PROTEIN TAXONOMY OF THE GULF OF MEXICO AND ATLANTIC OCEAN SEATROUTS, GENUS *CYNOSCION*

MICHAEL P. WEINSTEIN[1] AND RALPH W. YERGER[2]

ABSTRACT

Taxonomic relationships among the western North Atlantic seatrouts, genus *Cynoscion*, were investigated utilizing acrylamide gel electrophoresis. Several tissues (blood serum, eye lens, and muscle) were incorporated in this study to gain a better taxonomic overview than would be attainable with a single protein system.

Blood serum exhibited considerable variation in banding patterns. Because direct interspecific comparisons were not possible, a phenetic analysis was employed. Eye lens and muscle patterns, however, were directly comparable. Based on the overall results, three taxonomic conclusions may be drawn. First, with the exception of a single taxonomic distance (d_{jk}) value calculated in the phenetic analysis, the relationships established by electrophoresis reflect the phyletic relationships proposed by Ginsburg. This "aberrant" value is believed to result from the small sample size and the possibility of ecological convergence. Second, the data indicate that *Cynoscion nebulosus* is the most divergent of the four forms, supporting previous morphological and ecological conclusions. Third, as suggested by previous studies, the taxonomic status of *C. arenarius* as a distinct species is again questioned. Electrophoretic patterns indicate that it should be regarded as a subspecies of *C. regalis*.

Investigation of general protein systems has often proven useful in elucidating taxonomic relationships. Species-specific banding patterns have been reported for numerous taxa including fishes (Tsuyuki and Roberts 1965; Perrier et al. 1973). Nyman and Westin (1969) studied serum patterns of cottid fishes from the Baltic Sea and concluded that the patterns reflected the commonly accepted scheme. Species and group (genus, family, class) specificities have also been described for eye lens proteins of several fishes (Rabaey 1964, Bon et al. 1964, Cobb et al. 1968). Recently Eckroat (1974) compared members of the pike family (Esocidae) using this tissue. Myogens have proven particularly useful in reviews of several groups in the families Catostomidae (Tsuyuki, Roberts, and Vanstone 1965; Tsuyuki et al. 1967; Huntsman 1970), Salmonidae (Tsuyuki, Roberts, Vanstone, and Markert 1965; Tsuyuki et al. 1966) and Scorpaenidae (Tsuyuki et al. 1968; Johnson et al. 1972).

In this study we have investigated taxonomic affinities among the western North Atantic seatrouts, genus *Cynoscion*. Four species are currently recognized: spotted seatrout, *C. nebulosus* (Cuvier); weakfish, *C. regalis* (Bloch and Schneider); silver seatrout, *C. nothus* (Holbrook); and sand seatrout, *C. arenarius* Ginsburg. *Cynoscion arenarius* is restricted to the Gulf of Mexico; specimens have been captured from Campeche, Mexico, eastward to the southwest coast of Florida. *Cynoscion regalis* has been generally considered to be limited to the Atlantic coast. Guest and Gunter (1958) described its southernmost occurrence as the St. Lucie estuary, Fla. We now have evidence which conclusively proves its presence in the Gulf of Mexico.

Cynoscion nebulosus occurs from New York to Mexico (Bay of Campeche); its center of abundance is in Florida and the Gulf States (Pearson 1929). *Cynoscion nothus* is found from Chesapeake Bay, Md., to the Bay of Campeche but is uncommon at the southern extremity of its range. It is relatively abundant on the gulf coast and from the east coast of Florida to North Carolina.

Several tissues (blood serum, eye lens, and muscle) were utilized in order to achieve a better taxonomic overview. Since it is difficult (if not impossible) to construct a phylogeny solely on the basis of biochemical differences, our results have been compared with the existing phylogenetic schemes of Ginsburg (1929) who recognized *C. arenarius* and *C. regalis* as cognate species, and Mohsin (1973) who placed *C. arenarius* and *C. nebulosus* in one phyletic line, and *C. regalis* and *C. nothus* in another.

[1]Lawler, Matusky & Skelly Engineers, 415 Route 303, Tappan, NY 10983.
[2]Department of Biological Science, Florida State University, Tallahassee, FL 32306.

