Identification of Commercial Penaeid Shrimp Species by Isoelectric Focusing

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Abstract: Muscle proteins were isolated from 7 western Atlantic and 10 Pacific Ocean *Penaeus* species encompassing 6 subgenera utilizing isoelectric focusing. Protein banding patterns of individuals from the 17 species were distinguishable. A high resolution gradient of pH 4.5–5 was required to separate complex banding patterns and facilitate identification among species possessing similar banding patterns. Most species were characterized by the presence of 2–6 major protein bands in the pH 4.62–4.95 range. Densitometric analysis provided a graphic representation of differences in band migration, number, and intensity among the 17 *Penaeus* species. Due to the large number of protein bands and their proximity to each other, reference densitometric tracings were necessary to consistently distinguish all species. General protein patterns observed in this study appeared to substantiate recent systematic reclassifications within the genus *Penaeus*.

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Exotic penaeid shrimp species have received considerable attention from the U.S. aquaculture industry since the early 1970s and later, wildlife management agencies. Exotic species have demonstrated culture characteristics superior to native shrimps and have become established as the major species utilized by commercial aquaculture in the southeastern United States. Recent accidental releases into U.S. waters of exotic shrimps held under intensive culture conditions has generated concern among wildlife agencies (Balboa et al. 1991, S. Hopkins pers. commun.). The environmental and economic consequences of these introductions remain undetermined.

Only slight differences in morphology are exhibited among penaeid species (Holthius 1980) which could hinder recognition of exotics by untrained observers. Although unaltered adult shrimp can be distinguished using morphological and meristic characteristics, shrimp cannot be accurately identified solely from tail morphology. Wildlife management and consumer protection agencies need a reliable identification method for altered (i.e., headless) specimens.

Isoelectric focusing (IEF), a high resolution protein separation technique, is well suited for examination of general protein profiles of related species (Krzynowek and Wiggin 1979, Lundstrom 1979). Sarcoplasmic proteins separated by IEF have proven useful for distinguishing specimens of northern pink shrimp (*Penaeus duorarum*) and northern white shrimp (*P. setiferus*) (An et al. 1988, 1989). A comparison of IEF protein banding patterns among morphologically similar *Penaeus* species has not, to our knowledge, been performed.

The utility of water-soluble muscle proteins in elucidating taxonomic relationships is well documented in fish (Avise 1974, Buth 1984). However, literature of biochemical investigations into systematic issues of penaeid shrimps is lacking. Numerous taxonomic reclassifications have recently occurred within the genus *Penaeus* based on differences in life history and morphological characteristics (Holthius 1980).

Creation of a library of protein patterns ("fingerprints") of commercially important penaeid shrimp species could assist wildlife law enforcement officials and consumer protection agencies in shrimp identification. Further, a survey of general proteins provided by IEF may help clarify the systematics of the genus *Penaeus*. The objectives of this study were to establish an extensive library of diagnostic muscle protein patterns of commerically important penaeid species from the western Atlantic and Pacific Oceans using IEF and to provide insight into the systematics of the genus *Penaeus* utilizing muscle protein patterns.

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Methods

Species native to Texas were collected in fall 1990 by seine or trawl and frozen at −85° C until processed for IEF. Non-native species were shipped frozen from their respective collecting localities (Table 1); most were collected in fall 1990. Intact specimens were identified based on morphological characteristics described in taxonomic keys (Holthuis 1980, Perez-Farfante 1988). Muscle tissue was excised from lateral musculature of the sixth abdominal segment of each specimen. Tissue samples were homogenized in an equal volume of deionized water; after centrifugation supernatant was stored at −85° C for IEF.

