# ELECTROPHORETIC INVESTIGATION OF THE FAMILY SCORPAENIDAE

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

## ABSTRACT

Thirty-one species of three genera of the family Scorpaenidae were separated into 17 groups based on starch gel electrophoretic comparison of muscle proteins and six enzymatic systems. This study concluded that relatively greater similarity existed between the Pacific *Sebastes* and the Atlantic *Sebastes* than between either and the other genera. Ten of the 27 species of Pacific *Sebastes* tested had unique biochemical profiles which may be useful for identification of specimens.

The family Scorpaenidae contains several genera in the Pacific and Atlantic Oceans. On the Pacific coast of North America there are four genera—*Sebastes*,[2] *Sebastolobus*, *Scorpaena*, and *Scorpaenodes*. *The genus of Pacific Sebastes* contains over 50 species (Tsuyuki et al., 1968). In this genus new species and extensions of known distribution ranges have been described in recent years (Westrheim, 1965; Westrheim and Tsuyuki, 1967; Nishimoto, 1970; Tsuyuki and Westrheim, 1970). At present there are difficulties in showing taxonomic relations and, in some instances, in making positive identification of specimens using morphometric and meristic methods, although taxonomic relations can be obtained by biochemical methods. Starch gel electrophoresis—developed by Smithies (1955)—coupled with histochemical procedures (Hunter and Markert, 1957) is one of the best biochemical techniques for taxonomic studies.

Scorpaenid muscle proteins and hemoglobin were investigated by starch gel electrophoresis by Tsuyuki et al. (1968). They suggested that the electrophoretic evidence did not support the separation of the two genera *Sebastodes* and *Sebastes*. Chu (1968), using disk electrophoresis of muscle proteins, found different patterns in two out of eight species of *Sebastodes*. Altukhov and Nefyodov (1968) demonstrated serum protein differences between *Sebastes marinus* and *S. mentella* using agar gel electrophoresis.

This paper reports the findings of our investigation of proteins and six enzyme systems found in the skeletal muscle or liver of members of the family Scorpaenidae. Our study involved 27 species of Pacific *Sebastes*, 2 of the Atlantic *Sebastes*, and 1 each of *Sebastolobus* and *Helicolenus*. We present information on the relative biochemical similarity between genera and a key which separates 10 of the 27 Pacific *Sebastes* species studied. This was not a genetic study per se but a research which demonstrated repeatable biochemical differences between species. The observed constancy of biochemical characters examined within a species in samples taken at different ages, depths, and geographic locations is evidence that the reported differences between species are, indeed, genetic. Alternate explanations for such repeatable expression of proteins under the above conditions seem much less likely.

## MATERIAL AND METHODS

Sampling data including location, species, and number of individuals collected are given in Table 1.

Most samples were frozen quickly after capture, but in some instances were kept on ice for short periods; all samples were kept frozen at —20°C after receipt at the laboratory until tested. Extracts were prepared by mixing equal

[1] National Marine Fisheries Service, Northwest Fisheries Center, Seattle Laboratory, 2725 Montlake Boulevard East, Seattle, WA 98102.

[2] In this paper we follow the designation of Bailey (1970) and Chen (1971), considering *Sebastodes* as *Sebastes*. Members of the genus *Sebastes* that were collected along the Pacific Coast of North America are signified in this paper as Pacific *Sebastes*.

Manuscript accepted November 1971.
FISHERY BULLETIN: VOL. 70, NO. 2, 1972.

TABLE 1.—Location and number of specimens of Scorpaenidae collected, 1968-70.

| Species | Location[1] | | | | | | Total number of fish |
|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | |
| Pacific *Sebastes* | | | | | | | |
| *S. aleutianus* | 10 | -- | 6 | -- | -- | -- | 16 |
| *S. alutus* | 217 | -- | 843 | -- | -- | -- | 1,060 |
| *S. auriculatus* | -- | 76 | -- | -- | -- | -- | 76 |
| *S. aurora* | 3 | -- | -- | -- | -- | -- | 3 |
| *S. brevispinis* | 5 | -- | 40 | -- | -- | -- | 45 |
| *S. caenaematicus* | -- | -- | -- | -- | -- | 3 | 3 |
| *S. caurinus* | -- | 283 | -- | -- | -- | -- | 283 |
| *S. chlorostictus* | -- | -- | -- | -- | 1 | -- | 1 |
| *S. crameri* | 2 | -- | 16 | -- | -- | -- | 18 |
| *S. diploproa* | 14 | -- | -- | -- | -- | -- | 14 |
| *S. elongatus* | -- | 297 | 96 | -- | -- | -- | 393 |
| *S. entomelas* | 2 | -- | 2 | -- | -- | -- | 4 |
| *S. flavidus* | 8 | -- | -- | -- | -- | -- | 8 |
| *S. helvomaculatus* | 5 | -- | 19 | -- | -- | -- | 24 |
| *S. levis* | -- | -- | -- | -- | 1 | -- | 1 |
| *S. maliger* | -- | 25 | -- | -- | -- | -- | 25 |
| *S. melanops* | -- | 28 | -- | -- | -- | -- | 28 |
| *S. paucispinis* | 2 | -- | 15 | -- | 1 | -- | 18 |
| *S. pinniger* | -- | -- | 24 | -- | -- | -- | 24 |
| *S. proriger* | 9 | -- | 100 | -- | -- | -- | 109 |
| *S. reedi* | 1 | -- | 110 | -- | -- | -- | 111 |
| *S. ruberrimus* | 5 | 27 | 5 | -- | -- | -- | 37 |
| *S. rubivinctus* | 5 | -- | 34 | -- | -- | -- | 39 |
| *S. saxicola* | 5 | -- | -- | -- | 1 | -- | 6 |
| *S. wilsoni* | -- | -- | 1 | -- | -- | -- | 1 |
| *S. variegatus* | -- | -- | -- | -- | -- | 1 | 1 |
| *S. zacentrus* | 1 | -- | 37 | -- | -- | -- | 38 |
| Atlantic *Sebastes* | | | | | | | |
| *S. marinus* | -- | -- | -- | 9 | -- | -- | 9 |
| *S. viviparous* | -- | -- | -- | 10 | -- | -- | 10 |
| *Sebastolobus alascanus* | -- | -- | 100 | -- | -- | -- | 100 |
| *Helicolenus dactylopterus* | -- | -- | -- | 10 | -- | -- | 10 |

[1] A = Pacific Coast of Washington and Oregon, 1968-70; B = Puget Sound, Wash., 1968-70; C = Queen Charlotte Sound, B.C., Canada, June 1970; D = West Coast of Britain and Ireland, August 1970; E = Avila Beach, Calif., October 1970; F = Cape Ommaney, Alaska, April 1970.

volumes of tissue and phosphate-buffered physiological saline solution (pH 7.4) into uniform pastes with glass rods. The extracts were tested by electrophoresis without further treatment by (1) drawing them into ¼-inch × 3/16-inch filter paper inserts (Schleicher and Schuell grade S and S No. 470)[3], placed on the surface of the tissue-saline mixture, and (2) placing the inserts into starch gels.