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MATERIALS AND METHODS

Spotted seatrout were obtained by hook and line at seven localities from Corpus Christi, Tex., to Indian River, Fla. Weakfish were caught by hook and line in Peconic Bay, N.Y., and together with silver seatrout in otter trawls in Wassaw Sound, Ga. Sand seatrout were collected by hook and line at Pensacola, Fla., and by shrimp trawl in the vicinity of Carrabelle, Fla.

Preparation of serum and eye lens samples and electrophoretic methods are identical to those recently described by Weinstein and Yerger (in press). Samples were electrophoresed in 7% acrylamide gel, using a modified Davis (1964) technique. Diluting tissue preparations with 10% glycerol avoided the tedius requirement of producing three-layered gels, yet allowed highly satisfactory resolution.

Soluble muscle proteins were prepared by homogenizing 1-g tissue samples with 2 volumes of ice cold 0.05 M phosphate buffer (pH 7.4). Homogenates were centrifuged in a Sorvall[3] RC-2 refrigerated centrifuge at 20,000 rpm for 20 min. Fifty microliters of supernate were combined with an equal volume of 10% glycerol, and 50 μl of the mixture layered on each gel. During electrophoresis the dye band was allowed to migrate to within 0.5 cm of the end of each gel.

RESULTS

Serum Proteins

Although serum protein patterns varied intraspecifically both in the frequency of occurrence of particular bands, and occasionally in their composition (intensity), species specificity was evident. Differences among patterns were not so pronounced as to prevent assigning a given pattern to the proper taxon. Typical results obtained from the four seatrouts are shown in Figure 1, and are diagrammed in Figure 2. All bands observed in the total number of electrophoresed samples are indicated. Their position on the diagrams is also an accurate representation of the relative distances (on the gels) that each band migrated.

We follow the standard method of defining protein zones (α, β, γ, albumin, prealbumin). The various designations were derived from a population study on *C. nebulosus* (see Weinstein 1975).

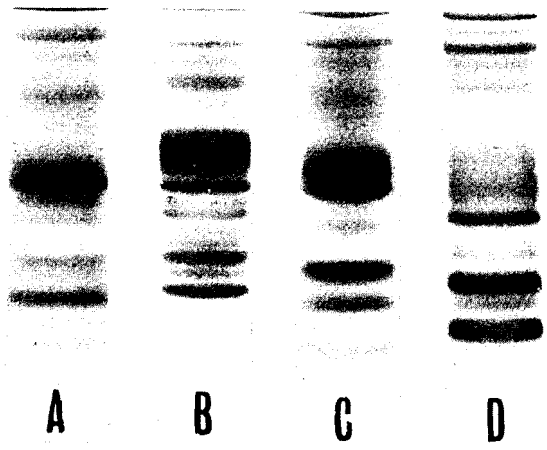

FIGURE 1.—Serum protein electropherograms derived from whole sera of four seatrouts. (A) *Cynoscion nothus*, (B) *C. arenarius*, (C) *C. regalis*, (D) *C. nebulosus*.

Because of the widespread variation observed in the blood serum patterns, direct comparison between the species investigated was difficult. In order to "sum" the intraspecific variation observed and subsequently to use the composites for direct comparison, the taxonomic distance (d_{jk}) measure of Sokal (1961) was utilized. In this formula

