AN ABSTRACT OF THE THESIS OF F[illegible]da Osborne Wray for the Master of Science in Biology presented May 14, 1975.

Title: An Electrophoretic Study of the Eye Lens Nuclear Proteins of Abudefduf amabilis (de Vis) and Abudefduf leucopomus (Lesson) (Pomacentridae)

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THEODORE D. ALLEN, Chairman, Thesis Committee

Variation in color patterns within a single species has caused many taxonomic problems. It was thought that Abudefduf amabilis (De Vis) and Abudefduf leucopomus (Lesson) were perhaps also color variants of the same species. Hybrids have been found on Guam and elsewhere in relatively small numbers and although it is indicative of interbreeding between these two species of Abudefduf it does not imply that they are the same species.

It was decided to use the technique of cellulose acetate electrophoresis to study the eye lens nuclear proteins. The eye lens nuclei have been used in many taxonomic studies because of their very stable character and have been found to be reliable indicators of species as well as breeding populations. Abudefduf glaucus (Cuvier and Valenciennes) and Abudefduf biocellatus (Quoy and Gaimard) which belong to the same subgenus, Chrysiptera, as Abudefduf amabilis and Abudefduf leucopomus

were used as controls since were readily distinguishable. Although all four species have the same protein band pattern, that is they all exhibit seven bands with the same migration distances, it was found by using the Kruskal-Wallis and Wilcoxon statistical tests that there is a significant difference (P=.05) between the four species in protein concentration of these bands. Thus four separate breeding populations are present and probably four different species.

TO THE GRADUATE SCHOOL

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CHAPTER I

INTRODUCTION

A commonly encountered problem in taxonomic studies is one in which two groups appear to be the same except for a variation in color. For example, Scarus, the parrotfish, shows several cases of sexual dimorphism in which the color patterns of male and female are so markedly different that these two sexes have been classified as two separate species (Randall, 1963). There may be other cases of dimorphism caused by sex, age, or environment, either questioned or still unnoticed in reef fishes. It has also been suggested that Abudefduf amabilis (De Vis) and Abudefduf leucopomus (Lesson) in the family Pomacentridae are perhaps dimorphic forms of a single species (R.S. Jones, pers. comm.; Allen, 1972; See Appendix). Fish showing intermediate color patterns have been observed in New Guinea, Fiji, Palau, the Solomon Sea, and on the Great Barrier Reef (Allen, 1972). These have also been observed in Guam in relatively small numbers (pers. observ.). It is known that the color variation is not a result of sexual dimorphism as ripe females of both color forms have been reported (Allen, 1972). The presence of mature females showing both color patterns indicates that the color variation is not the result of

changes in color pattern with age. The possibility remains, however, that the color variation in Abudefduf amabilis and A. leucopomus may be caused by environmental factors. A. leucopomus patterns were observed in individuals in calm water and A. amabilis patterns in individuals in fairly rough surge (Allen, 1972 and pers. observ.). Intermediates would presumably be found in any environment if the two extreme color patterns of A. amabilis and A. leucopomus interbreed. However, interbreeding is not evidence that the two are of the same species unless it is found that the hybrid is fertile. Breeding of these rare hybrids, like breeding of most tropical fish in the laboratory, might prove to be very difficult. Because of the anticipated difficulty of breeding the hybrids in the laboratory, it was decided to use electrophoresis of the eye lens nuclear proteins to see if this type of analysis might indicate whether A. amabilis and A. leucopomus belong to the same breeding population and therefore to the same species.

Electrophoresis of the eye lens nuclear proteins was chosen for the following four reasons. First, the eye lens nucleus is made of cells that have sclerosed and died during lens formation from the lack of intrinsic circulation; therefore, these proteins have been removed from sources of dietary protein and oxygen (Walls, 1942). Carbon-14 studies have not detected any turnover of the nuclear lens protein (and Bell, 1965).

No change in electrophoretic pattern has been observed with age (Smith, 1969 and Salvelinus fontinalis, Eckroat and Wright, 1969) or between right and left eyes (scombroid fishes, Barret and Williams, 1967; Smith, 1968; and Salvelinus fontinalis, Eckroat and Wright, 1969) or sex (Salvelinus fontinalis, Eckroat and Wright, 1969). Third, the nuclear proteins are highly concentrated as well as readily soluble (Wood and Burgess, 1961). Fourth, these proteins resist denaturation (Thunnus albacares, Smith, 1965; Sebastolobus alascanus, Smith, 1971b). Smith (1971b) studied the natural stability of the nuclear lens proteins. No change in the electrophoretic pattern of the eye lens nuclear protein was found in fish which had been frozen once; frozen, thawed, and refrozen; decomposed for five days at room temperature; stored outdoors in the shade for five days; and stored outdoors in the sun for five days.

Because of their excellent properties, eye lens nuclear proteins have been used in taxonomic studies of several species of fish. The electrophoretic band patterns have supported the results of traditional taxonomic studies of bluefin tuna (Smith, 1968); Scorpaenidae (Smith, 1968); yellowfin tuna and big eye tuna (Smith, 1970) and bluefin tuna, bonita, and albacore (Smith, 1971c). In one case, where speciation was doubtful, the electrophoretic study did not support previous taxonomy (Smith, 1968).

Not only has it been found possible to distinguish species electrophoretically but also to separate breeding populations within species (Salmo gairdneri, Smith, 1971a; Salvelinus fontinalis, Eckroat and Wright, 1969). Electrophoretic pattern differences were found in populations of the same species of tuna coming from California and Australia (Smith, 1969); bluefin tuna from various areas along the California coast (Smith and Clemens, 1973) and in ocean whitefish from Cedros Island, Baja California and Coronados Island, California (Smith and Goldstein, 1967).

The genes for eye lens nuclear proteins appear to be independent of other genes, and there is probably a simple Mendelian mode of inheritance (Smith, 1971a). Variation in protein patterns appears to be caused by incomplete dominance in Salvelinus fontinalis (Eckroat and Wright, 1969) and Salmo gairdneri (Smith, 1971a). Multiple alleles appear to be present at the locus for some eye lens nuclear proteins (Smith, 1971a

Because of the ural stability of the eye lens nuclear protein as obs ved in studies on a wide variety of fish (and it is med that pomacentrids would also show this stability) and the sensitivity of electrophoresis in separating species and b eeding populations of fish, it was decided to apply this technique to Abudefduf amabilis and A. leucopomus to d ermine if they do, indeed, belong to different breedi lations.