Electrophoresis in starch gel followed the methods of Kristjansson (1963). All but two of the biochemical systems were resolved well using a buffer system described by Markert and Faulhaber (1965). Lactate dehydrogenase and phosphoglucomutase were best resolved by using the buffer system described by Ridgway, Sherburne, and Lewis (1970). Gels consisted of 35 g starch plus 250 ml of buffer. A voltage of 300 was applied for 10 min; sample inserts were removed and 400 v applied until indicator dye markers reached a point 6 to 9 cm anodal to the origin. The gels were cooled during electrophoresis by placing ice packs on glass plates on top of the gels. After electrophoresis, bands reflecting enzyme activity were detected by the following methods:

Tetrazolium oxidase (TO) (after Brewer, 1967, and Johnson, Utter, and Hodgins, 1970b):
- 5 mg phenazine methosulfate (PMS)
- 3 mg p-nitro blue tetrazolium (NBT)
- 40 ml tris-citrate buffer (0.03 M tris, 0.005 M citric acid, pH7.0)

L-alpha-glycerophosphate dehydrogenase (αGPDH) (after Nyman, 1967, and Johnson, Utter, and Hodgins, 1970a):
- 5 mg PMS
- 3 mg NBT
- 5 mg NAD+
- 100 mg L-alpha-glycerophosphate
- 40 ml tris-citrate buffer

Lactate dehydrogenase (LDH):
- 10 mg PMS
- 5 mg NBT
- 5 mg NAD+
- 20 ml of 0.5 M sodium lactate solution
- 40 ml tris-citrate buffer

Peptidase A (after Lewis and Harris, 1967, and Lewis and Truslove, 1969):
- 10 mg DL valyl-leucine
- 1 mg horseradish peroxidase
- 5 mg 0-dianisidine in 10 ml acetone
- 0.5 ml M $MgCl_2$
- 1 mg *Bothrops atrox* venom
- 40 ml tris-citrate buffer

Phosphoglucomutase (PGM) (after Spencer, Hopkinson, and Harris, 1964):
- 100 mg glucose-1-phosphate (dipotassium salt)
- 5 mg NADP
- 5 mg PMS
- 3 mg NBT
- 20 units glucose-6-phosphate dehydrogenase

[3] Reference to trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

TABLE 2.—Classification of species of Scorpaenidae into various groups by means of biochemical characteristics.

| Species | Biochemical characteristics | | | | | | Biochemical group |
|---|---|---|---|---|---|---|---|
| | Muscle pattern[1] | TO | GPDH | LDH | Peptidase A | | |
| | | | | | I (Fast zone) | II (Slow zone) | |
| Pacific *Sebastes* | | | | | | | |
| *S. elongatus* | 2 | F | E | C | c | c | I[2] |
| *S. entomelas* | 2 | S | E | C | c | c | II[2] |
| *S. aurora* | 3 | F | E | C | b | d | III[2] |
| *S. chlorostictus* | 4 | F | E | B | a | c | IV[2] |
| *S. aleutianus* | 4 | F | E | C | c | c | V |
| *S. zacentrus* | 4 | F | E | C | c | c | V |
| *S. caurinus* | 4 | F | F, S | C | d | c | VI |
| *S. diploproa* | 4 | F | E | C | b | b | VII[2] |
| *S. helvomaculatus* | 4 | F | E | B | c | c | VIII[2] |
| *S. maliger* | 4 | F | F | C | d | c | VI |
| *S. ruberrimus* | 4 | F | E | C | c | c | V |
| *S. rubrivinctus* | 4 | F | E | C | c | c | V |
| *S. saxicola* | 4 | F | F | C | c | c | IX[2] |
| *S. auriculatus* | 4 | F | E, F | C | d | c | VI |
| *S. brevispinis* | 4 | F | E | C | c | c | V |
| *S. flavidus* | 4 | S | E | C | c | c | X |
| *S. melanops* | 4 | S | E | C | c | c | X |
| *S. pinniger* | 4 | S | E | C | c | c | X |
| *S. proriger* | 4 | S | E | C | c | c | X |
| *S. wilsoni* | 4 | S | -- | -- | -- | -- | -- |
| *S. variegatus* | 4 | S | E | C | a | c | XI[2] |
| *S. caenaematicus* | 4 | S | E | C | c | c | X |
| *S. alutus* | 4 | S | F, S | C | c | c | XII[2] |
| *S. crameri* | 4 | VS | E | C | c | c | XIII |
| *S. paucispinis* | 4 | VS | E | C | c | c | XIII |
| *S. reedi* | 4 | VS | E | C | c | c | XIII |
| *S. levis* | —[3] | F | E | C | a | c | XIV[2] |
| *Sebastolobus alascanus* | A | VS | D | A | b | a, e | XV[2] |
| Atlantic *Sebastes* | | | | | | | |
| *S. marinus* | B | S | E, F | B | c | c | XVI |
| *S. viviparous* | B | S | E | B | c | c | XVI |
| *Helicolenus dactylopterus* | G | S, VS | C | B | e | b | XVII[2] |

[1] Modified after Tsuyuki et al., 1968.
[2] Species with unique biological characteristics.
[3] Pattern of the single specimen tested has not been described.