$$\overline{d_{12}^2} = \frac{1}{n} \sum_{i=1}^{n} (\xi_{i1} - \xi_{i2})^2$$

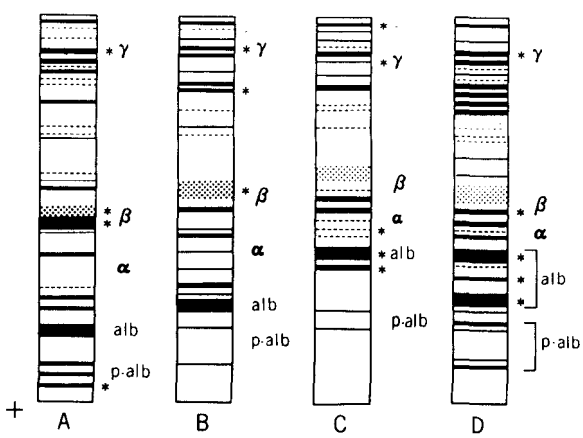

FIGURE 2.—Diagrammatic representation of protein bands occurring in serum electropherograms of four seatrouts. Protein zones are as follows: γ = immunoglobin zone; β = β-globulin zone; α = α-globulin zone; alb = albumins; p-alb = prealbumins. * indicates band present in 100% of samples. (A) *Cynoscion nothus*, (B) *C. arenarius*, (C) *C. regalis*, (D) *C. nebulosus*.

[3]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

all of the bands observed on the gels were taken as "characters" and their percent occurrence as "character states." The data utilized in computing taxonomic distances are summarized in Table 1, and the results of such an analysis in Table 2. All data were "standardized" to have a mean of 0 and a variance of 1 (indicated by ξ). Values of d are interpreted as follows, "The larger the distance, the smaller the degrees of association or correlation between taxa." (Sokal 1961). Based on the calculated distances (Table 2), *C. nebulosus* has diverged to a larger extent than any other member of the genus and differs from the others by about the same order of magnitude. *Cynoscion nothus* and *C. regalis* apparently share a closer relationship in blood protein patterns than do *C. regalis* and *C. arenarius*, a result comparable to that based on osteological similarity (Mohsin 1973). It also seems apparent that the close correlation between *C. nothus* and *C. regalis* (44.0) and between *C. arenarius* and *C. regalis* (54.7) should imply a similar distance between *C. nothus* and *C. arenarius*. Such is not the case; and the value 63.8 apparently indicates that their differences are even greater than their similarities.

TABLE 1.–Percent occurrence of banding patterns derived from whole serum samples of seatrouts (*Cynoscion*). A dash indicates the absence of that band.

Serum band	*C. nothus* n = 34	*C. arenarius* n = 19	*C. regalis* n = 19	*C. nebulosus* n = 500
1	81.1	83.3	100	74.5
2	70.3	75.0	7.7	—
3	81.1	91.7	76.9	96.5
4	—	100	30.8	—
5	100	50.0	84.6	100
6	8.1	—	—	—
7	54.1	—	100	—
8	67.6	75.0	84.6	30.0
9	10.8	—	—	92.4
10	—	—	—	23.9
11	86.5	50.0	92.3	18.4
12	—	—	—	88.9
13	78.4	100	53.8	94.3
14	—	8.3	76.9	11.2
15	—	—	—	67.3
16	78.4	50.0	15.4	70.6
17	59.5	50.0	—	76.0
18	45.9	—	—	66.4
19	—	—	—	63.9
20	43.2	—	92.3	46.1
21	24.3	100	—	—
22	24.3	—	—	—
23	—	—	—	89.1
24	100	—	15.4	—
25	—	—	84.6	—
26	—	91.7	69.2	100
27	100	—	38.5	89.8
28	48.6	16.7	100	20.0
29	—	91.7	15.4	90.4
30	94.6	66.7	100	100
31	—	—	—	8.4
32	—	91.7	—	100
33	75.7	91.7	100	—
34	24.3	16.7	—	100
35	91.9	50.0	92.3	13.8
36	—	—	—	77.6
37	78.4	66.3	23.0	24.8
38	—	—	—	2.1
39	73.0	91.7	—	80.5
40	21.6	—	—	—
41	100	—	—	—

TABLE 2.–Taxonomic distances (d_{jk}) calculated for interspecific comparisons among four seatrouts (*Cynoscion*). The larger the value, the smaller the degree of association or correlation between taxa (Sokal 1961).