Because Abudefduf biocellatus (Quoy and Gaimard) and A. glaucus (Cuvier and Valenciennes) belong to the same subgenus (subgenus Chrysiptera) as A. amabilis and A. leucopomus but are clearly distinguishable as separate species they were used as standards to determine the expectable range of variation in electrophoretic patterns between distinct species. Three other pomacentrid species in different genera (Dascyllus aruanus, Pomacentrus albofasciatus, and P. melanopterus) were also run to give some indication of the range of electrophoretic variation to be found within the family.

TABLE 1

THE MEAN STANDARD LENGTH, THE MEAN EYE LENS NUCLEI WEIGHT AND SAMPLE SIZE OF THE POMACENTRIDS STUDIED

	Sample Size	Mean Standard Length (mm)	Mean Wet Weight of Eye Lens Nuclei (gm)
Abudefduf amabilis	33	43	.0059
Abudefduf leucopomus	6	35	.0032
[illegible] biocellatus	9	48	.0124
Abudefduf glaucus	20	49	.0088
Dascyllus aruanus	24	33	.0090
Pomacentrus albofasciatus	8	38	.0087
Pomacentrus melan[illegible]rus	4	54	.0094

to give an equal volume of solution and tissue when centrifuged (Smith, 1969). The nuclei were then macerated using a glass rod. Each tube was covered with parafilm and placed in the refrigerator at 5°C for a 24-hour period. During this time the proteins were solubilized (Smith, 1969).

After 24 hours the tubes were removed and centrifuged at a force of about 500 G for three to five minutes. A two microliter aliquot of the cleared extract was removed with a micropipette and was then pipetted in a straight line across the center of a 1" by 6" cellulose acetate strip. The strips were soaked in a solution containing 0.1 M urea and 0.023 M sodium borate at a pH of 8.6 (Barret and Williams, 1967) and were blotted lightly before the extract was applied. The strips were placed in the electrophoresis chamber built after Audubert and de Mende (1960) which had been filled with one liter of the urea-sodium borate buffer solution. Following an equilibration period of 30 minutes, an electric current of 300 Volts, 7 milliamps was applied for 20 minutes causing the proteins to migrate in bands. The distance migrated was determined by protein size based on the number of amino acids in the protein and by protein charge based on type of amino acids and their sequence in the protein. At the end of the run, the strips were removed and stained for eight minutes in a five percent trichloroacetic acid solution containing 0.5 percent Ponceau S. The amount of stain absorbed by each band was

proportional to the amount of protein present. The strips were then transferred to two successive baths of seven percent glacial acetic acid, each for a period of five to eight minutes for destaining. The strips were cleared in cyclohexanone as described by Beckman Instruction Manual for the Microzone Electrophoresis Cell, 1965. The cleared strips were scanned on a Beckman Model RB-IN-6 densitometer to produce a graph of stain intensity versus distance migrated reflecting the types and amounts of protein present.

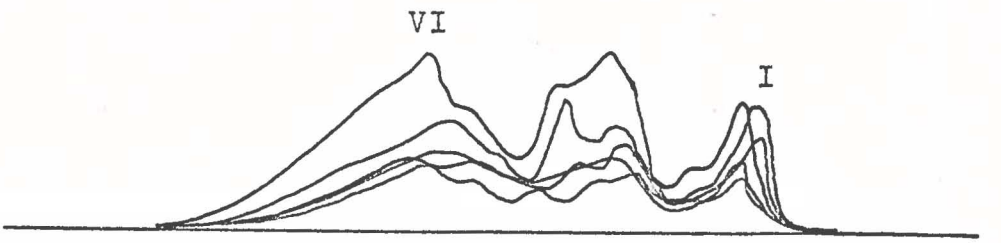
CHAPTER III

RESULTS

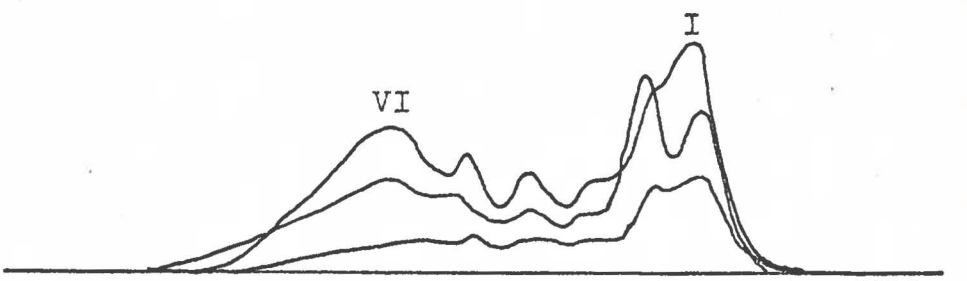
Figures 1 and 4 show overlapping densitometer scans (actual size) of the electrophoretic patterns obtained from runs on several specimens of each species. These patterns were selected to illustrate the wide variation of quantity of protein present in each band within one species.

Figures 2 and 5 are histograms of migration distances for each of the bands. To reduce variation of migration distances measured for each band from the densitometer scans, the data was transformed by dividing the distance migrated for each band by the total migration distance. This transformation eliminated many of the overlaps caused by variation in total distances. Band I was arbitrarily assigned a distance of 0 because it was a sharp distinctive band and was used as a reference point for measuring band distances. There was still some overlap which can be seen in Figures 2 and 5; however, when the stained patterns were examined the bands were readily distinguishable from each other.