0.5 ml 1 M $MgCl_2$
40 ml tris-citrate buffer

Isocitrate dehydrogenase (ICDH):

(a) NADP dependent (after Opher, Leonard, and Miller, 1969):
30 mg DL sodium isocitrate
5 mg NADP
0.5 ml 1 M $MgCl_2$
5 mg PMS
5 mg NBT
40 ml tris-citrate buffer

(b) NAD + dependent:
Same formulation as NADP dependent, but substituting 10 mg NAD + for 5 mg NADP

Muscle protein detected by nonspecific protein staining using 1% nigrosin-buffalo black in solution of 1:4:5 acetic acid:methanol:water and destained with a 1:4:5 solution of acetic acid, methanol, and water.

## ENZYME AND PROTEIN PHENOTYPES

### TETRAZOLIUM OXIDASE (TO)

Interspecific variation of TO was previously reported in the genus *Sebastes* (Pacific) by Johnson et al. (1970b), where three anodal mobilities were observed in 15 species studied: Fast (F), Slow (S), and Very Slow (VS). These findings are expanded in the present study (Table 2, Figure 1). The F band occurred in 15 of the 27 Pacific *Sebastes* species; the S band was present in 9 species and the VS band in 3 species. Only the S band occurred in both Atlantic *Sebastes* species. The VS band was found

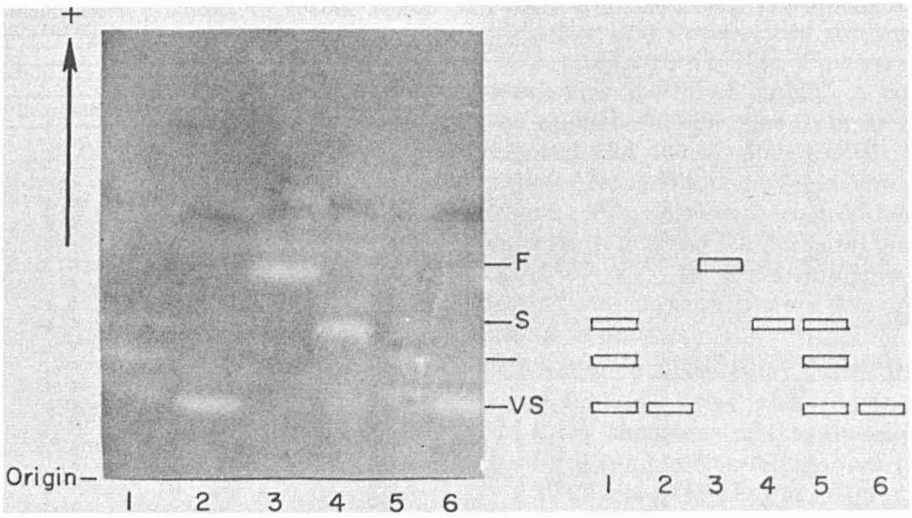


FIGURE 1.—Band in starch-gel illustrating the four tetrazolium oxidase phenotypes, F, S, S-VS, and VS, detected in the family Scorpaenidae. The following samples are shown: 1, 5 *Helicolenus dactylopterus* (S-VS), 2 *Sebastes reedi* (VS), 3 *Sebastes caurinus* (F), 4 *Sebastes alutus* (S), and 6 *Sebastes reedi* (VS).

in *Sebastolobus alascanus*.[4] *Helicolenus dactylopterus* was polymorphic for the S and VS bands; of the 10 samples tested, two exhibited a three-banded phenotype having the S and VS bands in addition to another band of intermediate mobility, whereas the rest had only the single S band. The three-banded phenotype suggests that two TO alleles are segregating in *Helicolenus* and that tetrazolium oxidase functions as a dimer in scorpaenids. This interpretation is consistent with TO polymorphisms observed in salmonids (Utter, 1971) where three-banded phenotypes were observed in heterozygous rainbow trout (*Salmo gairdneri*) and chinook salmon (*Oncorhynchus tshawytscha*).

## L-ALPHA -GLYCEROPHOSPHATE DEHYDROGENASE ($\alpha$GPDH)

Evidence for a polymorphic dimer having two alleles—Fast (F) and Slow (S)—were described in *S. alutus* (Johnson et al., 1970a). In addition to the F and S bands, three faster $\alpha$GPDH bands have been observed among the scorpaenids that we have tested: E, D, and C,[5] listed according to increasing mobility (Figure 2 and Table 2). Additional $\alpha$GPDH bands invariably occurred, regardless of phenotype, when electrophoresis proceeded beyond a 6-cm anodal migration of the dye marker. These bands are presumably artifacts of electrophoresis and did not alter our interpretation of enzyme variations. This phenomenon was also noticed by McCabe, Dean, and Olson (1970) in $\alpha$GPDH variants of skipjack tuna (*Katsuwonus pelamis*).

In Pacific *Sebastes*, 19 species were monomorphic for the E band. *S. auriculatus* was polymorphic for the E and F bands. *S. caurinus* as well as *S. alutus* were polymorphic for F and S bands. *S. maliger* and *S. saxicola* were monomorphic for the F band. In the Atlantic *Sebastes*, *S. viviparous* was monomorphic for the E band and *S. marinus* was polymorphic for the E and F bands. The D and C bands were monomorphic *Sebastolobus alascanus* and *Helicolenus dactylopterus*, respectively.

[4] We used liver extracts of this species for detection of TO activity because muscle extracts failed to develop TO bands. We assume that this is a valid comparison because of parallel TO activity between liver and muscle observed in other scorpaenid species. All other scorpaenid enzymes tested were extracted from skeletal muscle.

[5] The separation of $\alpha$GPDH bands C and D depends on optimal electrophoretic conditions.