Species compared	d_{jk}
C. nothus versus *C. arenarius*	63.8
C. nothus versus *C. regalis*	44.0
C. nothus versus *C. nebulosus*	78.5
C. arenarius versus *C. regalis*	54.7
C. arenarius versus *C. nebulosus*	67.3
C. regalis versus *C. nebulosus*	72.9

Eye Lens Proteins

Eye lens preparations exhibited considerable uniformity of pattern (Table 3, Figure 3). Four bands designated by arabic numerals were shared in common by the seatrouts; however, the amount of protein in each band differed significantly. For example, *C. regalis* had a greater protein concentration in band 1 than did any of the others. The quantity of protein in this band was not observed to differ significantly in any of the samples processed. Bands 1 and A in *C. nebulosus* (n = 275)

TABLE 3.–Percent occurrence of banding patterns derived from eye lens nuclei of seatrouts (*Cynoscion*). A dash indicates the absence of that band.

Band	*C. nothus* n = 35	*C. arenarius* n = 12	*C. regalis* n = 16	*C. nebulosus* n = 275
1	100	100	100	100
A	—	—	—	12.7
2	100	100	100	100
3	100	100	100	100
B	—	—	—	14.1
C	—	33.0	—	—
4	100	100	100	100
D	17.0	42.0	—	59.7
E	—	—	—	19.0

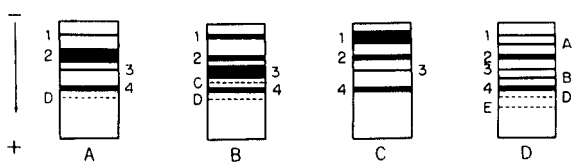

FIGURE 3.–Diagrammatic representation of protein bands occurring in eye lenses of four seatrouts. Arabic numerals indicate bands shared by all taxa (similar electrophoretic mobility). Letters indicate bands that are either unique or not shared by all members of the genus. (A) *Cynoscion nothus*, (B) *C. arenarius*, (C) *C. regalis*, (D) *C. nebulosus*.

together contained approximately the same quantity of protein as found in band 1 of *C. arenarius*. We believe that band 1 (100% occurrence) in *C. nebulosus* contains at least one protein which exhibits polymorphism. Since other proteins (frequency 100%) in this band mask the identity of the protein in question, it is not possible at the level of sensitivity of this system to distinguish the mode of inheritance for this polymorphism. The same situation seems to be true of bands 3 and B in this species.

Band 1 is consistently found in lower concentration in *C. nothus* (n = 35), but the reverse is true of band 2, which exhibits continuously greater concentration than the comparable band in any other seatrout. Band 3 is found in the highest concentration in *C. arenarius* (n = 12); a slightly lower concentration occurs in the composite of bands 3 and B in *C. nebulosus*, and a still lower concentration is found in *C. regalis* and *C. nothus*. Band 4 is present in approximately the same concentration in all four species. It should be emphasized that these are average values; small intraspecific differences were noted from sample to sample.

Qualitative pattern differences were also noted. Bands A and B are unique to *C. nebulosus*. A third band, designated C, was found in 2 of 12 samples of *C. arenarius*, but not in any other species. A fourth variant, designated D, was found in *C. nebulosus*, *C. arenarius*, and *C. nothus*, but not in *C. regalis*. Lastly, a band migrating farthest anodally in *C. nebulosus* was designated E. These qualitative as well as quantitative differences in eye lens patterns are summarized in Table 3.

Myogens

Electropherograms derived from soluble muscle proteins provided the most clearly discernible measure of biochemical relationship. Compared with serum patterns, only minor intraspecific variations were evident. A typical grouping from the four seatrouts is shown in Figure 4, and the patterns are diagrammed in Figure 5. The broken lines indicate two minor bands that occurred in a variable manner and in relatively low frequencies; hence, they were not considered further. All other bands occurred in 100% of the samples and are designated as comprising the typical species-specific patterns. A remarkable degree of similarity in the patterns is obtained for *C. regalis* and *C. arenarius*; they share not only 12 and 13 bands in

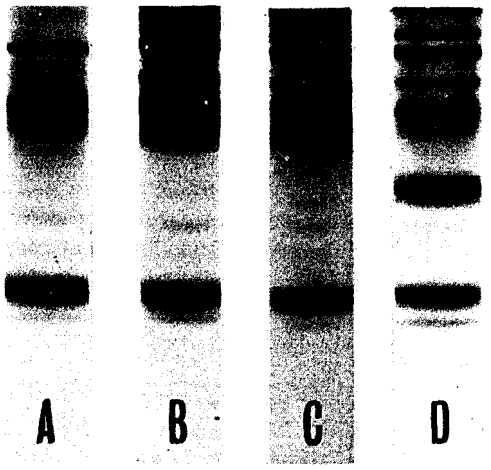

FIGURE 4.—Electropherograms derived from protein extracts of epaxial musculature. (A) *Cynoscion nothus*, (B) *C. arenarius*, (C) *C. regalis*, (D) *C. nebulosus*.