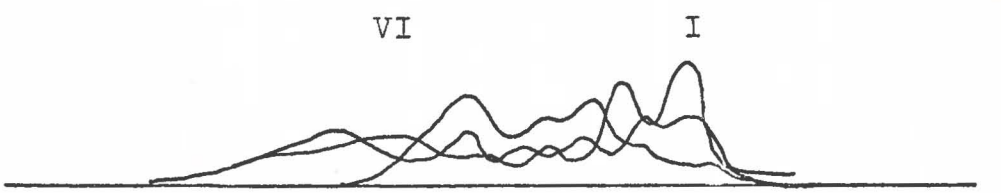
The average transformed migration distances and variation and mean of percent of protein present in each band can be seen in Figures 3 and 6 and Tables 2 and 3. Migration distance reflects size and type of protein present.



Dascyllus aruanus



Pomacentrus albofasciatus



Pomacentrus melanopterus

Figure 1. Overlapping densitometer scans (actual size) showing the variation in percent protein occurring in each band within [illegible] of the species. Bands I and VI are marked.

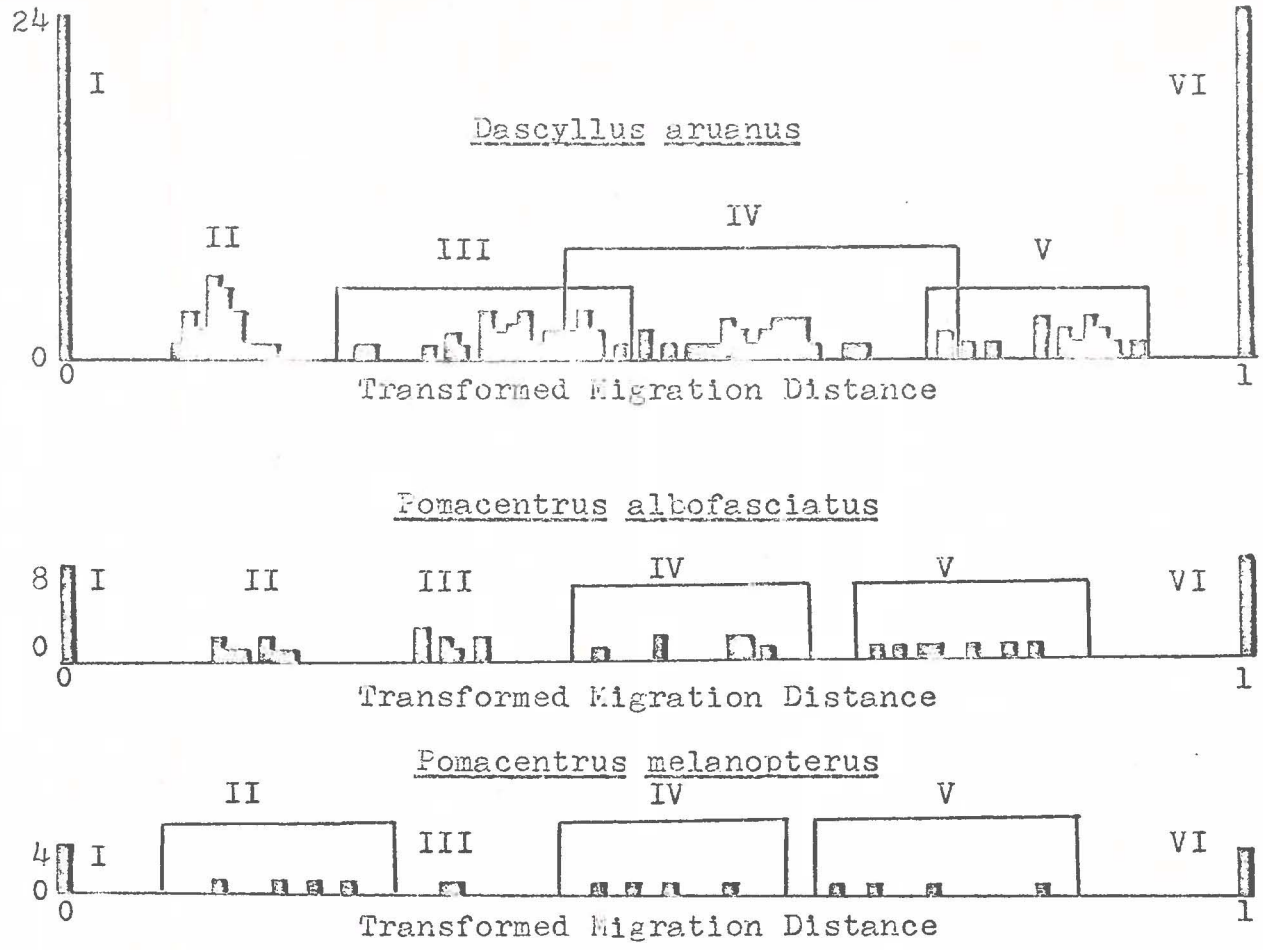


Figure 2. Histogram of transformed migration distances for each of the six protein bands. Note that there is variation in the distance migrated occurring in each individual band.