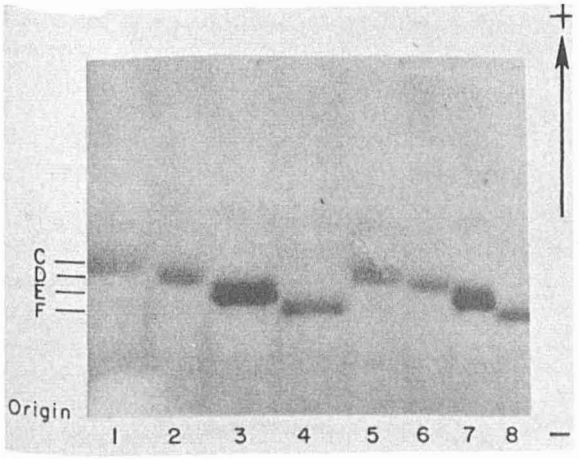


FIGURE 2.—Bands in starch-gel illustrating four L-alpha glycerophosphate dehydrogenase phenotypes (C, D. E, F) detected in the family Scorpaenidae. The following samples are shown: 1, 5 *Helicolenus dactylopterus* (C), 2, 6 *Sebastolobus alascanus* (D), 3, 7 *Sebastes rubrivinctus* (E), and 4, 8 *Sebastes alutus* (F).

## LACTIC DEHYDROGENASE (LDH)

Muscle LDH was resolved as a single anodal band in each scorpaenid species we tested. This agrees with studies of Wilson, Kitto, and Kaplan (1967), who found single anodal bands of muscle LDH in two scorpaenid species, *Sebastes marinus* and *Scorpaenopsis gibbosa*. The electrophoretic mobilities were distinct in each species. LDH bands of three different mobilities (A, B, and C) were found in our sampling (Figure 3 and Table 2). No polymorphisms were detected. All but two Pacific *Sebastes* species expressed the C band. The B band was found in *S. helvomaculatus* and *S. chlorostictus*. The B band was found in two Atlantic *Sebastes* species and *Helicolenus dactylopterus*. Only *Sebastolobus alascanus* expresses the LDH A band.

## PEPTIDASE

Peptidase staining occurred in two anodal regions for all species tested (Figure 4, Table 2). Both regions are developed with the dipeptide valyl-leucine, which is the specific substrate for peptidase A in mammals (Lewis and Harris, 1967; Lewis and Truslove, 1969). We have therefore called these regions peptidase A-I and peptidase A-II.

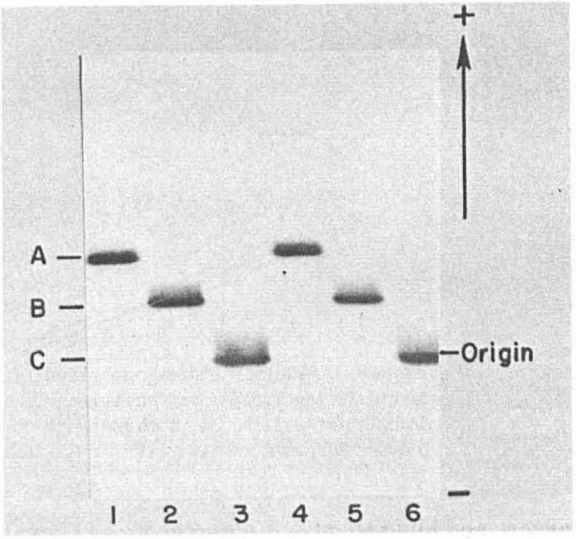


FIGURE 3.—Bands in starch-gel illustrating the three phenotypes of lactate dehydrogenase detected in the family Scorpaenidae. The following species are shown: 1, 4 *Sebastolobus alascanus* (A), 2, 5 *Sebastes helvomaculatus* (B), and 3, 6 *Sebastes alutus*.

Five different bands (a, b, c, d, e) were observed in the peptidase A-I (fast) zone. In Pacific *Sebastes*, peptidase A-I bands were expressed as follows: $I^a$ - *S. chlorostictus, S. levis,* and *S. variegatus*; $I^b$ - *S. caurinus, S. auriculatus*, and *S. maliger*. *S. marinus* had band $I^c$ as did 9 of the 10 *S. viviparous* tested; *Sebastolobus alascanus* had band $I^b$; and *H. dactylopterus* had band $I^e$. The aberrant *Sebastes viviparous* sample had a single $I^d$ band but corresponded to *S. viviparous* in all other systems tested. The significance of the variant is unclear. It may reflect an intraspecies genetic variant (although multiple bands would be expected if this were the case) or perhaps a sibling species. Because only muscle samples were available for Atlantic *Sebastes*, identification of subtle morphological differences between individuals was not possible.

Bands of five different mobilities (a, b, c, d, e) were also observed in the peptidase A-II (slow) zone. Band $II^c$ was expressed in all but two Pacific *Sebastes* tested; band $II^d$ was found in *S.*

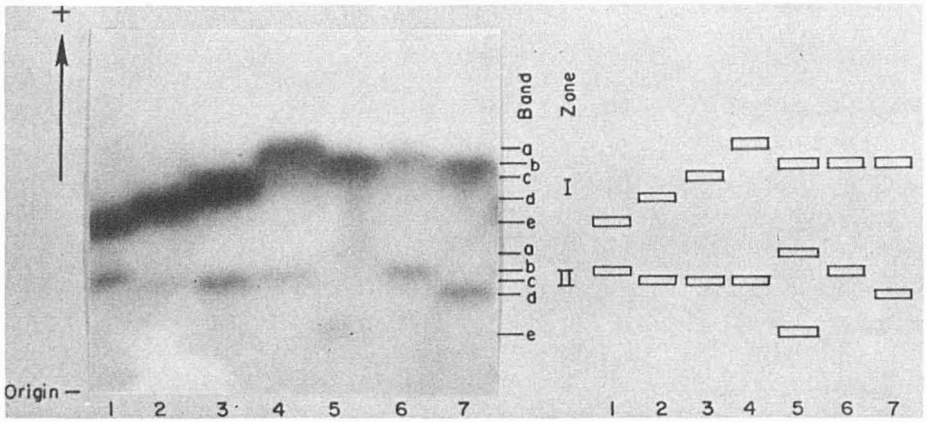


FIGURE 4.—Bands in starch-gel illustrating the various phenotypes of Peptidase A detected in the family Scorpaenidae. The following species are shown: 1 *Helicolenus dactylopterus* ($II^b$, $I^e$), 2 *Sebastes caurinus* ($II^d$, $I^c$), 4 *Sebastes variegatus* ($II^c$, $I^a$), 5 *Sebastologus alascanus* ($II^{a,e}$ $I^b$), 6 *Sebastes diploproa* ($II^b$, $I^b$), and 7 *Sebastes aurora* ($II^d$, $I^b$).

