Sciara 1987).

The observations of *M. tarapacana* at the WFGB reported here are the northernmost confirmed sightings in the western North Atlantic Ocean. Yet, monthly underwater observations conducted since 1992 at the FGBNMS indicate that *M. tarapacana* is rare at the WFGB coral reef and would probably be best observed during the warmest weeks of the year at the FGBNMS, around the time when corals are spawning en masse. Like the area Notarbartolo-di-Sciara and Hillyer (1989) studied in Venezuela, the WFGB possibly serves as an important feeding habitat for mobulid rays including *M. birostris, M. hypostoma,* and *M. tarapacana.*

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DIFFERENTIATION OF POSTLARVAE OF *PENAEUS AZTECUS* AND *PENAEUS DUORARUM* FROM THE GULF OF MEXICO USING ISOELECTRIC FOCUSING.—Commercial landings of wild penaeid shrimp in the U.S.A. have been relatively stable over the past 5 yr, whereas consumption of and demand for shrimp continue to increase (Rosenberry, 1995). Although much of the additional demand has been met by farm-reared shrimp from abroad, there has also been increased pressure placed on our endemic wild stocks. Consequently, natural populations must be carefully managed to prevent overfishing and depletion. Management of wild shrimp populations requires accurate assessments of population numbers and recruitment information. Management practices would be facilitated by the accurate identification of commercially important species at all life history stages.

The shrimp industry in the Gulf of Mexico is based primarily on three species of the family

Penaeidae: *Penaeus duorarum* Burkenroad (pink shrimp), *Penaeus aztecus aztecus* Ives (brown shrimp), and *Penaeus setiferus* Linnaeus (white shrimp). Juvenile and adult *P. aztecus* and *P. duorarum* can be identified by the presence of grooves that flank the carina of the sixth abdominal segment (Williams, 1984). Postlarvae of these species can be identified by the presence of spines on the dorsal carina of the sixth abdominal segment (Ringo and Zamora, 1968). All developmental stages of *P. setiferus* lack such grooves and spines, allowing them to be easily distinguished from the other two species. Reliable differentiation of *P. aztecus* and *P. duorarum* from the Gulf of Mexico during postlarval and juvenile stages, however, is much more difficult. Unlike the cooler waters of the Atlantic coast, where the spawning of the two species is separated temporally, the warm waters of the Gulf of Mexico extend the spawning seasons, allowing them to overlap. Shrimp of both species, at various developmental stages, can be found almost year-round in common nursery areas. This diversity in environmental conditions creates a greater plasticity in external morphological features (Farfante, 1969) than is seen in other areas where spawning is restricted to a single season (Williams, 1959).

In the absence of external morphological features, species identification can often be determined using biochemical or molecular markers. Structural characteristics of proteins can potentially be used in species identification. For example, separation of proteins using isoelectric focusing (IEF) techniques has the potential to produce banding patterns that are species-specific. The separation of total proteins using IEF has already been used to identify several commercially important fish species (Lundstrom, 1977, 1980, 1981; Harvey and Fries, 1987; Whitmore, 1986; Whitehead and Harvey, 1989) and other crustaceans, including adult shrimp of wild and cultured species (An et al., 1988, 1989; Balboa et al., 1991; King et al., 1992) and crayfish (Powell et al., 1995). It is one of only four techniques currently accepted by the Association of Official Analytical Chemists (AOAC) for the identification of fish species (Haynes, 1988). The objective of the present study was to develop biochemical markers, using IEF, that can be used to identify and separate *P. aztecus* and *P. duorarum* at the postlarval and juvenile stages regardless of season. Once these markers have been established, the technique will be used to support studies that will describe seasonal morphological variability for these species and develop reliable descriptive methods for their identification.