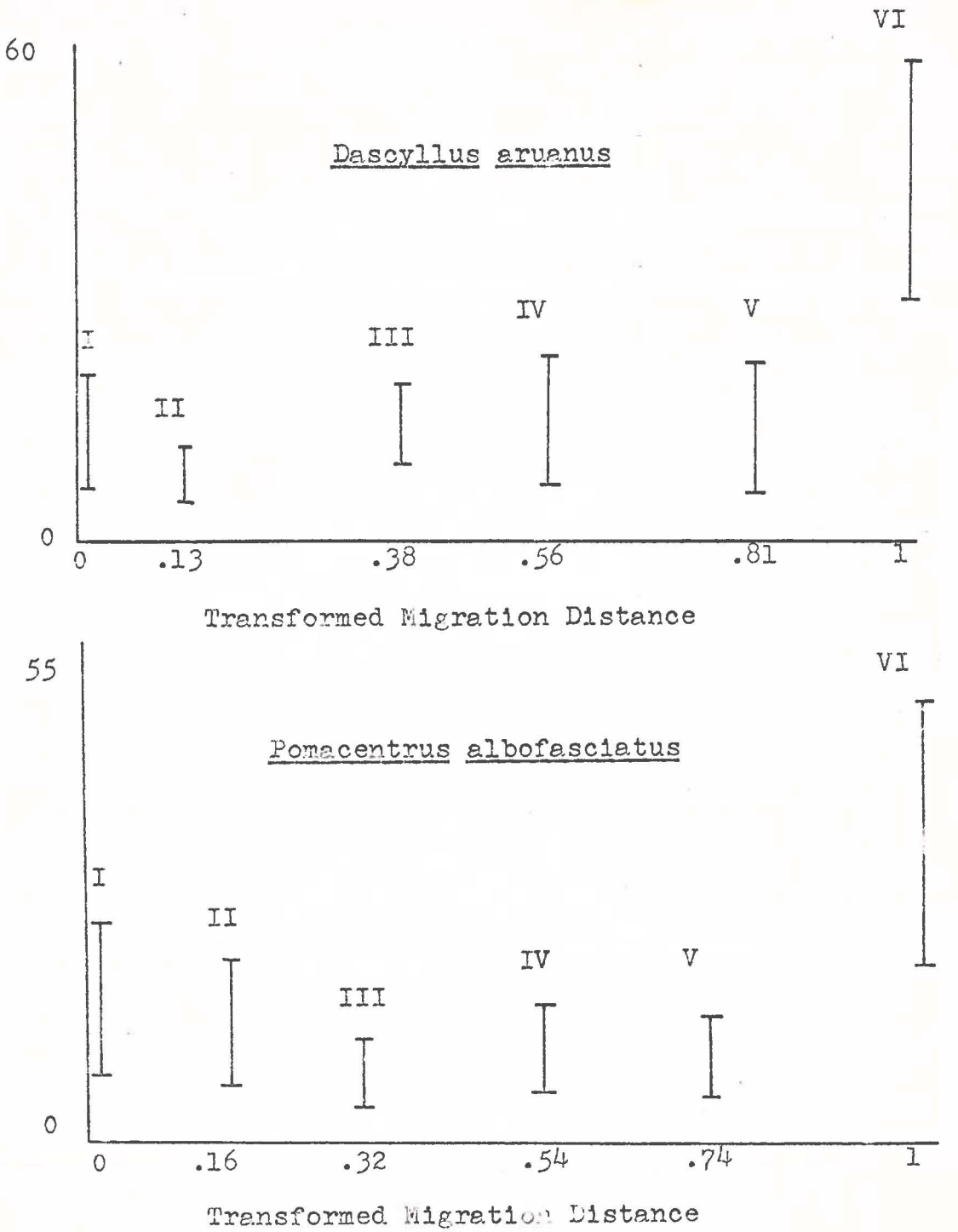
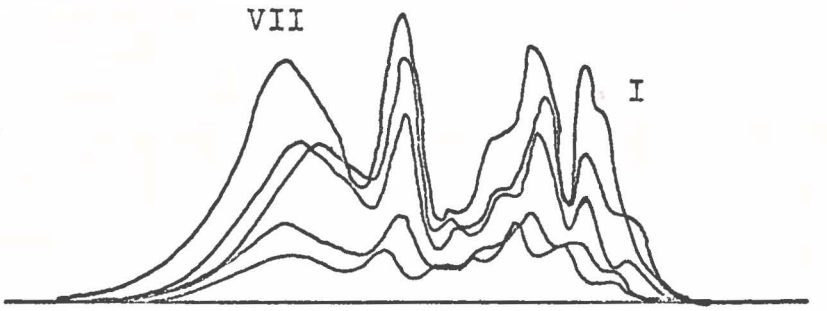
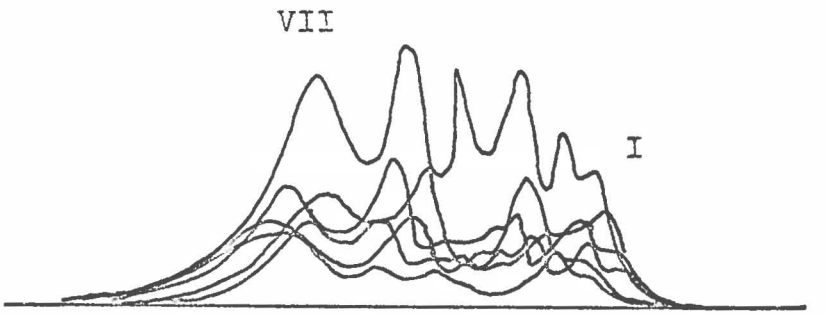


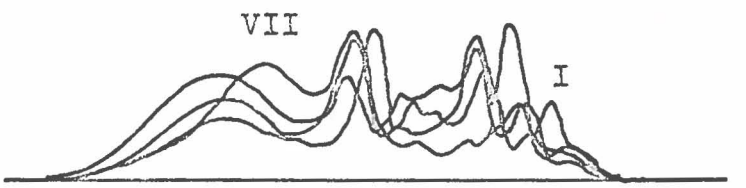
Figure 3. Range of percent protein based on one standard deviation for the six protein bands.