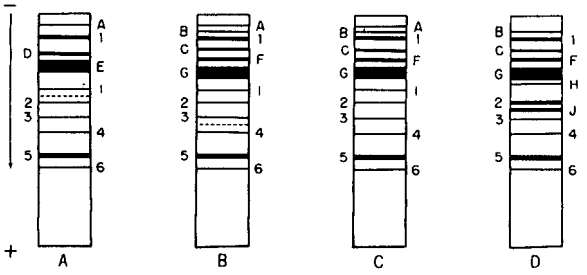

FIGURE 5.—Diagrammatic representation of the protein bands occurring in myogen extracts of four seatrouts. (A) *Cynoscion nothus*, (B) *C. arenarius*, (C) *C. regalis*, (D) *C. nebulosus*.

common (as indicated by electrophoretic mobility and sieving characteristics), but also compare favorably in the quantities of protein comprising each band (Figure 6). Although some variation occurred in relative peak heights from sample to sample (within a species), the densitometer tracings shown in Figure 6 are representative of each species. A close relationship clearly exists between *C. regalis* and *C. arenarius* both in the distance of migration and in the quantity of protein making up the individual bands (Figures 5, 6). Although the other two species shared the same general generic pattern, they varied in the composition of several major bands. *Cynoscion nebulosus* always has a high concentration in band 2 in each of the 12 samples processed, and has a second band immediately adjacent of the same thickness (denoted J on Figure 5). These bands (2 and J) were not resolved as separate peaks in a series of densi-

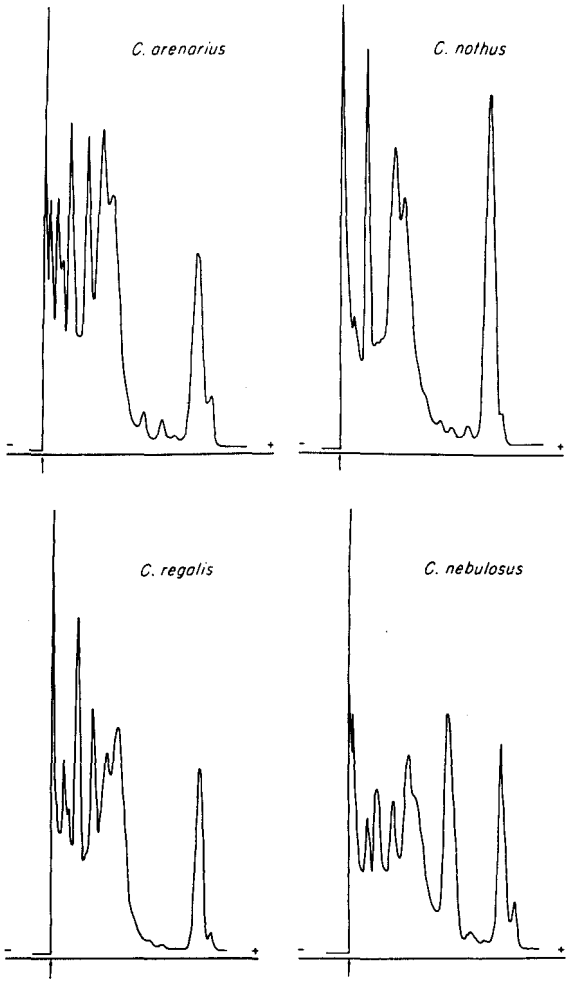

FIGURE 6.—Densitometer tracings of representative myogen patterns of four seatrouts (*Cynoscion*). Intensity of particular bands are indicated by relative peak heights.

tometer tracings; however, observation of gels and photographs clearly indicated their double nature. Band 1 was not present in any of the samples of *C. nebulosus*; however, a band designated as H occurred in a more cathodal direction (above position I).