IEF was performed on 0.25-mm polyacrylamide gels following the methods of King et al. (1991) employing 0.8 ml pH 4.5–5 and 0.2 ml pH 3–10 of ampholytes (Serva Biochemicals, Paramus, N.J.). Gels were cast on support film (GELBOND, Pharmacia LKB Instruments, Piscataway, N.J.) and allowed to polymerize for 1

Table 1. General collecting localities of western Atlantic and Pacific penaeid species subjected to isoelectric focusing of sarcoplasmic proteins in a pH 4.5–5 gradient.

Common name[a]	Scientific name	Collection site	*N*
Western Atlantic			
Northern white shrimp	*Penaeus* (*Litopenaeus*) *setiferus*	Matagorda Bay, Texas	50
Southern white shrimp	*P.* (*Litopenaeus*) *schmitti*	Margarita Island, Venezuela	43
Northern pink shrimp	*P.* (*Farfantepenaeus*) *duorarum*	Matagorda Bay, Texas	50
Southern pink shrimp	*P.* (*Farfantepenaeus*) *notialis*	Margarita Island, Venezuela	6
Northern brown shrimp	*P.* (*Farfantepenaeus*) *aztecus*	Matagorda Bay, Texas	50
Southern brown shrimp	*P.* (*Farfantepenaeus*) *subtilis*	Margarita Island, Venezuela	12
Redspotted shrimp	*P.* (*Farfantepenaeus*) *brasiliensis*	Margarita Island, Venezuela	4
Eastern Pacific			
Pacific white shrimp	*P.* (*Litopenaeus*) *vannamei*	Parita Gulf, Panama	100
Pacific blue shrimp	*P.* (*Litopenaeus*) *stylirostris*	Parita Gulf, Panama	10
Western white shrimp	*P.* (*Litopenaeus*) *occidentalis*	Guaymas, Mexico	20
Yellowleg shrimp	*P.* (*Farfantepenaeus*) *californiensis*	Guaymas, Mexico	12
Indo-western Pacific			
Giant tiger prawn	*P.* (*Penaeus*) *monodon*	Bataan, Philippines	20
Green tiger prawn	*P.* (*Penaeus*) *semisulcatus*	Tel Aviv, Israel	15
Kuruma prawn	*P.* (*Marsupenaeus*) *japonicus*	Nagoya, Japan	4
Fleshy prawn	*P.* (*Fenneropenaeus*) *chinesis*	Butuan, Philippines	4
Indian white prawn	*P.* (*Fenneropenaeus*) *indicus*	Butuan, Philippines	20
Aloha prawn	*P.* (*Melicertus*) *marginatus*	Oahu, Hawaii	5

[a]Common names according to Holthuis (1980).

hour. Protein separation was achieved with an anode solution of 0.5 M acetic acid solution, pH 3 and a cathode solution of 0.5 M sodium hydroxide, pH 12. Gels were focused on flatbed units cooled to 10° C. Current (mA) was adjusted to a starting voltage of 200 volts; final voltage was limited to 1,200 volts. Initial power was set at 4 watts. Gels were prefocused for 20 minutes. Protein extracts were loaded using 10-mm filter paper wicks saturated with sample. Gels were focused until no decrease in resistance (mA) was observed for 10 minutes. Gels were fixed for 5 minutes with 12.5% trichloroacetic acid / 4% sulfosalicylic acid, washed with 100 ml of 40% methanol / 10% glacial acetic acid, and stained with 5% Coomasie blue R-250 (Serva Biochemicals). Gels were de-stained with wash solution and air dried at room temperature.

Isoelectric points (pI) of shrimp protein bands were indirectly assigned by comparison with pI values produced by standard protein markers (beta-lactoglobulin, pI 5.1; and trypsin inhibitor, pI 4.6; Serva Biochemicals). Gels were scanned utilizing an UltroScan XL Laser densitometer employing the GelScan 2.0 software package (Pharmacia LKB Instruments). Shrimp species were distinguished by identification of unique protein bands.