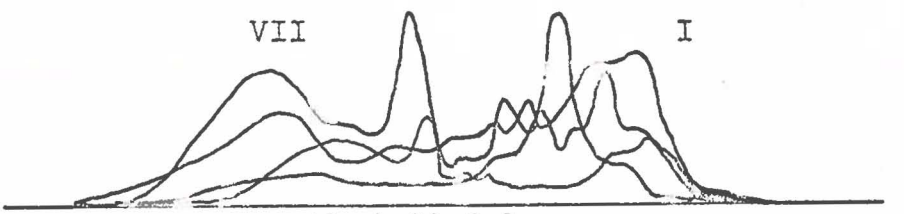
Abudefduf amabilis



Abudefduf biocellatus

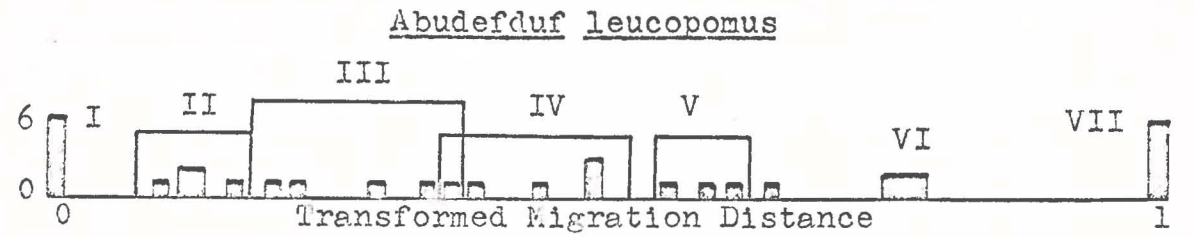


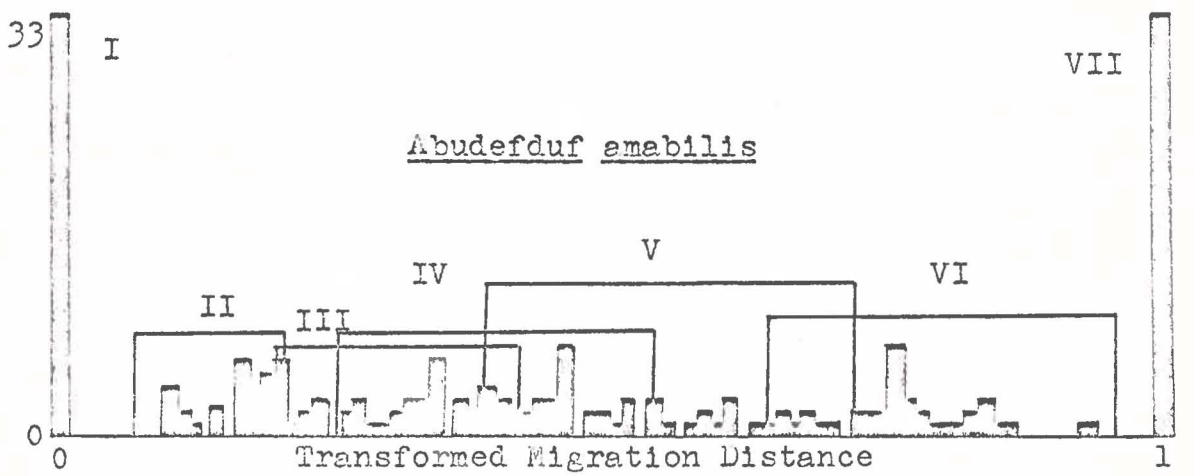
Abudefduf glaucus



Abudefduf leucopomus

Figure 4. Overlapping densitometer scans (actual size) showing the variation in percent protein occurring in each band within each of the species. Bands I and VII are marked.


Abudefduf leucopomus
6
0
I
II
III
IV
V
VI
VII
0
Transformed Migration Distance
1


33
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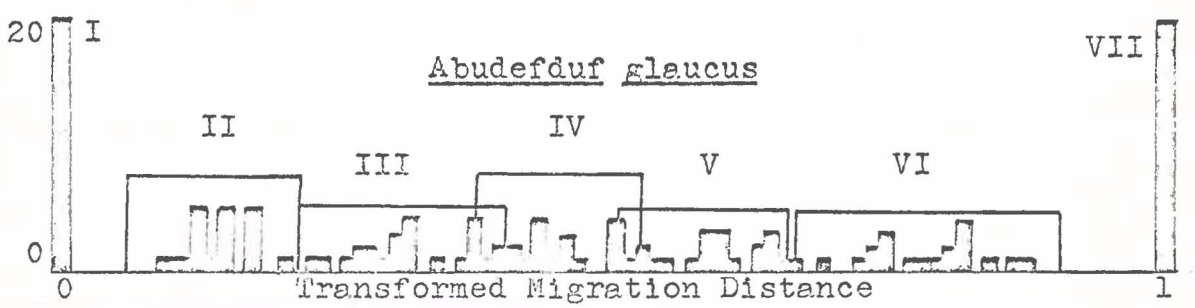
VII
Abudefduf amabilis
II
III
IV
V
VI
0
Transformed Migration Distance
1


20
0
I
VII
Abudefduf glaucus
II
III
IV
V
VI
0
Transformed Migration Distance
1

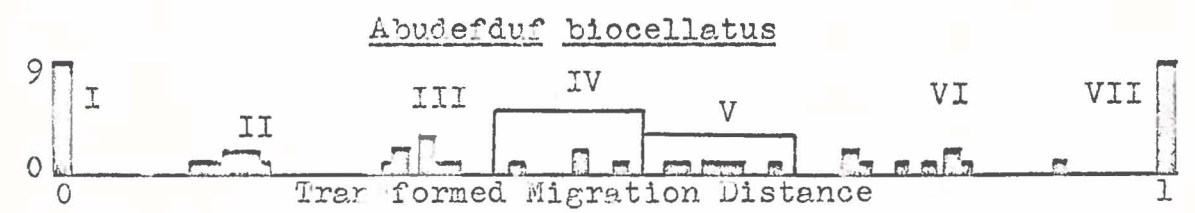

Abudefduf biocellatus
9
0
I
II
III
IV
V
VI
VII
0
Trar formed Migration Distance
1

Figure 5. Histogram showing transformed migration distances for each of the seven protein bands. Note that there is variation in the distance migrated occurring in each individual band. Distances were measured from Band I.