*Materials and methods.*—*Penaeus duorarum* (n = 33) ranging in size from 8 to 60 mm total length (TL) were collected from Davis Bayou, MS, and Perdido Key, Key Biscayne, Pensacola, and Tampa Bay, FL, in the spring and fall of 1993. *Penaeus aztecus* (n = 33) of similar size were collected from Davis Bayou, MS, and Perdido Key and Pensacola, FL, in the spring and fall of 1993. A series of laboratory-reared shrimp of known parentage were used to determine the presence of protein bands for a wide range of developmental stages.

Protein samples were prepared from the tail muscle of shrimp. The head and sixth abdominal segment from each shrimp were removed and frozen at −70 C for later morphological examination. The tail, with the shell intact, was then used for protein extraction; proteins were extracted successfully from postlarval shrimp as small as 8 mm TL (this was not attempted for smaller postlarvae).

Water-based protein extractions were performed on ice and frozen immediately after preparation. Samples were prepared by placing a known weight of tail muscle in a 1.5-ml microfuge tube and adding distilled water to obtain a final dilution of 1:3 (w:v). For small tissue samples, a standard volume of 30 µl of distilled water was added. The tissue was then homogenized using a plastic rod fitted to the tube. The samples were placed in a water bath sonicator with ice for 10 sec. The homogenate was centrifuged in an Eppendorf microcentrifuge for 20 min at 8,800 RCF. The resulting supernatant was transferred to a new tube, frozen, and stored at −20 C.

*IEF.*—IEF gels were prepared based on a modification of Whitmore's (1990) protocol (Powell et al., 1995). The protein content of the samples was determined with a Bio-Rad Bradford protein assay using bovine serum albumin as the standard. Sample volumes were adjusted to yield 10 mg of protein per sample application. Samples were applied directly to the gel surface using an applicator mask (Pharmacia Biotech, Piscatawasy, NJ) that formed wells on the gel surface. A broad pH range (3–10) was initially used to identify regions containing numerous protein bands. A suitable narrow pH range (3–6) was then selected to maximize the separation of protein bands in the region of interest.

Gels were visually inspected to identify banding pattern differences and were also scanned using a Ultrascan XL enhanced laser densitometer (LKB Pharmacia). Densitometric data were collected and stored using the GelScan XL program (version 2.1). This information


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was used by the program to construct standard curves based on the migration distances of proteins of known isoelectric point (pI). The pIs of unknown proteins were then determined from this curve.

*Results.*—Laboratory-reared *P. aztecus* postlarvae, juveniles, and adults were differentiated from those of *P. duorarum* based on the presence or absence of a protein band in all age classes examined. This protein band was located at pI 5.41 in *P. aztecus* and at pI 5.09 in *P. duorarum* (Fig. 1A). The same proteins were present in wild-caught shrimp and could be used for the identification of postlarval, juvenile, and adult *P. aztecus* and *P. duorarum* regardless of their location or season of capture (Fig. 1B). The presence or absence of protein bands was clearly visible in the chromatographic representations of the banding patterns produced by laser densitometry (Fig. 2). The protein bands are represented as single peaks on the chromatograph. The location, height, and width of each individual peak corresponds to the pI value, staining intensity, and width of the protein band on the gel, respectively. These chromatographs are easily stored for future comparison in species identifications.

*Discussion.*—IEF is a reliable biochemical technique for the identification and separation of closely related aquatic species. IEF of total proteins has been adopted by the AOAC as one of the four currently accepted techniques for species identification (Haynes, 1988). Although most commonly employed for the identification of fish, IEF has also been successfully used to verify the occurrence of Pacific white shrimp in lower Laguna Madre, TX (Balboa et al., 1991), to separate morphologically similar penaeid species from the western Atlantic Ocean and the eastern and Indo-western Pacific Ocean, and to substantiate recent systematic reclassifications within the genus *Penaeus* (King et al., 1992). We have also tested the accuracy of IEF procedures developed in our laboratory in a blind study of 18 adult *P. aztecus* and *P. duorarum* shrimp that could be identified using available keys. The shrimp were identified by a National Marine Fisheries taxonomist (samples provided by Harriet Perry, Gulf Coast Research Laboratory, Ocean Springs, MS) and sent to our laboratory with only numerical identifications. Resulting protein banding patterns corroborated the morphological identifications of all individuals. Since the protein bands used to differentiate the species are located in different orientations relative to β-lactoglobin (pI 5.1), a commercially available protein standard, we were able to distinguish *P. duorarum* and *P. aztecus* electrophoresed on different gels without the use of authenticated shrimp protein of either species. Densitometric scans of protein banding patterns allows reproducible and quantitative analysis of gels and provides a convenient computer database for long-term storage of electrophoretic data that can be easily referenced by other researchers.