Bands D and E in *C. nothus* are slightly displaced; i.e., they have a slightly different electrophoretic mobility from their "counterparts" (F and G) in the other three species. This difference could be an artifact, but duplicate experiments indicate otherwise. *Cynoscion nothus* also lacks bands B and C found in the other species. Band A is absent in *C. nebulosus*, but present in the other seatrouts.

DISCUSSION

Morphological Taxonomy

In his review of the seatrouts of the Atlantic and Gulf coasts of the United States, Ginsburg (1929) recognized *C. nebulosus* as the most distinctive morphologically on the basis of its color pattern and its scaleless dorsal and anal fins. *Cynoscion nebulosus* also differs ecologically from the other *Cynoscion;* it is primarily an estuarine form while the others have a closer affinity to the marine environment.

The remaining species are less easily distinguished. Of the many criteria used, size and color are most important. *Cynoscion regalis* is readily recognized in the adult stage by the longitudinal rows of small spots on its back, which produce a mottled appearance. The paler, *C. arenarius* of the gulf lacks conspicuous pigmentation. *Cynoscion nothus* is similar in color to *C. arenarius*, but differs in several other respects including vertebral and anal-fin ray counts. *Cynoscion nothus* may not attain as large a maximum size as *C. arenarius*, although this observation may be a sampling artifact. Gunter (1945) noted that *C. nothus* occurs at slightly greater depths than the other seatrouts. Therefore, the main populations of *C. nothus* may not have been adequately sampled.

Taxonomically, the status of *C. arenarius* has never been satisfactorily resolved. Guest and Gunter (1958) accorded full species rank for *C. arenarius*, as does the current list of the American Fisheries Society (Bailey et al. 1970), and the recent investigation by Mohsin (1973). However, the original description leaves room for considerable doubt. Ginsburg (1929) stated in a footnote that, "An unbiased study of the data here presented shows, I believe, that there is room for difference of opinion as to the degree of difference between this form [*C. arenarius*] and *ragalis* [*regalis*] from the Atlantic coast—whether they should be regarded as species or subspecies." Furthermore, by Ginsburg's (1938) own criteria of the "arithmetical" definition of a species, the 18% intergradation of the most "divergent" character (the number of articulated dorsal rays) would give the two forms only subspecific status.

Protein Taxonomy

Our primary purpose in this study has been to

provide biochemical evidence for the taxonomic relationship among four members of the genus *Cynoscion* (including the degree of divergence), and to compare this information with existing phylogenetic schemes. Although no attempt has been made to construct a phylogeny based on biochemical data, qualitative differences (and similarities) allow some taxonomic conclusions to be drawn.

Serum Proteins

Environmentally induced changes in blood serum components have been well substantiated (Thurston 1967). This evidence, nonetheless, would not preclude blood serum patterns from being a useful taxonomic tool if one additional step is taken. It is obvious that the classical morphologists in comparing populations of animals (or plants) are including the influence of the environment in the range of variation they are describing. For example, it is commonly observed that counts of meristic characters (fin rays, scales, etc.) increase in the northerly direction of the animal's range (in the Northern Hemisphere). This, however, will not affect the conclusions drawn as long as sufficient samples are taken to cover the full (or nearly so) range of variation in the population. Once adequate samples are obtained, accurate modes may be calculated for each character and the relationship between two forms established. Within this framework utilization of highly variable patterns such as that found for serum proteins are justified.

In this study we have been able to sample only a relatively small number of each species, with the exception of *C. nebulosus* (Table 2). Hence, any conclusions regarding the biochemical relationship among the four taxa must be provisional.