*aurora* and band $II^b$ in *S. diploproa*. Band $II^c$ was found in both Atlantic *Sebastes* species, and *Helicolenus dactylopterus* possessed band $II^b$. Two bands representing the extremes of peptidase A-II mobilities—$II^a$ and $II^e$—were expressed in all *Sebastolobus alascanus* individuals tested. These bands are presumed to be fixed rather than polymorphic because of their invariant expression and may reflect gene duplication.

## PHOSPHOGLUCOMUTASE (PGM)

PGM polymorphism was reported in *Sebastes alutus*, where two allelic bands—A and B—were described (Johnson, Utter, and Hodgins, 1971). In extending these observations here to additional scorpaenid species a third band—A′—has also been found which migrates somewhat faster than the A band (Figure 5).

PGM is the most polymorphic of the scorpaenid enzymes that we have investigated (Table 3). In Pacific *Sebastes* polymorphism was found in 10 species for the A and B bands and in 1 species for the A and A′ bands. Twelve species of Pacific *Sebastes* were monomorphic for the A band, one for the B band, and one for the A′ band. In other scorpaenid species, *Sebastes marinus* was polymorphic for the A and B bands, and *S. viviparous* was monomorphic for

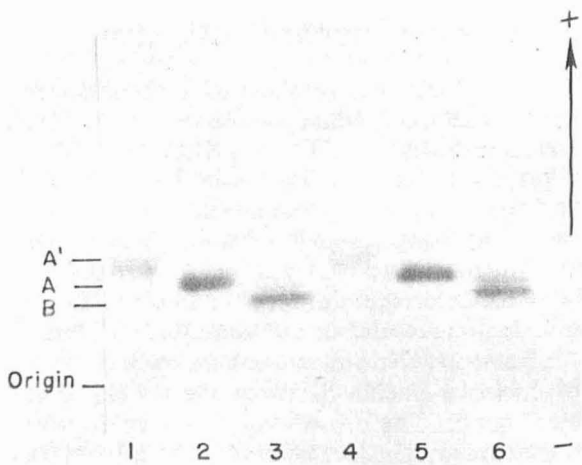


FIGURE 5.—Bands in starch-gel illustrating three mobilities of phosphoglucomutase detected in the family Scorpaenidae. The following species are shown: 1, 4 *Sebastolobus alascanus* (A′), 2, 5 *Sebastes caurinus* (A), and 4, 6 *Sebastes reedi* (B).

the A band. *H. dactylopterus* was polymorphic for the A and B bands, and *Sebastolobus alascanus* was monomorphic for the A′ band. We assume that these variants reflect allelic differences although further study is needed for some species. Also, the limited number of samples

tested for some species that were listed as monomorphic are too few to preclude the possibility of polymorphism.

## ISOCITRATE DEHYDROGENASE, NADP DEPENDENT (ICDH NADP)

We tested for both NAD- and NADP-dependent ICDH in the 31 species studied and found activity only for the latter form. It is assumed that this represents cytoplasmic ICDH activity (Opher et al., 1969). Two anodal mobilities of ICDH were detected: the band of *H. dactylopterus* migrated slightly faster than the band of the other species (Figure 6). No activity was detectable in extracts of *Sebastolobus alascanus*. Activity was highly labile in all species, requiring testing on the same day that the extraction was made. It may be that *S. alascanus* has an even more labile form of ICDH than the other species tested.

TABLE 3.—Phosphoglucomutase phenotypes in muscle samples from species of Scorpaenidae.[1]

| Species | Phenotypes | | | | |
|---|---|---|---|---|---|
| | B | AB | A | AA′ | A′ |
| Pacific *Sebastes* | | | | | |
| *S. aleutianus* | -- | + | + | -- | -- |
| *S. alutus* | + | + | + | -- | -- |
| *S. auriculatus* | -- | -- | + | -- | -- |
| *S. aurora* | -- | + | + | -- | -- |
| *S. brevispinis* | + | + | + | -- | -- |
| *S. caurinus* | -- | -- | + | -- | -- |
| *S. chlorostictus* | -- | -- | -- | -- | + |
| *S. crameri* | -- | + | + | -- | -- |
| *S. elongatus* | + | + | + | -- | -- |
| *S. entomelas* | -- | -- | + | -- | -- |
| *S. flavidus* | -- | -- | + | -- | -- |
| *S. helvomaculatus* | -- | -- | + | + | + |
| *S. levis* | -- | -- | + | -- | -- |
| *S. maliger* | -- | -- | + | -- | -- |
| *S. melanops* | -- | -- | + | -- | -- |
| *S. paucispinis* | -- | + | + | -- | -- |
| *S. pinniger* | -- | + | + | -- | -- |
| *S. proriger* | + | + | + | -- | -- |
| *S. reedi* | + | -- | -- | -- | -- |
| *S. ruberrimus* | -- | -- | + | -- | -- |
| *S. rubivinctus* | -- | -- | + | -- | -- |
| *S. saxicola* | -- | -- | + | -- | -- |
| *S. zacentrus* | -- | -- | + | -- | -- |
| *S. caenaematicus* | -- | + | + | -- | -- |
| *S. variegatus* | -- | -- | + | -- | -- |
| Atlantic *Sebastes* | | | | | |
| *S. marinus* | -- | + | + | -- | -- |
| *S. viviparous* | -- | -- | + | -- | -- |
| *Sebastolobus alascanus* | -- | -- | -- | -- | + |
| *Helicolenus dactylopterus* | + | -- | + | -- | -- |

[1] PGM in our samples of *S. diploproa* and *S. wilsoni* did not develop.

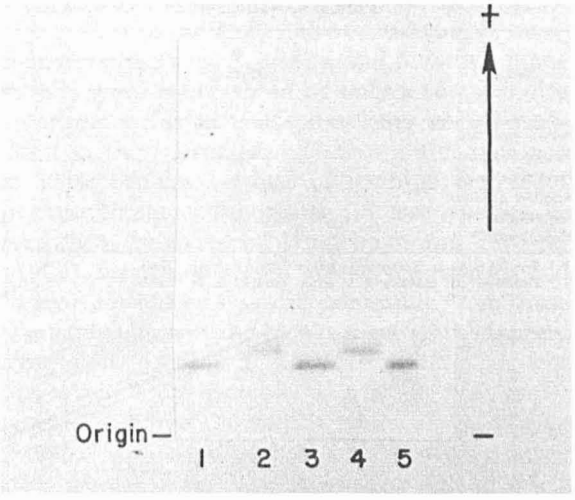


FIGURE 6.—Isocitric dehydrogenase (NADP dependent) bands found in the family Scorpaenidae. Samples 1, 3, 5 are *Sebastes alutus* and samples 2, 4 are *Helicolenus dactylopterus*.

