ELECTROPHORETIC COMPARISON OF FIVE SPECIES OF PANDALID SHRIMP FROM THE NORTHEASTERN PACIFIC OCEAN

ALLYN G. JOHNSON, FRED M. UTTER, AND HAROLD O. HODGINS[1]

ABSTRACT

Pandalid shrimp from off Alaska, Washington, and Oregon were investigated using starch-gel electrophoresis. Each species was found to be polymorphic for phosphoglucomutase, and the general protein patterns separated them into two groups—one consisting only of *Pandalus hypsinotus* and the other containing *P. borealis, P. goniurus, P. jordani*, and *Pandalopsis dispar*.

A key based on biochemical characters was developed which could separate the five pandalid species investigated.

The increase of commercial fishing for shrimp along the Pacific coast of North America in recent years has stimulated interest in the biology and identification of species and population units. Ronholt (1963) reported on the distribution and relative abundance of five species of pandalid shrimp from the northeastern Pacific Ocean. Butler (1965) presented a comprehensive report on the growth, reproduction, and distribution of pandalid shrimp in British Columbia waters, demonstrating the importance of inlets and bays to this group of crustaceans. Several reports on sampling techniques, diel vertical migration, and population movements have occurred which emphasize the need for additional information on the biology of pandalid shrimp for optimal utilization of this resource (Barr and McBride, 1967; Barr, 1970, 1971; Gotshall, 1972).

One of the more promising techniques for the detection of population units is the biochemical genetic approach, utilizing starch-gel electrophoretic separation of proteins coupled with histochemical staining procedures (Hunter and Markert, 1957). This method has been widely used and successfully applied to fisheries problems (reviewed by de Ligny, 1969, 1972).

This paper reports our application of starch-gel electrophoresis to separation of species and populations of five species of shrimp which occur along the coast of the northeastern Pacific ocean.

MATERIALS AND METHODS

Five species of adult pandalid shrimp from two genera were investigated; *Pandalopsis dispar, Pandalus borealis, P. goniurus, P. hypsinotus,* and *P. jordani*. All samples except those of *P. jordani* and one collection of *P. hypsinotus* were obtained from Marmot and Kazakof Bays of Kodiak Island, Alaska, during May 1972, and identified by personnel of the National Marine Fisheries Service at Kodiak, Alaska. These samples were shipped frozen to our laboratory where they were kept at −15°C until tested. Two collections of *P. jordani* were obtained off Coos Bay and Astoria, Oreg., in 1971 and identified by personnel of the Fish Commission of Oregon, shipped to us frozen and kept at −15°C until tested. Additional samples of *P. jordani* and *P. hypsinotus* were obtained during December 1972 from Bellingham Bay, Wash.

Extracts of muscle tissue were prepared by mixing equal volumes of tissue and 2% phenoxyethanol in distilled water into uniform pastes with glass rods. The starch-gel electrophoretic procedure followed the methods reported by Johnson, Utter, and Hodgins (1972). The buffer system used was described by Ridgway, Sherburne, and Lewis (1970). After electrophoresis the gels were sliced into four horizontal slices and stained for phosphoglucomutase (PGM), lactate dehydrogenase (LDH), tetrazolium oxidase (TO), peptidase (Johnson et al., 1972), malate dehydrogenase NAD and NADP (MDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Shaw

[1]Northwest Fisheries Center, National Marine Fisheries Service, NOAA, 2725 Montlake Boulevard East, Seattle, WA 98112.

Manuscript accepted November 1973.
FISHERY BULLETIN: VOL. 72, NO. 3, 1974.

and Prasad, 1970), naphthyl acetate and propionate esterases (modified after Utter, Stormont, and Hodgins, 1970), and general protein (Johnson et al., 1972).

RESULTS AND DISCUSSION

Bands of identical electrophoretic mobility for a given enzyme system were observed in all species for the following systems: MDH (NADP), one anodal band; MDH (NAD), one anodal band; peptidase (valyl-leucine), three anodal bands; TO, one anodal band; and GAPDH, three anodal bands.

Esterase patterns varied among species; however, the patterns were weak and not completely repeatable. Gasser and Rowlands (1972) noted similar problems in interpreting esterase patterns from human serum and related the differences to nongenetic causes. We have, therefore, excluded them from further consideration in this study. Esterases have been found useful in studies of other invertebrates and may indeed be of use in studies of pandalid shrimp if reliable methods can be developed for stabilizing expression of patterns (Manwell and Baker, 1970; Barlow and Ridgway, 1971).