Although the blood patterns of the species of *Cynoscion* are somewhat more variable than has been reported for many fishes and other vertebrates, we can present evidence for relationships among the Gulf of Mexico and Atlantic Ocean seatrouts. The Taxonomic distances calculated for members of this genus are listed in Table 2. The value (54.7) for the alleged cognates, *C. arenarius* and *C. regalis*, is surpassed only by the value (43.9) for *C. nothus* and *C. regalis*. Only 10 bands of the 41 present were unique to one of the four species; 7 were found in *C. nebulosus*, 2 in *C. nothus*, and 1 in *C. regalis*. *Cynoscion arenarius* did not display any species-specific bands. Therefore, a considerable portion of the differences among the four seatrouts, as expressed by d_{jk}, are generated by different percentage compositions of the serum proteins.

The similar values obtained for *C. regalis* and *C. nothus* may be interpreted in three ways: 1) these species may actually be more closely related than are *C. regalis* and *C. arenarius*; 2) similar environmental selection pressures have produced an example of ecological convergence; 3) sample size may be insufficient to yield accurate results. Three of the 19 samples of *C. regalis* were taken from the same estuary (Wassaw Sound) as were all samples of *C. nothus*; the remaining sera from *C. regalis* were collected in an estuary (Peconic Bay) sharing several physical and chemical parameters with Wassaw Sound (Odum et al. 1974). Thus, a measure of ecological convergence may be involved. Similar reasoning might explain the d_{jk} calculated for *C. arenarius* versus *C. nothus*; the value (63.8) might be reduced if several other gulf populations of *C. nothus* were added to the total sample.

It could be argued that the much larger sample of *C. nebulosus* ($n = 500$) was responsible for most of the difference in the taxonomic distance value since rare bands are being included. This could only be the case for band 38 which occurred in only 2.1% of the specimens sampled. The values (percent occurrence) of the remaining six unique bands (8%, 23%, 66%, 67%, 89%, 89%) argue against this possibility. The average value of 72.8 is therefore taken to mean that *C. nebulosus* is the most divergent of the four species investigated. Possible reasons for this observation have been elaborated previously.

A significant observation in our study is that relatively few species-specific (i.e., unique) proteins have been detected, a phenomenon not without precedence, however (Lewontin 1974). In a study of 10 species of *Drosophila*, the number of unique proteins ranged from 2.6 to 28.2%, with an average of 14.3% (Hubby and Throckmorton 1968). Our own figures compare favorably with these: *C. nebulosus*, 23%; *C nothus*, 7%; *C. regalis*, 5%; and *C. arenarius*, 0%.

Eye Lens Proteins

In a review of intraspecific variation in lens proteins, Day and Clayton (1973) detected no polymorphisms and concluded that observed differ-

ences were almost wholly quantitative rather than qualitative. Data from other studies indicate two further conclusions. First, lens proteins on the whole express a high degree of conservatism. Secondly, in cases where evidence of polymorphisms have been obtained, fishes have been most often implicated. Smith and Goldstein (1967), Smith (1969, 1971), and Smith and Clemens (1973) reported intraspecific variations in the lens patterns of numerous species. Barrett and Williams (1967) detected a polymorphism in the lens proteins of the bonito *Sarda chiliensis*. Eckroat and Wright (1969) and Eckroat (1973) provided direct evidence of polymorphisms in the eye lens of the brook trout, *Salvelinus fontinalis*, and demonstrated simple Mendelian inheritance for several characters.

Previous observations for eye lens proteins and the conclusions stated above are reflected in our work on the patterns derived from the genus *Cynoscion*. Lens protein patterns displayed considerable convervatism among the four seatrouts. Four bands from a total of eight occur in all taxa and are probably high molecular weight α- and β-crystallins. Only a single band (E in *C. nebulosus*, Figure 3) is unique and is found in either very low frequency or not at all in four of the seven estuaries sampled. Its relatively high frequencies in Corpus Christi, Galveston, and Florida Bay (36, 39, and 50%, respectively) indicate a possible relationship to high turbidity and low light intensities characteristic of these three areas.