## MUSCLE PROTEIN

A satisfactory separation of muscle protein bands was obtained by permitting the dye marker to migrate 9.0 cm anodally from the origin. These bands were separated into two regions—A and B (Figure 7).

Distinct protein patterns occurred in region A, which differ between genera as well as within the genus *Sebastes* (Pacific) (Table 4). *S. aurora* has a unique pattern (bands 1, 4) which differed from the other Pacific *Sebastes* species (bands 1, 3). The intergeneric differences in region A were: *Sebastes* (Pacific)—bands 1, 4 and 1, 3; *Sebastes* (Atlantic)—bands 2, 6; *Helicolenus*—bands 3, 7; and *Sebastologus*—5, 7. A band (X) which migrated more anodally than band 7 was found in some *Sebastes alutus*. We assume this band (X) to be an artifact as it did not appear in repeated tests. The most anodal band (8) was found in all samples tested. Corresponding region A patterns were not described by Tsuyuki et al. (1968) in instances where the same species were tested and may arise from differences in methodology such as buffer systems (Rasmussen, 1969).

TABLE 4.—Intergeneric comparison of muscle protein bands of Scorpaenids.

| Genus | Subgroup[1] | Protein bands | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Region B[2] | | | | | | | | | | | | | | | Region A | | | | | | | |
| | | a | b | c | d | e | f | g | h | i | j | k | l | m | n | o | p | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Pacific *Sebastes* | 2 | + | + | + | -- | -- | -- | -- | -- | -- | + | + | -- | + | -- | -- | + | + | -- | + | -- | -- | -- | -- | + |
| Pacific *Sebastes* | 3 | -- | -- | -- | -- | -- | + | -- | + | -- | -- | + | -- | + | -- | -- | + | + | -- | -- | + | -- | -- | -- | + |
| Pacific *Sebastes* | 4 | + | + | + | -- | -- | -- | -- | -- | + | -- | + | -- | + | -- | -- | + | + | -- | + | -- | -- | -- | -- | + |
| Atlantic *Sebastes* | -- | + | + | + | -- | -- | -- | -- | -- | + | -- | + | -- | + | -- | -- | + | -- | + | -- | -- | -- | + | -- | + |
| *Sebastolobus* | -- | -- | -- | -- | -- | -- | -- | -- | -- | + | -- | -- | + | -- | -- | + | -- | -- | -- | -- | -- | + | -- | + | + |
| *Helicolenus* | -- | + | + | + | -- | -- | -- | -- | -- | + | -- | + | -- | + | -- | -- | + | -- | -- | -- | -- | -- | -- | + | + |

[1] Pacific *Sebastes* subgroups after Tsuyuki et al., 1968.
[2] Alphabetical classification after Tsuyuki et al., 1968.

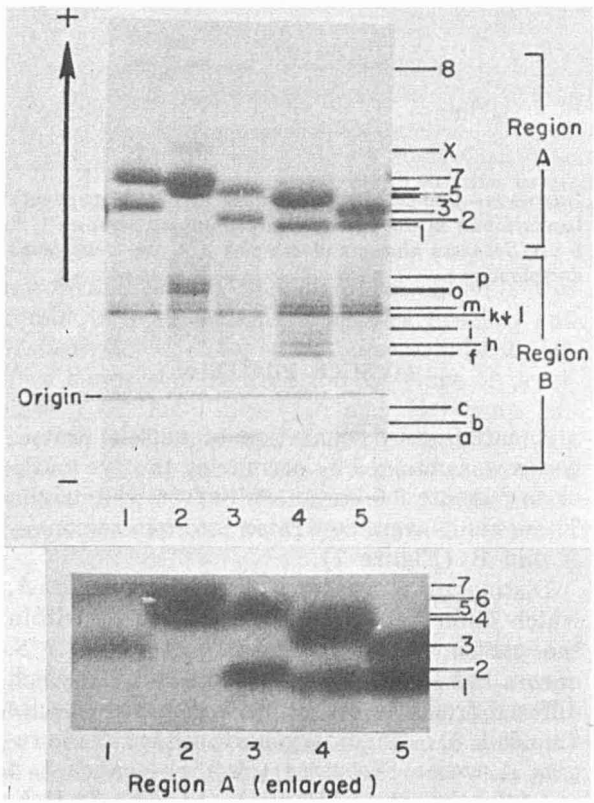


FIGURE 7.—Muscle protein bands in starch-gel: 1 *Helicolenus dactylopterus* (A-3, 7), 2 *Sebastolobus alascanus* (A-5, 7), *Sebastes marinus* (A-2, 6), 4 *Sebastes aurora* (A-1, 4), and 5 *Sebastes alutus* (A-1, 3).

The protein patterns in region B were similar to those described by Tsuyuki et al. (1968), who described 16 bands (a-p) that varied between genera and species. Three cathodally migrating bands (a, b, c) occurred in Pacific *Sebastes* (except *S. aurora*), Atlantic *Sebastes*, and *Helicolenus*. Bands b and c stained weakly in our gels and failed to show in some individuals (Figure 7). The slowest anodal bands were f and h which occurred only in *S. aurora*. Band i occurred in all species tested except *S. aurora*, *S. elongatus*, and *S. entomelas*. On the other hand, *S. entomelas* and *S. elongatus* were the only species having the j band, bands j and k being polymorphic in *S. elongatus* (first reported by Tsuyuki et al., 1968). Band k was present in all genera but *Sebastolobus*, which—in turn—was the only genus expressing band l. Similarly, bands m and p—present in other genera—were absent in *Sebastolobus*, which uniquely expressed band o. Our methods were unable to detect band q, reported by Tsuyuki et al. in Atlantic *Sebastes* and *Sebastolobus*.