Lactate dehydrogenase was expressed as one anodal band—with a broad, faint-staining area anodal to it—in each species. The bands of *P. jordani, P. borealis,* and *P. goniurus* had an identical mobility slightly more anodal than the bands of *P. hypsinotus* and *Pandalopsis dispar.*

The general protein patterns observed are shown in Figure 1. *Pandalus hypsinotus* had bands C, E, F, and G; *P. jordani* and *P. borealis,* bands A, B, D, and G; and *P. goniurus* and *Pandalopsis dispar*, bands A, B, and G. The B band, although qualitatively invariable, varied considerably in intensity in all species expressing it. A greater degree of difference in the protein pattern was observed between *Pandalus hypsinotus* (processing three unique bands) and the other four species than was observed among these four species.

Phosphoglucomutase (PGM) was polymorphic in all five species. Two-banded phenotypes (Figure 2) were observed in some individuals of all species, presumably reflecting heterozygous individuals, and the pattern suggests that the active PGM enzyme in shrimp is a monomer (Shaw, 1964). This agrees with reports of PGM polymorphisms found in vertebrates (see Johnson, Utter, and Hodgins, 1971). A diagrammatical representation of the allelic forms found within the five species (Figure 3) shows the six allelic bands that were observed and designated (in decreasing anodal mobility) A, B, C, D, E, and F. The distribution of these alleles, as indicated in Figure 3, was *P. hypsinotus*, A, B, E, and F; *P. goniurus*, A and B; *P. borealis*, C, E, and F; *P. jordani*, B, C, and E; and *Pandalopsis dispar*, C, D, and E. The phenotypic distributions of PGM in the five species of shrimp along with gene frequencies, Hardy-Weinberg calculations and collections data are presented in Tables 1 and 2. With the exception of *Pandalus hypsinotus*, the phenotypic distributions of PGM of the collections of shrimp species did not deviate significantly

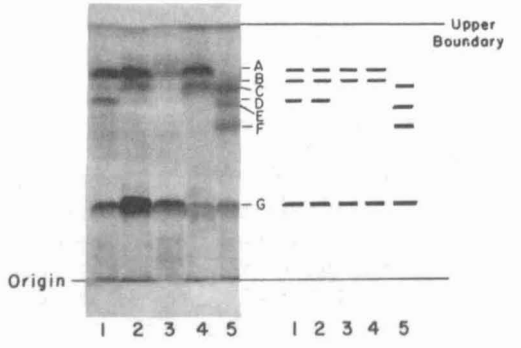

FIGURE 1.—Electrophoretic patterns on starch gel of muscle protein of five species of shrimp from the northeastern Pacific Ocean. Numbers below the patterns indicate the following species: 1. *Pandalus jordani*, 2. *P. borealis*, 3. *P. goniurus*, 4. *Pandalopsis dispar*, and 5. *Pandalus hypsinotus.*

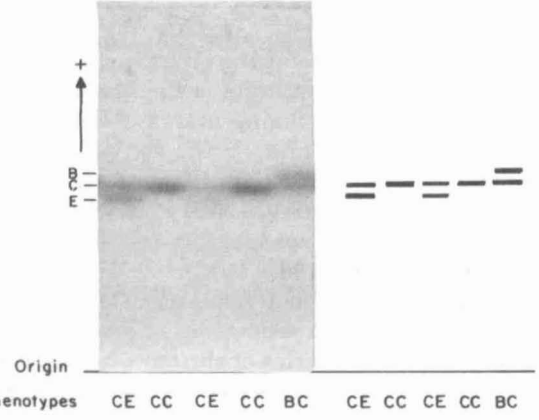

FIGURE 2.—Phosphoglucomutase phenotypes of *Pandalus jordani* in starch gels suggesting monomeric configuration of this enzyme.

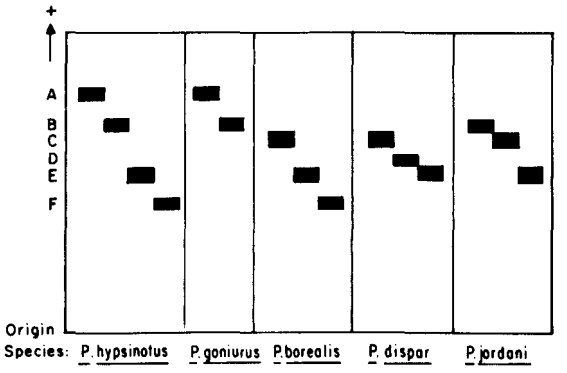

FIGURE 3.—Diagrammatical representation of the alleles of phosphoglucomutase in five species of pandalid shrimps as shown by the technique of starch-gel electrophoresis.

from expected Hardy-Weinberg values, and the gene frequencies of intraspecific samples were similar over the geographic range samples for *P. jordani*.