King et al. (1992) used high-resolution gels in the pH range of 4.5–5.0 to characterize and separate 17 species of penaeid shrimp. Densitometric scans of those gels were able to distinguish these species by the presence of protein bands in the pH range of 4.62–4.95. They observed a high degree of interspecific variability and no intraspecific variation among major protein bands in that pH range. However, they did not report on possible effects of sex, stage of development, or seasonality on protein banding patterns. In the present study, IEF gels in the pH range of 3–6 were used. We observed the most striking differences in protein banding patterns between *P. duorarum* and *P. aztecus* shrimp in the pH range of 5–5.5. Intraspecific variability among the major protein bands in that pH range was not observed. Results from our study have demonstrated that IEF can be used for the identification and separation of *P. duorarum* and *P. aztecus* from the northern Gulf of Mexico regardless of sex, stage of development, or season.

Identification of penaeid postlarvae is difficult owing to plasticity of morphological features. Christmas et al. (1966) observed substantial intraspecific morphological variation among penaeid postlarvae collected in Mississippi coastal waters at different times of the year and concluded that morphological differences useful for separating *P. aztecus* and *P. duorarum* postlarvae in the spring are not useful later in the year. Researchers from several laboratories involved in monitoring recruitment of penaeid shrimps from the north-central Gulf of Mexico have also experienced difficulties in separating postlarval *P. aztecus* and *P. duorarum,* particularly with specimens collected during the late summer and fall months (KCS, pers. obs.). Unlike identifications based on morphology, which are often limited to juvenile and mature specimens, IEF of total proteins permits the reliable identification of postlarval *P. aztecus* and *P. duorarum* regardless of intraspecific morphological variations. Lavery and Staples (1990) used allozyme electrophoresis to separate postlarvae of morphologically similar species of prawns from the Gulf of Car-