## COMPARISON OF VARIATION BETWEEN GENERA

A comparison of the total variation between genera suggests some possible relations. The greatest similarity was between the Pacific *Sebastes* and Atlantic *Sebastes* where all the electrophoretic patterns of the Atlantic *Sebastes* were found in one or more species of the Pacific *Sebastes*, except for the protein bands of region A. Pacific *Sebastes* and *Sebastolobus* exhibited common bands for PGM, TO, peptidase A-I, and protein B-i. Pacific *Sebastes* and *Helicolenus* shared common bands for LDH, PGM, and protein bands of region B. *Helicolenus* and one species of Pacific *Sebastes* possessed a common peptidase A-II band. *Helicolenus* and *Sebastes* had common bands in LDH, PGM, and protein region B. *Helicolenus* and *Sebastolobus* shared only pro-

tein bands B-i and A-7. Only protein band B-i was common to *Sebastolobus* and the Atlantic *Sebastes* (Tables 2, 4, and 5).

When the total amount of common patterns between genera is considered, we agree with Tsuyuki et al. (1968) that there is relatively greater similarity between the Pacific *Sebastes* and the Atlantic *Sebastes* than between either and the other genera studied. *S. aurora* was found to have relatively the same degree of difference between itself and the other Pacific *Sebastes* species as there was between the Atlantic *Sebastes* and the Pacific *Sebastes*. This agrees with the findings of Tsuyuki et al. (1968) who suggested that *S. aurora* should possibly be elevated to the generic level because of its degrees of difference. The interpretation of similarity based on electropherograms must be done with caution as only amino acid substitutions which change the net charge of the polypeptide chain can be detected.

TABLE 5.—Summary of intergeneric enzymatic similarity in Scorpaenidae.[1] X indicates the occurrence of common bands between one or more species of the genera compared.

| Genus and enzyme | Genera | | | |
|---|---|---|---|---|
| | Pacific *Sebastes* | Atlantic *Sebastes* | *Sebastolobus* | *Helicolenus* |
| Pacific *Sebastes* | | | | |
| TO | | X | X | -- |
| αGDPH | | X | -- | -- |
| LDH | | X | -- | X |
| Peptidase A-I | | X | X | -- |
| Peptidase A-II | | X | -- | X |
| ICDH | | X | -- | -- |
| PGM | | X | X | X |
| Atlantic *Sebastes* | | | | |
| TO | X | | -- | -- |
| αGDPH | X | | -- | -- |
| LDH | X | | -- | X |
| Peptidase A-I | X | | -- | -- |
| Peptidase A-II | X | | -- | -- |
| ICDH | X | | -- | -- |
| PGM | X | | -- | X |

[1] No common bands were found between *Sebastolobus* and *Helicolenus*.

## VARIATION WITHIN PACIFIC *SEBASTES*

Combining the enzyme and protein variations in the Pacific *Sebastes* resulted in 10 of the 27 Pacific *Sebastes* species having unique biochemical profiles (Table 2). These species were *S. elongatus*, *S. entomelas*, *S. aurora*, *S. chlorostictus*, *S. diploproa*, *S. helvomaculatus*, *S. saxicola*, *S. variegatus*, *S. alutus*, and *S. levis*. Some species were represented by only a few samples —therefore further sampling may reveal variation in these profiles. PGM was not included in these profiles because of its high degree of polymorphism in the genus. A new species, *S. reedi*, was reported by Westrheim and Tsuyuki (1967) that resembles *S. crameri*, *S. alutus*, and *S. proriger* but was readily separable from these when morphology and biochemical methods were employed. Our study found that *S. reedi* and *S. crameri* were identical with respect to muscle protein and five enzyme systems but differed in PGM. This suggests that *S. reedi* may be more closely related to *S. crameri* than to the other species.

Three species, *S. caurinus*, *S. maliger*, and *S. auriculatus*, had profiles that differed only in the enzyme αGPDH, which was monomorphic in *S. maliger* (F band) but polymorphic for the F and S bands in *S. caurinus*. All three species have the peptidase A-I[d] band which was found in no other *Sebastes* species. These three species are very similar in morphology and habitat preferences. In certain areas of Puget Sound, Wash., hybridization between the three may occur, whereas in other areas they remain separate because of behavioral differences.[6] Investigation of biochemical and morphological characteristics of these species may provide valuable information on the processes of speciation.

The amount of polymorphism of αGPDH and PGM in the family Scorpaenidae could prove to be useful for the identification of breeding populations and verification of species and subspecies. On the basis of morphometric data, Barsukov (1964) suggested that two subspecies exist in *Sebastes alutus* (*S. a. alutus*—off the Pacific coast of North America and *S. a. paucispinus*—from Honshu Island, Japan, to perhaps Bristol Bay, Alaska). Westrheim (1970) suggested that *S. alutus* had a southern and a northern type of fish off the coast of North America—the south-

[6] C. R. Hitz, National Marine Fisheries Service, Fishery Biologist, Exploratory Fishing and Gear Research Base, Seattle, Wash., personal commun., April 1971.

ern type south of Dixon Entrance and the northern type North of Dixon Entrance and in the Gulf of Alaska. Differences in gene frequencies would add to the support of their separations. This approach may also prove useful in studying complexes such as found in *S. aleutianus*, *S. reedi*, and *S. diploproa* (Tsuyuki et al., 1968).

## SUMMARY

An investigation of muscle protein and six enzymatic systems by starch-gel electrophoresis was presented. Samples of 31 species of three genera of the family Scorpaenidae were compared which resulted in the conclusion that a relatively greater similarity existed between the Pacific *Sebastes* and the Atlantic *Sebastes* than between the other genera.

Ten of the 27 species of Pacific *Sebastes* had unique profiles when the systems were compared.

## ACKNOWLEDGMENTS

We are especially grateful to the following persons who provided samples and valuable information: Dr. I. Barrett and S. Kato (National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, Calif.), C. R. Hitz, B. G. Patten, H. H. Shippen, K. E. Thorson, and K. D. Waldron (National Marine Fisheries Service, Northwest Fisheries Center, Seattle, Wash.), Dr. A. C. DeLacy (University of Washington, Seattle, Wash.), Dr. A. Jamieson (Ministry of Agriculture, Fisheries and Food, Lowestoft, Suffolk, England), and S. J. Westrheim (Fisheries Research Board of Canada, Nanaimo, B.C., Canada).