The collections of *P. hypsinotus* from Alaska showed highly significant deviation from Hardy-Weinberg expectations. Variation from expected Hardy-Weinberg proportions has also been observed for PGM variants of Pacific ocean perch, *Sebastes alutus* (Johnson et al., 1971) and related to depth of capture. Gene frequencies were similar for shallow- and deepwater collections; however, deepwater collections deviated significantly from expected Hardy-Weinberg frequencies. Further sampling stratified by depth (or other measurable variables) may reveal similar relationships for PGM variants of *P. hypsinotus*.

Inspection of the allele frequencies of the Alaskan and Washington collections of *P. hypsinotus* showed marked differences, especially with the A and E alleles (Table 1). These differences indicate that PGM variation may be useful in population identification of *P. hypsinotus*. Bullini and Coluzzi (1972) have presented evidence for selection of PGM alleles over a broad geographic range in mosquitoes *Aedes aegypti* and *A. mariae*. Additional sampling of *P. hypsinotus* may reveal a similar phenomenon as that found in the mosquito species.

The general protein, LDH, and PGM patterns observed in the five species were used to produce a key by which the species can be separated (Figure 4). This type of key should prove useful in application to shrimp identification problems.

CONCLUSION

The general protein patterns separated the species examined into two groups, one consisting only of *P. hypsinotus* and the other containing the remaining species of *Pandalus* and *Pandalopsis dispar*.

All five species of shrimp studied were polymorphic for PGM, with *Pandalus hypsinotus* possessing the greatest number of alleles (four

TABLE 1.—Phosphoglucomutase phenotypes of *Pandalus hypsinotus* taken off Alaska and Washington, 1971-72.[1]

Location	Sample size	Phenotypes of PGM									
		AA	AB	AE	AF	BB	BE	BF	EE	EF	FF
Alaska:											
Marmot Bay and Kazakof Bay	89	1 (1.8)	15 (9.0)	4 (8.5)	4 (4.1)	11 (11.5)	20 (21.5)	7 (10.3)	9 (10.2)	18 (9.8)	0 (2.3)
Washington:											
Bellingham Bay	80	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.9)	15 (14.6)	0 (0.1)	55 (55.5)	8 (8.9)	1 (0.0)

Location	Allelic frequencies				Test Data		
	A	B	E	F	df	χ^2	P
Alaska:							
Marmot Bay and Kazakof Bay	0.140	0.360	0.337	0.163	6	17.23	[2]$0.01>P>0.001$
Washington:							
Bellingham Bay	0.000	0.106	0.831	0.063	—	—	—
Contingency test comparing allelic composition of Alaska and Washington samples					3	89.64	$P<0.001$

[1]In parentheses are the expected values of a Hardy-Weinberg distribution.
[2]Chi-square test of Hardy-Weinberg distribution.

TABLE 2.—Phosphoglucomutase gene frequencies of four species of pandalid shrimp taken off Alaska, Oregon, and Washington; 1971-72.

Species and location	Sample size	Gene frequencies						Test data[1]		
		A	B	C	D	E	F	df	χ^2	P
Pandalus goniurus										
Alaska:										
Marmot Bay and Kazakof Bay	94	0.016	0.984	—[2]	—	—	—	1	2.34	$0.2>P>0.1$
Pandalus jordani										
Oregon:										
Coos Bay	150	—	0.010	0.980	—	0.010	—	3	0.00	$P>0.99$
Astoria	151	—	0.003	0.990	—	0.007	—	3	0.02	$P>0.99$
Washington:										
Bellingham Bay	79	—	0.000	1.000	—	0.000	—	3	0.00	$P>0.99$
Pandalus borealis										
Alaska:										
Marmot Bay and Kazakof Bay	418	—	—	0.059	—	0.934	0.007	3	7.60	$0.1>P>0.05$
Pandalopsis dispar										
Alaska:										
Marmot Bay	269	—	—	0.011	0.981	0.008	—	3	0.00	$P>0.99$

[1]Chi-square test of Hardy-Weinberg distribution.
[2]Indicates not observed in this species.

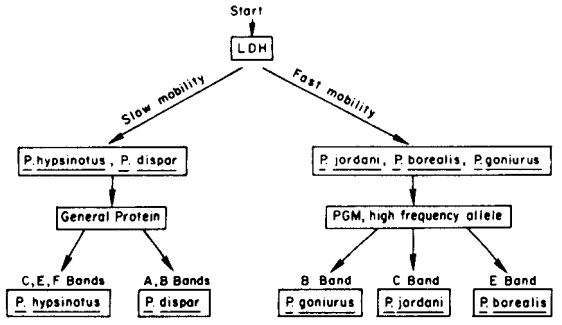

FIGURE 4.—Key to five species of pandalid shrimp based on three biochemical characters.

alleles). This polymorphism may prove useful for separation of breeding groups and as genetic markers in shrimp culturing experiments.

All five species could be identified based on a biochemical key that was developed.

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