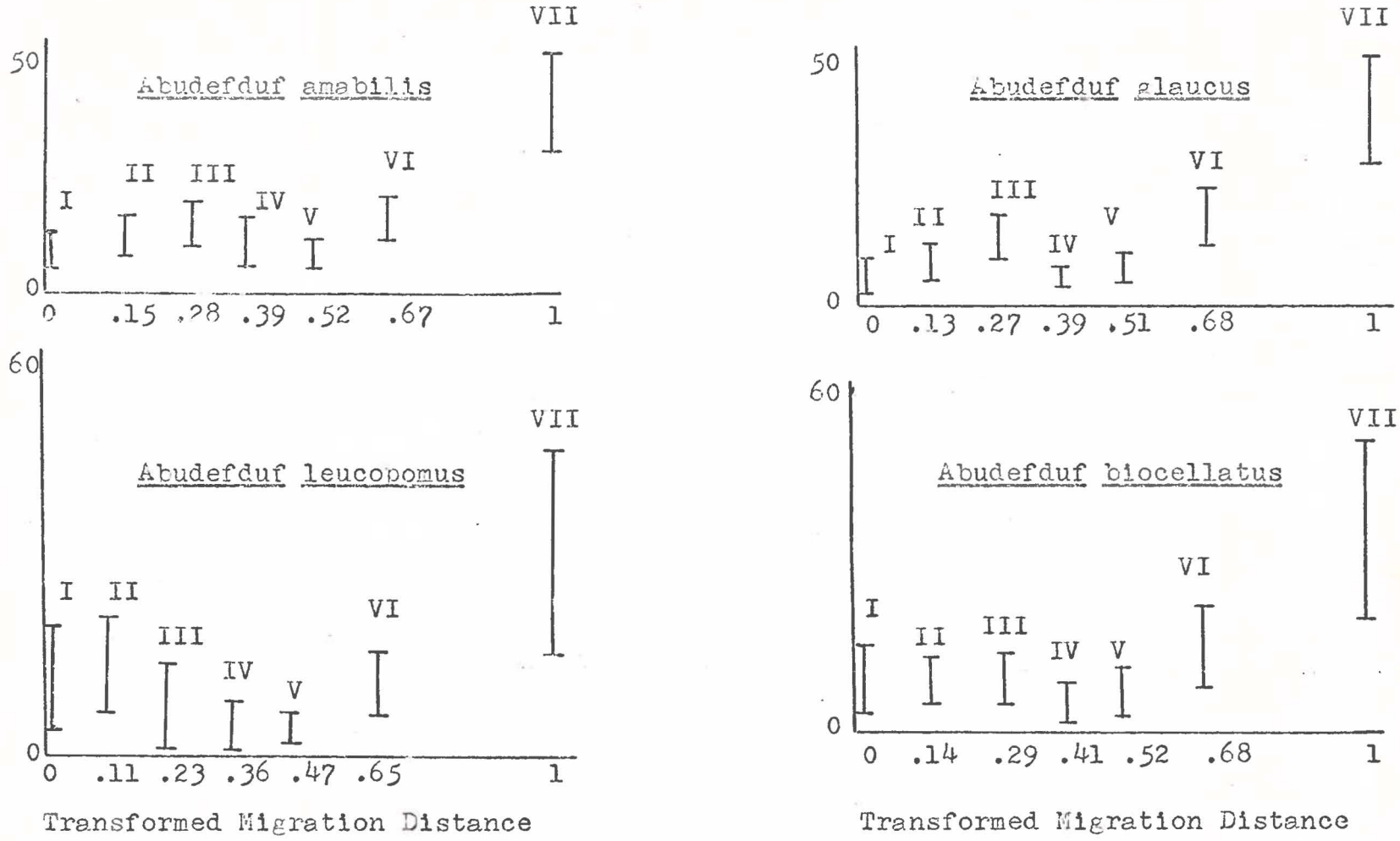


Figure 6. Range of percent protein based on one standard deviation for the seven protein bands in the four species of Abudefduf studied.

TABLE 2

AVERAGE PERCENT PROTEIN, STANDARD DEVIATION OF PERCENT PROTEIN, AVERAGE TRANSFORMED MIGRATION DISTANCE AND STANDARD DEVIATION OF TRANSFORMED MIGRATION DISTANCE

Band Number and Species	Average Percent Protein	S.D. of Percent Protein	Average Transformed Migration Distance	S.D. of Transformed Migration Distance
Band I				
Dascyllus aruanus	13.0	6.8	0.0	-
Pomacentrus albofasciatus	17.7	9.0	0.0	-
Pomacentrus melanopterus	-	-	0.0	-
Band II				
Dascyllus aruanus	7.8	3.4	.13	.03
Pomacentrus albofasciatus	14.8	7.4	.16	.06
Pomacentrus melanopterus	-	-	.19	.12
Band III				
Dascyllus aruanus	13.8	4.6	.38	.09
Pomacentrus albofasciatus	8.6	4.1	.32	.12
Pomacentrus melanopterus	-	-	.33	.32
Band IV				
Dascyllus aruanus	14.0	6.7	.56	.13
Pomacentrus albofasciatus	11.4	5.1	.54	.21
Pomacentrus melanopterus	-	-	.50	.29
Band V				
Dascyllus aruanus	13.2	8.0	.81	.21
Pomacentrus albofasciatus	10.5	4.6	.74	.31
Pomacentrus melanopterus	-	-	.72	.42
Band VI				
Dascyllus aruanus	43.2	14.3	1.0	-
Pomacentrus albofasciatus	37.2	16.1	1.0	-
Pomacentrus melanopterus	-	-	1.0	-

TABLE 3

PERCENT OCCURRENCE, AVERAGE PERCENT PROTEIN, AND STANDARD DEVIATION OF PERCENT PROTEIN, AVERAGE TRANSFORMED MIGRATION DISTANCE AND STANDARD DEVIATION OF TRANSFORMED MIGRATION DISTANCE

Band Number and Species	Percent Occurrence	Average Percent Protein	S. D. of Percent Protein	Average Transformed Migration Distance	S.D. of Transformed Migration Distance
Band I					
Abudefduf amabilis	100.00%	8.0	3.8	0.0	-
Abudefduf leucopomus	100.00%	14.0	10.3	0.0	-
Abudefduf glaucus	100.00%	6.0	3.6	0.0	-
Abudefduf biocellatus	100.00%	11.1	7.0	0.0	-
Band II					
Abudefduf amabilis	100.00%	11.3	4.0	.15	.05
Abudefduf leucopomus	100.00%	16.4	9.7	.11	.05
Abudefduf glaucus	100.00%	9.4	3.7	.13	.04
Abudefduf biocellatus	100.00%	10.8	4.7	.14	.05
Band III					
Abudefduf amabilis	96.97%	13.9	4.8	.23	.07
Abudefduf leucopomus	60.00%	8.6	9.1	.23	.15
Abudefduf glaucus	100.00%	13.8	4.2	.27	.07
Abudefduf biocellatus	88.89%	11.1	4.9	.29	.11
Band IV					
Abudefduf amabilis	90.91%	10.3	4.6	.39	.09
Abudefduf leucopomus	60.00%	4.8	5.1	.36	.21
Abudefduf glaucus	100.00%	6.3	2.3	.39	.10
Abudefduf biocellatus	44.44%	6.2	4.0	.41	.24