## LITERATURE CITED

ALTUKHOV, JU. P., AND G. N. NEFYODOV.
1968. A study of blood serum protein composition by agar-gel electrophoresis in types of redfish (genus *Sebastes*). Int. Comm. Northwest Atl. Fish., Res. Bull. 5:86-90.

BAILEY, R. M. (chairman).
1970. A list of common and scientific names of fishes from the United States and Canada. Am. Fish. Soc., Spec. Publ. 6, 150 p.

BARSUKOV, V. V.
1964. Vnutrividovaya izmenchivost' u Tikhookeanskogo morskogo okunya (*Sebastodes alutus* Gilbert) [Intraspecific variability in the Pacific rockfish (*Sebastodes alutus* Gilbert)]. Tr. Vses. Nauchn.-issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 49 (Izv. Tikhookean. Nauchn.-issled. Inst. Morsk. Rybn. Khoz. Okeanogr. 51): 231-252. (Transl., 1968, *in* Soviet fisheries investigations in the northeast Pacific, Part II, p. 241-267. Avail. Natl. Tech. Inf. Serv., Springfield, Va., TT 67-51204.)

BREWER, C. J.
1967. Achromatic regions of tetrazolium stained starch gels: inherited electrophoretic variation. Am. J. Hum. Genet. 19:674-680.

CHEN, L.
1971. Systematics, variation, distribution, and biology of rockfishes of the subgenus *Sebastomus* (Pisces, Scorpaenidae, *Sebastes*). Bull. Scripps Inst. Oceanogr. Univ. Calif. 18, 115 p.

CHU, R.
1968. Identification of commercially used fish found in the Pacific by disk electrophoresis. J. Assoc. Off. Anal. Chem. 51:743-746.

HUNTER, R. L., AND C. L. MARKERT.
1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels. Science (Wash., D.C.) 125:1294-1295.

JOHNSON, A. G., F. M. UTTER, AND H. O. HODGINS.
1970a. Electrophoretic variants of L-alpha-glycerophosphate dehydrogenase in Pacific ocean perch (*Sebastodes alutus*). J. Fish. Res. Board Can. 27:943-945.
1970b. Interspecific variation of tetrazolium oxidase in *Sebastodes* (rockfish). Comp. Biochem. Physiol. 37:281-285.
1971. Phosphoglucomutase polymorphism in Pacific ocean perch, *Sebastodes alutus*. Comp. Biochem. Physiol. 39 B:285-290.

KRISTJANSSON, F. K.
1963. Genetic control of two per-albumins in pigs. Genetics 48:1059-1063.

LEWIS, W. H. P., AND H. HARRIS.
1967. Human red cell peptidase. Nature (Lond.) 215:351-355.

LEWIS, W. H. P., AND G. M. TRUSLOVE.
1969. Electrophoretic heterogeneity of mouse erythrocyte peptidases. Biochem. Genet. 3:493-498.

MARKERT, C. L., AND I. FAULHABER.
1965. Lactate dehydrogenase isozyme patterns of fish. J. Exp. Zool. 159:319-332.

MCCABE, M. M., D. M. DEAN, AND C. S. OLSON.
1970. Multiple forms of 6-phosphogluconate dehydrogenase and alpha-glycerophosphate dehydrogenase in the skipjack tuna, *Katsuwonus pelamis*. Comp. Biochem. Physiol. 34:755-757.

NISHIMOTO, J.
1970. Western range extension of the rosethorn

rockfish, *Sebastes helvomaculatus* (Ayres). Calif. Fish Game 56:204-205.

NYMAN, C.
1967. Protein variations in Salmonidae. Rep. Inst. Freshwater Res. Drottingholm 47:5-38.

OPHER, A. W., J. W. LEONARD, JR., AND J. M. MILLER.
1969. Isoenzymes of isocitrate dehydrogenase in cytoplasm of human cells. Exp. Med. Surg. 27: 256-266.

RASMUSSEN, D. I.
1969. Molecular taxonomy and typology. BioScience 19:418-420.

RIDGWAY, G. J., S. W. SHERBURNE, AND R. D. LEWIS,
1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc. 99:147-151.

SMITHIES, O.
1955. Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. Biochem. J. 61:629-641.

SPENCER, N., D. A. HOPKINSON, AND H. HARRIS.
1964. Phosphoglucomutase polymorphism in man. Nature (Lond.) 204:742-745.

TSUYUKI, H., AND S. J. WESTRHEIM.
1970. Analyses of the *Sebastes aleutianus—S. melanostomus* complex, and description of a new scorpaenid species, *Sebastes caenaematicus*, in the northeast Pacific Ocean. J. Fish. Res. Board Can. 27:2233-2254.

TSUYUKI, H., E. ROBERTS, R. H. LOWES, W. HADAWAY, AND S. J. WESTRHEIM.
1968. Contribution of protein electrophoresis to rockfish (Scorpaenidae) systematics. J. Fish. Res. Board Can. 25:2477-2501.

UTTER, F. M.
1971. Tetrazolium oxidase phenotypes of rainbow trout (*Salmo gairdneri*) and Pacific salmon (*Oncorhynchus* spp.). Comp. Biochem. Physiol. 39 B:891-895.

WESTRHEIM, S. J.
1965. Northern range extensions for four species of rockfish (*Sebastodes goodei, S. helvomaculatus, S. rubrivinctus,* and *S. zacentrus*) in the North Pacific Ocean. J. Fish. Res. Board Can. 22:231-235.
1970. Survey of rockfishes, especially Pacific ocean perch, in the northeast Pacific Ocean, 1963-66. J. Fish. Res. Board Can. 27:1781-1809.

WESTRHEIM, S. J., AND H. TSUYUKI.
1967. *Sebastodes reedi*, a new scorpaenid fish in the northeast Pacific Ocean. J. Fish. Res. Board Can. 24:1945-1954.

WILSON, A. C., G. B. KITTO, AND N. O. KAPLAN.
1967. Enzymatic identification of fish products. Science (Wash., D.C.) 157:82-83.