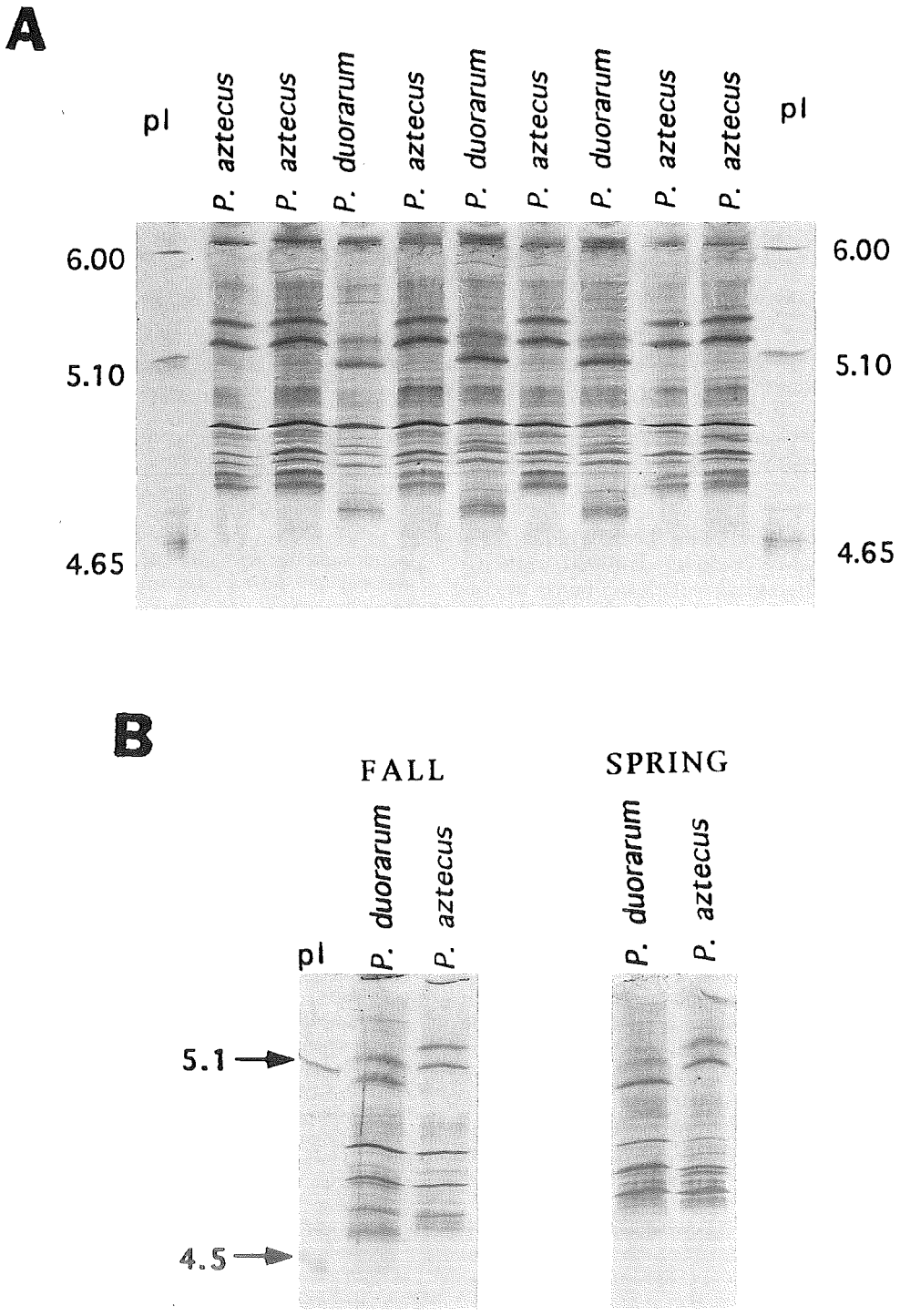


Fig. 1. Isoelectric focusing gel showing species-specific protein banding patterns. Lanes labeled pI show protein standards of known isoelectric points; the specific pI value for each standard protein band is indicated by the number next to the band. (A) Individual adult *Penaeus duorarum* and *P. aztecus*. (B) Spring and fall postlarval *P. duorarum* and *P. aztecus*. The protein bands indicated by the arrows in the first lane are standards of known isoelectric points.


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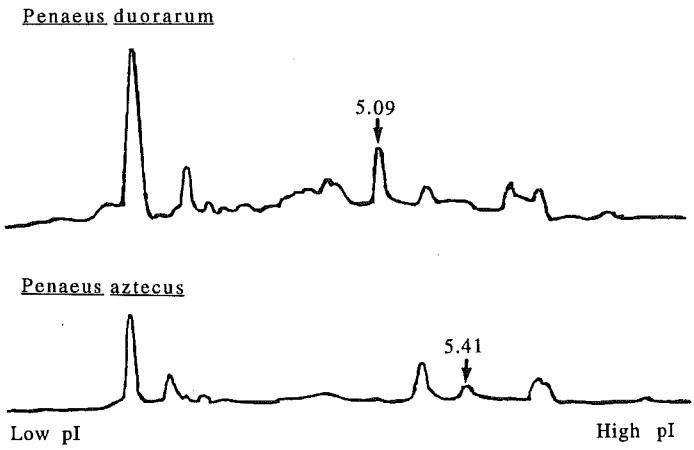