Results and Discussion

Muscle proteins were isolated from 7 western Atlantic and 10 Pacific Ocean *Penaeus* species encompassing 6 subgenera, utilizing IEF (Table 1). Protein banding

patterns of individuals from the 17 species were distinguishable. A high resolution gradient of pH 4.5–5 was required to satisfactorily separate complex banding patterns and facilitate identification among species possessing similar banding patterns (Figs. 1, 2). A high degree of interspecific variability in protein banding patterns was observed. Intraspecific variation was absent from major (most dense) protein bands; some variation was observed in minor protein bands. Most species were characterized by the presence of 2–6 major protein bands and several minor bands in the pH 4.62–4.95 range. Densitometric analysis provided a graphic representation of differences in band migration, number, and intensity among the 17 *Penaeus* species (Figs. 3, 4). Due to the large number of protein bands and their proximity to each other, reference densitometric tracings were necessary to consistently distinguish all species. Although variation occurred in relative peak heights (absorbance) from sample to sample, the migration distance (from anode) and the ratio of absorbance values among bands were consistent within a species.

Western Atlantic Penaeid Shrimp

Little similarity existed between general protein banding patterns of the northern white (*P. setiferus*) and southern white shrimps (*P. schmitti*); no protein bands were shared between the species (Figs. 1, 3). Until 1967, they were considered conspecific based on morphological similarities (Holthuis 1980). In addition, hybrids have been artificially produced between these species (J. L. Lester pers. commun.). The protein banding patterns presented in this study suggest they are distinct species. Banding patterns of the southern white shrimp were more similar to consubgenerics from the eastern Pacific (Table 1; Figs. 1, 2) than to the northern white shrimp. The 2 most morphologically similar species, the northern pink shrimp (*P. duorarum*) and southern pink shrimp (*P. notialis*), also exhibited the most similar protein banding patterns (Figs. 1, 3). Compared to other species, the northern pink and southern pink shrimps exhibited minor protein band divergence. Southern pink shrimp exhibited a major band (pI = 4.93) slightly more acidic than the corresponding band in northern pink shrimp (pI = 4.94); migration and absorbance

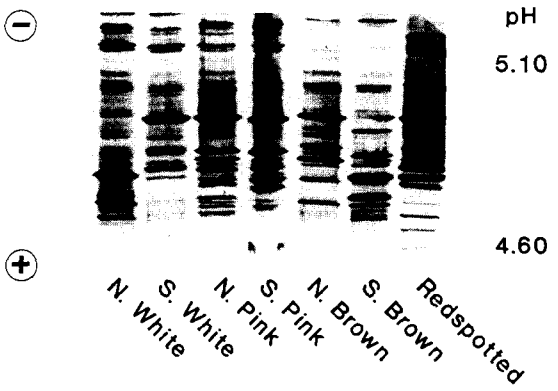

Figure 1. Muscle protein patterns from 7 commercially important western Atlantic *Penaeus* species subjected to thin-layer isoelectric focusing in a pH 4.5–5 gradient. Scientific names are provided in Table 1.

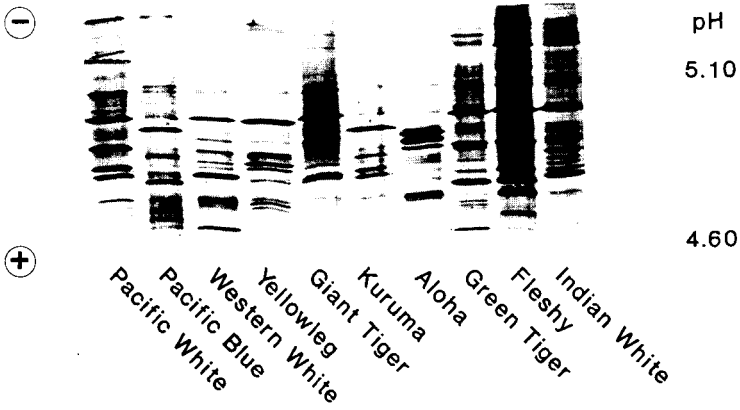

Figure 2. Muscle protein patterns from 10 commercially important eastern and indo-western Pacific *Penaeus* species subjected to thin-layer isoelectric focusing in a pH 4.5–5 gradient. Scientific names are provided in Table 1.