Band Number and Species	Percent Occurrence	Average Percent Protein	S.D. of Percent Protein	Average Transformed Migration Distance	S. D. of Transformed Migration Distance
Band V					
Abudefduf amabilis	48.48%	7.3	3.3	.52	.14
Abudefduf leucopomus	80.00%	4.2	3.3	.47	.24
Abudefduf glaucus	94.40%	7.8	3.1	.51	.13
Abudefduf biocellatus	66.67%	8.4	5.3	.52	.23
Band VI					
Abudefduf amabilis	100.00%	15.6	4.2	.67	.13
Abudefduf leucopomus	100.00%	13.0	6.6	.65	.33
Abudefduf glaucus	100.00%	18.2	5.4	.68	.16
Abudefduf biocellatus	100.00%	17.7	8.1	.68	.25
Band VII					
Abudefduf amabilis	100.00%	38.0	9.7	1.0	-
Abudefduf leucopomus	100.00%	39.2	20.3	1.0	-
Abudefduf glaucus	100.00%	39.1	10.7	1.0	-
Abudefduf biocellatus	100.00%	42.1	16.7	1.0	-

Dascyllus aruanus, Pomacentrus albofasciatus, and P. melanopterus were used as additional controls. These three species showed a difference in the number of protein bands (six) present as well as a difference in migration distances of these bands from the four species of Abudefduf showing that the technique would indeed separate species.

Band I, the reference point, was seen in several different forms on the densitometer scans. It occured in Abudefduf amabilis as a shoulder of Band II or as a small peak. Rarely did it form a distinctive peak. Band I appeared in the same form in A. leucopomus. In A. biocellatus Band I formed one of the largest peaks and was distinctive in many cases. It was also frequently seen in conjunction with Band II as a shoulder. This was also the case in A. glaucus. A. leucopomus had the highest average percent protein present in Band I and A. glaucus the lowest (Table 3).

Band II was present in all four species of Abudefduf. In A. amabilis it was found generally to be a peak of varying size, only rarely did it occur as a shoulder of Band III. Band II in A. leucopomus occurs both as a peak and shoulder of Band I, i.e. these two bands are often found in conjunction. In A. glaucus Band II was predominately found as a small peak but also as a shoulder of Band III. This was also the case in A. biocellatus.

Abudefduf leucopomus again had the highest average percent protein (16.4). The lowest percentage of protein was in A. glaucus (9.4).

Band III was a distinctive band in all four species of Abudefduf. It occured only 60 percent of the time in A. leucopomus and also had the lowest percentage of protein in A. leucopomus (8.6). The highest concentration of protein was in A. amabilis and A. glaucus and in both of these, Band III was generally present (Table 3).

Bands IV and V were small bands forming shoulders with the adjacent bands, Bands III and VI, respectively. Band IV was present in A. leucopomus 60 percent of the time and in A. biocellatus 44 percent of the time. It was always present in A. glaucus and 90 percent of the time it appeared in A. amabilis. A. leucopomus had the lowest percent of protein and A. amabilis the highest (Table 3). Band V was present 48 percent of the time in A. amabilis (lowest occurrence) and 94 percent of the time in A. glaucus (highest occurrence). The highest percentage of protein was found in A. biocellatus which had a 66 percent occurrence and the lowest in A. leucopomus with an 80 percent occurrence of the band.

Band VI was a distinctive peak in all four species of Abudefduf. It was present all of the time in all species. The highest concentration of protein was in A. glaucus and the lowest in A. leucopomus, 18.2 and 13.0 respectively.

Band VII contrasts sharply with Band VI in shape. It is a broad flat curve and was again found in all four species. The variation of protein between the four species was considerably less in this band. Abudefduf biocellatus had the highest concentration (42.1) and A. amabilis the lowest (38.0).

Migration distances for each of the seven bands were very similar for all four of the species of Abudefduf. It was, therefore, necessary to determine if significant differences could be found between A. amabilis, A. leucopomus, A. glaucus, and A. biocellatus based on the percentages of protein found in each of the bands. All four sets of data from Abudefduf were first tested using the Kruskal-Wallis tests for more than two samples (Sokal and Rohlf, 1969). This is a ranked statistical test. Each of the seven bands was tested comparing all four species. With the exception of Band VII, it was found that there was a significant difference between the four species. It was then necessary to test two samples at a time. For this the Wilcoxon Two Sample test was used (Sokal and Rohlf, 1969); it is also a ranked test. A total of six different statistical comparisons between the four species was made for each of the seven bands (Table 4). Again it was found in all six tests that Band VII showed no significant difference for each of the interspecies comparisons. Band VI, on the other hand, showed the highest number of significant statistical differences.

TABLE 4

RESULTS OF THE WILCOXON TWO SAMPLE TEST. P= .05 SHOWED NO SIGNIFICANCE DIFFERENCE

Statistical Comparison	Band I	Band II	Band III	Band IV	Band V	Band VI	Band VII
Abudefduf leucopomus x Abudefduf biocellatus	>.05	<.025	>.05	>.05	>.05	<.01	>.05
Abudefduf glaucus x Abudefduf leucopomus	<.005	<.005	>.05	>.05	<.025	<.001	>.05
Abudefduf biocellatus x Abudefduf glaucus	<.025	>.05	<.01	<.025	>.05	>.05	>.05
Abudefduf amabilis x Abudefduf leucopomus	<.05	<.025	>.05	<.025	>.05	<.01	>.05
Abudefduf glaucus x Abudefduf amabilis	<.025	<.05	>.05	<.005	<.0005	<.005	>.05
Abudefduf amabilis x Abudefduf biocellatus	>.05	>.05	<.025	<.0005	>.05	<.025	>.05

The remaining five bands showed either two or four significant differences in the six comparisons. The results of the interspecies comparisons can be seen in Table 4. Four bands were shown to be significantly different between Abudefduf amabilis and A. leucopomus; the highest number of significant differences was found between A. amabilis and A. glaucus (five) and the lowest between A. leucopomus and A. biocellatus (two). Between A. glaucus and A. biocellatus three bands were shown to be significantly different.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Seven bands were found to be present in the four species of Abudefduf studied: A. amabilis, A. leucopomus, A. biocellatus, and A. glaucus. However, only six bands were found to be present in Dascyllus and Pomacentrus species studied. This indicates that the species of Abudefduf studied have one more gene for eye lens nuclear protein than is found in Dascyllus and Pomacentrus. The species of Abudefduf studied are, therefore, different from the species in the other two genera tested.