Fig. 2. Chromatograph produced by densitometric scanning of isoelectric focusing gels. The arrow indicates the peak produced by the protein band used for identification of each species.

pentaria, Australia. Using this technique, 95% of the postlarvae could be identified, while existing morphological techniques failed to correctly identify 15% of the individual postlarvae examined. Because of the difficulties in identifying postlarval penaeids, protein electrophoretic techniques such as IEF should be periodically employed to verify identifications as a means of quality control in laboratories involved in research on recruitment or routine monitoring of penaeid shrimp postlarvae. With modifications to the sample preparation procedures described in this study, IEF protein fingerprints have been obtained on individual penaeid larvae as small as 1.0 mm (Cline and Stuck, 1991). Protein electrophoretic techniques have also been developed for the identification of individual planktonic marine organisms as small as 250μm (Hu et al., 1992). Accurate identification of shrimp at a wide range of developmental stages will allow researchers to better understand distribution and recruitment patterns of penaeid shrimp. This information will be useful in the development of management strategies to protect these commercially important species.

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SALINITY TOLERANCE OF FOUR ECOLOGICALLY DISTINCT SPECIES OF *FUNDULUS* (PISCES: FUNDULIDAE) FROM THE NORTHERN GULF OF MEXICO.—Fishes that use estuaries during all or a portion of their life history provide an ideal opportunity to study the influence of abiotic factors on distribution and abundance as they relate to the highly variable conditions in that environment. Often, closely related species segregate along abiotic estuarine gradients (see Martin, 1988; Dunson et al., 1993), and this is most obvious when congeneric species exhibit species-specific salinity tolerances. One of the most abundant groups of estuarine resident fishes in the Western Hemisphere are members of the Fundulidae, with 28 species in the genus *Fundulus*. By nature, this group is extremely euryhaline (Nordlie and Walsh, 1989; Perschbacker et al., 1990; Toepfer and Barton, 1992), but has been found to segregate along salinity gradients (Weisberg, 1986; Peterson and Ross, 1991).

Our understanding of the relationship between salinity and fundulid occurrence and abundance stems mainly from distributional studies, although photoperiod has been shown to affect salinity selection/preference in *F. grandis* (Fivizzani and Meier, 1978; Spieler et al., 1976). *Fundulus grandis* occurs between 0.1 and 76.0 (Simpson and Gunter, 1956; Renfro, 1960), *F. m. similis* between 0.8 and 76.1‰ (Kilby, 1955; Simpson and Gunter, 1956), *F. chrysotus* between 0 and 24.7‰ (Kilby, 1955), and *F. notti* between 0 and 10.0‰ (Peterson and Ross, 1991). Because a more accurate understanding of estuarine community assembly requires quantification of the direct effects of salinity, we became interested in salinity tolerance of fundulids. Specifically, we wanted to determine the salinity tolerance of four fundulids that distribute themselves along salinity gradients, which, in our area, range from 0 to ~28‰. To achieve this, we quantified acute (7‰, 14‰, 21‰, 28‰, and 35‰) and chronic (salinities up to 70‰) salinity exposures for *F. notti* and *F. chrysotus*, two species that use tidal freshwater and oligohaline sections of the Biloxi Bay estuary, MS, and *F. grandis* and *F. m. similis*, two euryhaline fundulids found lower in the estuary.

*Materials and methods.*—Field collections and laboratory adjustment protocols: The four species of fundulids were collected from Biloxi Bay and Old Fort Bayou, MS, and transported to the laboratory in water from the collection site between October and November. These postspawning fundulids (Greeley et al., 1986, 1988) ranged between 30 and 50 mm total length (TL), and we used both male and female fish in the experiments. The fish were left in coolers with high aeration overnight to allow the water to adjust to room temperature. Fish were then transferred to holding tanks with salinities equal to those in which they were caught (salinities varied depending on location and date collected). *Fundulus chrysotus* and *F. notti* were held at 0‰. Preliminary data


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