Although intensity patterns did not vary significantly within a species (with the exception of two bands involved in a suspected polymorphism in *C. nebulosus*), the quantities of protein in bands with the same mobility were quite different and species-specific (Figure 3). The selective forces which control the quantity of protein present in a given band are not easily recognized. The geographic ranges of these four species overlap considerably althouth their centers of abundance are quite different. *Cynoscion nothus* is found farther offshore than its congeners; *C. nebulosus* is primarily restricted to the estuarine habitat. All seatrouts probably experience a similar range of water color and turbidities in their respective habitats. None is considered to be more diurnal or nocturnal than the others. Their temperature ranges overlap considerably. Therefore, it is somewhat puzzling as to the cause of the common observation that variations in patterns both within a species and between them is restricted mainly to intensity differences. Presently the advantages of different proportions of crystallins and other eye lens protein in a particular species are poorly known.

Myogen Proteins

The general application of myogen proteins to systematic studies has been reviewed by Tsuyuki (1974). Perhaps no other tissue investigated has displayed such an overall lack of intraspecific variations. Only a few species of fishes have exhibited detectable polymorphisms (e.g., Nyman 1967; Tsuyuki et al. 1968; Gray and McKenzie 1970), and it is noteworthy that most of these are "tetraploid" species. The majority of investigations on other forms reveal virtually no intraspecific variation, an observation in direct contrast with other protein systems which generally display polymorphisms. Various estimates of proportions of polymorphic alleles in vertebrate species are placed at from 10 to 20% (Selander and Kaufman 1973). The constancy maintained in myogen proteins in the presence of selective forces is indeed remarkable.

The general conservatism displayed in myogen patterns was observed in our own work, but with several important differences. As previously described, *C. nothus* and *C. nebulosus* differed in the presence or absence of one or more major (by staining intensity) bands. Band J (Figure 5), unique in *C. nebulosus*, is found in all samples, and produces a large characteristic peak on densitometer tracings. The absence of several bands, notably B and C (Figure 5), characterizes *C. nothus*.

On the basis of myogen patterns, we suggest that *C. arenarius* and *C. regalis* are more closely related to each other than are any other combination of species under consideration and should be treated as conspecific. Thus, we reject the phylogeny based on slight osteological differences proposed by Mohsin (1973). The gulf form (*C. arenarius*) should be recognized as a subspecies of *C. regalis*, a conclusion strengthened by recent confirmation of specimens of *C. regalis* from the Gulf of Mexico.

Earlier reports of *C. regalis* in the gulf generally lacked documentation, or were misidentifications of *C. arenarius*. The report of Jordan and Eigenmann (1889) from Mobile Bay, Ala., was based on specimens of *C. arenarius*, a form not recognized

until 45 yr later. Rivas (1954) mentioned the weakfish in the gulf but provided no specific data. Hutton et al. (1956) reported *C. regalis* from Boca Ciega Bay at St. Petersburg, Fla., but Springer and Woodburn (1960) listed only *C. arenarius* from Tampa Bay. No specimens of *C. regalis* from the gulf are in the reference fish collection of the Department of Natural Resources at the St. Petersburg Marine Laboratory (Moe et al. 1966).

Two adult *C. regalis* (266 and 298 mm standard length) were captured by personnel from the Marco Ecology Laboratory in the vicinity of Marco Island, on the southwest coast of Florida on 21 July 1972 (Florida State University Fish Collection, catalog number 24023). The documentation of the weakfish in the Gulf of Mexico together with the extremely close morphological and biochemical characteristics shared by *C. regalis* and *C. arenarius* suggest that gene exchange between the Atlantic Ocean and gulf coast populations is feasible although we have no proof of their interbreeding. Nevertheless, the evidence points to the same series of events which characterize the evolutionary history of other marine geminate species in Florida. When the peninsula split the ancestral population into two, the Gulf population differentiated from that in the Atlantic (see Ginsburg 1952; Walters and Robins 1961). Whether or not isolation was complete or only partial, the present distribution indicates that at least one form (*C. regalis*) has been successful in moving around the tip of the peninsula into southeastern gulf waters and in establishing secondary contact with the other (*C. arenarius*). The status of *C. arenarius* should be investigated in depth. Perhaps an extensive enzyme study would be appropriate, the results of which could be compared by statistical analyses (Avise 1974) to determine the level of differentiation between two forms.

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