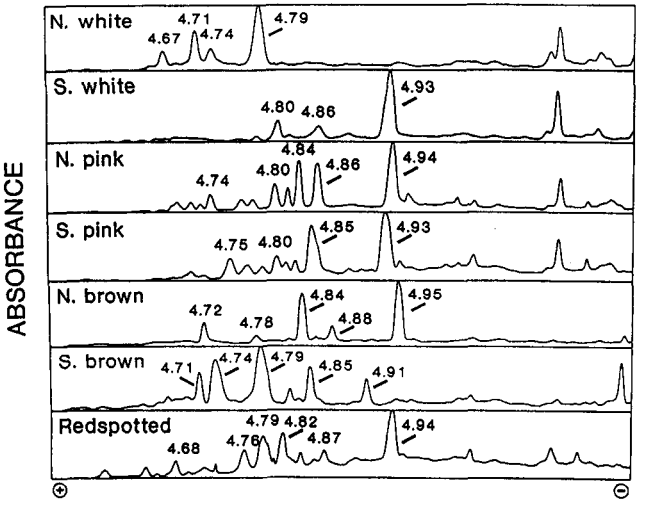

Figure 3. Densitometric tracings and isoelectric points for representative muscle protein bands of 7 commercially important western Atlantic *Penaeus* species subjected to isoelectric focusing in a pH 4.5–5 gradient. Band intensity (absorbance) is indicated by relative peak height. Migration distance is relative to anode (+) electrode strip. Scientific names are provided in Table 1.

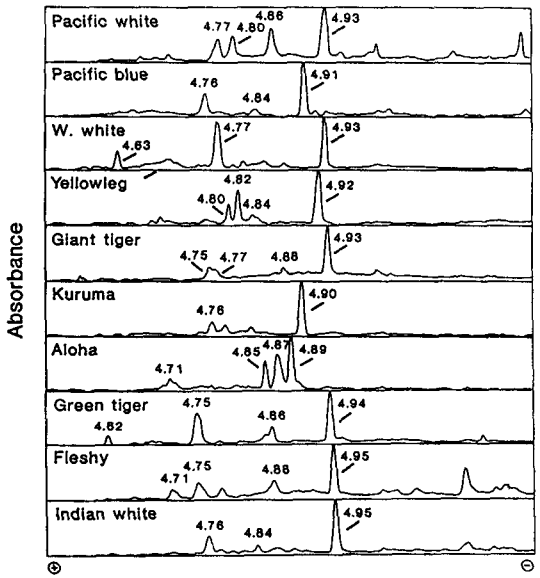

Figure 4. Densitometric tracings and isoelectric points for representative muscle protein bands of 10 commercially important eastern and indo-western Pacific *Penaeus* species subjected to isoelectric focusing in a pH 4.5–5 gradient. Band intensity (absorbance) is indicated by relative peak height. Migration distance is relative to anode (+) electrode strip. Scientific names are provided in Table 1.

differences were observed in some minor bands. This degree of differentiation suggests speciation may have been relatively recent or it may be incomplete.

The observed biochemical similarity between the pink shrimps corresponds well with historical taxonomic classifications of these putative species. Until reclassification as distinct species (Perez-Farfante 1988), the pink shrimps were considered conspecific (subspecies) (Perez-Farfante 1967) based upon morphological characteristics. Further IEF analysis or an allozyme survey among geographic populations throughout the range of each putative species is needed to determine if they are reproductively isolated.