Electrophoretic migration distances for each of the seven bands for all four species were similar (Table 3 and Figure 6) indicating that the same types of protein are found in all four species. There is a high degree of variation in the amount of protein in each band from species to species. Inter[illegible]cies variation in protein can be seen in Figure 6. The [illegible]mary difference between the four species is the amoun[illegible] of protein present in each of the seven bands and also in the high rates of absence of a band in some species. Bands III, IV, and V were the only bands to sho[illegible]bsences (Table 3).

In all cas[illegible] at least two bands were statistically distinguishable f[illegible] each other in all six statistical comparisons.

Smith (1971a), in working with Salmo gairdnerii, pointed out that evolutionary rates of change for one amino acid is at the rate of seven to ten million years for blood and cytochrome-c. No rates have been postulated for the eye lens nuclear proteins. Since the patterns are similar between these four species, the divergence is occurring with the amounts of protein found in each band; this could be either the formation of a band or the loss of a band. Since these proteins are set down in early development and not turned over nor do they fluctuate with age, these differences are probably genetic differences. If the quantities of protein are different and this difference is not because of age, there are probably different frequencies of alleles at the loci. These most likely function in a fashion of incomplete or co-dominance. Also the high rate of absence of Bands IV and V indicate the absence of the alleles coding for these two bands in some individuals.

Between Abudefduf leucopomus and A. biocellatus, two bands were found to be statistically distinguishable which was the lowest number of statistical differences found. There is not doubt that these two species are, indeed, separate species: they differ in coloration, in habitat, and in external morphology. Between the two control species, A. biocellatus and A. glaucus, there were three bands which were statistically significantly different; there is no doubt that these are two species.

On the other hand, Abudefduf amabilis and A. leucopomus, which have been thought to be ecological color variants of the same species showed four out of seven bands to be statistically different. Thus, four distinct breeding populations appear to be present. It is highly possible that A. amabilis and A. leucopomus, which overlap in habitat, are still diverging but have not separated to the point that they cannot still interbreed as evidenced by the occurrence of individuals with intermediate color patterns. Both A. amabilis and A. leucopomus inhabit the same locality on the reef, the surge zone. A. amabilis prefers the reef flat and areas most subjected to wave action. A. leucopomus, however, was always observed in holes or channels where the surge is absent, that is in deeper water on the reef flat. The few intermediates observed appeared in the same sort of habitat with A. leucopomus (pers. observ.).

The Hardy-Weinberg Law states that unselected alleles in a freely interbreeding population will have a binomial distribution. When the action of the genes is additive as in the case of incomplete dominance, the gene frequencies and the protein content of the bands may have complex distributions. Nevertheless, in a freely interbreeding population, the samples should not be statistically distinguishable as they are for A. amabilis and A. leucopomus. The interspecies comparison of these two showed four bands to be statistically different.

This was a higher number of bands that were statistically different than between two distinguishably separate species, Abudefduf leucopomus and A. biocellatus in which only two bands were statistically different. Between the two control species, A. biocellatus and A. glaucus, three bands were found to be significantly different. Thus four separate breeding populations are present and most likely four separate species.

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APPENDIX

The following are color descriptions of live specimens according to Woods and Schultz (1960) of the four species of Abudefduf studied.

Abudefduf leucopomus (Lesson) has a black spot at the dorsal edge of the base of the caudal fin which is prominent at all ages. A narrow blue stripe passes from the snout along the dorsal edge of the eye, widening behind the eye and continuing to the base of the soft dorsal. Here it surrounds the large oblong black spot. The upper sides are brown and the lower, pale yellowish. The belly is white and the pelvic, anal, and caudal fins are pale hyaline in color. No dusky color is found on the distal portion of the caudal fin. There are 12 or 13 soft dorsal rays and 12 or 13 soft anal rays.

Abudefduf amabilis (De Vis) is brown and usually has a narrow white transverse bar from the fifth to seventh dorsal spine to the anus. There is a second white ring around the anterior part of the caudal peduncle. These white areas may be absent. The entire basal third of the caudal fin is black. The pectoral area is always pale; pelvics and anals are dark brown or black, and the middle caudal rays are dusky. There are 11 or 12 soft dorsal rays and 12 soft anal rays.

Both Abudefduf leucopomus and A. amabilis have a pale yellow spot on their opercles.

Abudefduf glaucus (Cuvier and Valenciennes) is plain pale greyish tan in color; bluish or whitish lower sides with two faint inverted V-shaped pale areas. The anus is black, contrasting sharply with the surrounding area. Young have a narrow bluish line from the snout across the top of the eye along the base of the dorsal breaking up into blue dots. This disappears with age. There are 12 soft dorsal rays and 12 soft anal rays.

Abudefduf biocellatus (Quoy and Gaimard) has a brown to black body, with or without a white wedge-shaped transverse bar under the fifth to seventh dorsal spines. The lower sides and belly are brownish and the back bears a large black ocellus (except in large adults), just below the base of the last four dorsal spines and a smaller black spot at the base of the posterior rays. Specimens from 20 to 50 mm may have a narrow pale blue line from the snout across the top of the eye along the dorsal base to the ocellus, sometimes this is absent. There are 13 soft dorsal rays and 13 soft anal rays.

The pale spot on the opercles is lacking in both Abudefduf biocellatus and A. glaucus.