Before 1939, the northern brown (*P. aztecus*), southern brown (*P. subtilis*), and redspotted shrimps (*P. brasiliensis*) were taxonomically classified as conspecific under *P. brasiliensis* (Holthuis 1980). However, in 1967 the northern brown and southern brown shrimps were classified as conspecific (subspecies) and distinct from the redspotted shrimp (Perez-Farfante 1967). Recently the northern brown and southern brown shrimps were reclassified as distinct species (Perez-Farfante 1988). The distinct protein patterns observed for these 3 species (Figs. 1, 3) suggest the latest classification is correct (i.e., 3 distinct species).

The northern white shrimp and southern brown shrimp exhibited the most distinctive banding patterns among western Atlantic species (Figs. 1, 3). Both species exhibited major protein bands considerably more anodal than other species, yet were easily distinguishable from each other.

Attempts to collect the only remaining commercially important western Atlantic *Penaeus* species, the São Paulo shrimp (*P. paulensis*), were unsuccessful.

Pacific Penaeid Shrimp

Due to large geographic distances separating the ranges of the eastern and indo-western Pacific penaeid species, protein banding patterns from each region are presented separately.

Eastern Pacific.—Protein banding patterns of the 4 eastern Pacific *Penaeus* species were easily distinguishable. Several diagnostic differences in major protein bands were observed among the 3 members of the subgenus *Litopenaeus:* Pacific white shrimp (*P. vannamei*), Pacific blue shrimp (*P. stylirostris*), and western white shrimp (*P. occidentalis*). The yellowleg shrimp (*P. californiensis*) exhibited a similar banding pattern to some Atlantic consubgenerics, exhibiting 2 well-separated major bands and several minor bands.

All 4 species are commerically important to the Pacific Coast of Central and South America (Holthuis 1980). Populations of the Pacific white and Pacific blue shrimp comprise the majority of the New World aquaculture industry. The capability to positively identify these species as U.S. imports of either industry will provide a powerful tool to regulatory and enforcement agencies.

Indo-Western Pacific.—The aloha prawn (*P. marginatus*) exhibited the most distinctive banding pattern of all the Pacific penaeid species. This species possessed 3 tightly grouped major bands with pI values slightly lower than the cathodal-most band in the remaining 5 species. The more anodal protein bands present in the 5 remaining species are either absent or correspond to 1 of the 3 tightly grouped bands in aloha prawn.

The fleshy prawn (*P. chinensis*) and the Indian white prawn (*P. indicus*), members of the subgenus *Fenneropenaeus* exhibited clearly distinct protein banding patterns. Taxonomic questions as to the distinctness of these 2 species have been raised previously (Holthuis 1980). Divergent protein banding patterns observed between the 2 species in the present study suggest they are distinct species.

The fleshy prawn comprises the majority of the Asian aquaculture industry and has been implicated in mislabeling of imported shrimp as domestic product (D. M. Soignet pers. commun.). The capability to positively identify this species provides consumer protection agencies with a powerful enforcement tool.

The giant tiger prawn (*P. monodon*) and the green tiger prawn (*P. semisulcatus*), members of the subgenus *Penaeus,* exhibited similar, yet distinct banding patterns. The giant tiger prawn exhibited 2 bands positioned more cathodal than the corresponding band(s) in the green tiger prawn; they exhibited similar pI values for the most cathodal major band. The similarity in protein banding patterns may represent a relatively recent speciation event or convergent evolution.

The Kuruma prawn (*P. japonicus*) was easily distinguished by its single major protein band appearing slightly anodal to most indo-western Pacific species. The absence of other major protein bands may have been the result of extended storage of tissue samples. Attempts to acquire additional samples were unsuccessful.

Conclusions

This study suggests that in tissues possessing soluble muscle protein, IEF is a robust technique for penaeid species identification. A library of protein profiles (densitometric tracings) of 17 western Atlantic and Pacific penaeid shrimp now exists that can provide wildlife and consumer protection agencies with an accurate means of species identification. In addition, general protein patterns observed in this study appeared to substantiate recent systematic reclassifications within the genus *Penaeus*.

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