NOTES

DISTRIBUTION OF MELANIN IN THE COLOR PATTERN OF *DELPHINUS DELPHIS* (CETACEA; DELPHINIDAE)

Previous studies of cetacean pigmentation have been concerned with the description of color patterns and the possibilities for their evolutionary production and their adaptive significance. Mitchell (1970) identified four basic color patterns among the Delphinidae: saddled, as exemplified by some species of *Stenella*; spotted, as seen in *Stenella plagiodon*; striped, as seen in *Stenella coeruleoalba*; and crisscross, as seen in *Delphinus delphis*. Naming the crisscross pattern as the most complex, Mitchell used it to establish a terminology for elements of the color patterns. One of his conclusions concerning the evolutionary development of the patterns was that the saddled pattern is most primitive, since it is closest to generalized countershading and because one may hypothetically derive the other three patterns from it by addition of certain features, emphasis of some features, and de-emphasis of others.

Perrin (1972) compared the color pattern of a partially albinistic whitebelly spinner (*S. longirostris*) with that of a normally pigmented individual and showed that the normal color pattern may be described in terms of two independently produced but interacting pigmentation systems or components, only one of which had developed in the partially albinistic individual. Using the two-component approach, he analyzed the color patterns of other delphinids, including *Delphinus* spp., and proposed pattern homologies among the species. He suggested that the more generalized of the two pigmentation systems involves the cape (terminology of Perrin 1970) and its accessory stripes, eye and gape marks, and dorsal fin and fluke colorations. This is overlaid by a second component system that he named the "dorsal overlay system." He proposed that partial overlapping of the two produces the four-part crisscross pattern in *Delphinus*.

If two discrete interacting pigmentation systems are involved in the color pattern of *Delphinus*, that fact should be evidenced in the microstructure of the skin. Previous histological study of *Delphinus* skin provides only a description of the general microscopic anatomy. Stiglbauer (1913) described the microstructure of the skin of *Delphinus delphis* in great detail, including the existence of large dermal papillae and epidermal pegs with granular inclusions of pigment that he identified as melanin. His sample, however, was from the back of a single animal, and he did not have the opportunity to compare the distributions of pigment in different parts of the color pattern. Sokolov (1962) commented very briefly on the pigmentation of two specimens of *Delphinus*, stating that the epidermis on the back and below the dorsal fin was moderately pigmented, on the side of one animal was lightly pigmented, and along the side of the other animal and on the bellies of both was unpigmented. This paper reports the results of comparative microscopic examination of skin samples taken from various areas of the color pattern.

Materials and Methods

Skin samples, each about 5 cm square, were taken from various areas of the bodies of two animals as shown in Figure 1. The porpoise were collected at San Diego, Calif. One animal (field no. WFP 125, adult female, 176 cm, 61 kg) had been frozen for several months before dissection, and the other (WFP 221, adult male, 185 cm, 83 kg, Figure 2) was sampled about 1 h after death.

One centimeter-square specimens were fixed in 10% Formalin,[1] and imbedded in paraffin. Sections were cut 8 μm thick and stained with Schmorl's ferricyanide for malinin. This method stains melanins a dark blue or blue-green, while other epidermal and dermal tissue is stained light green.

The prepared sections were examined under a light microscope. Pigment densities were scored at 125 diameters magnification.

Results

General microscopic anatomy of the skin of *Delphinus* is simple when compared with that of terrestrial mammals and fits the description of cetacean skin given by Simpson and Gardner

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

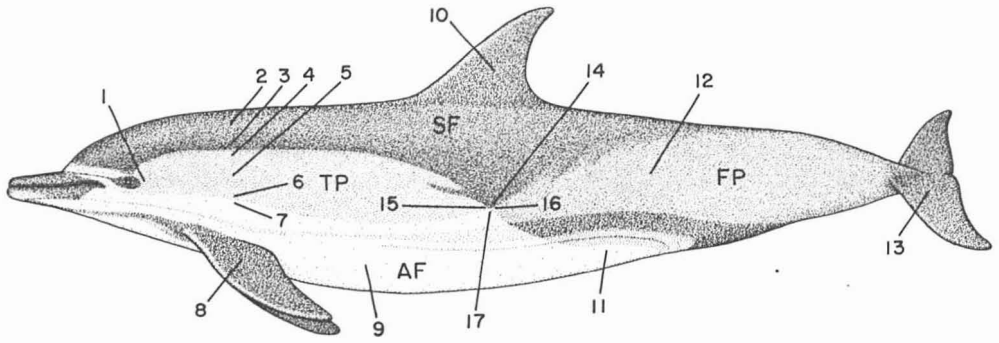

FIGURE 1.—Major pattern features (terminology of Mitchell 1970) and locations from which skin samples were taken: SF = spinal field (black), TP = thoracic patch (buff), FP = flank patch (gray), AF = abdominal field (white).

(1972). The epidermis generally lacks hair (a few hairs are present on the snout in early development but disappear before or shortly after birth), cuticular keratin, and accessory glands. Its thickness varies, being greatest in the ventral region and least on the flippers, dorsal fin, and flukes. The superficial layer of the epidermis, about 10 cells thick, shows considerable flattening of cells. The epidermis consists almost entirely of polyhedral prickle cells, many of which show clear, distended infranuclear cytoplasm. This clear cytoplasm at first appears as holes in the tissue when it is viewed in section, but upon closer examination one may identify the nucleoli inside the clear areas. The epidermis interlocks with the dermis by interdigitation of epidermal rete pegs and dermal papillae (Figure 3), and the dermis grades into the subcutaneous blubber layer. The dermis varies in thickness and density and is composed largely of collagen fibers. The dermal papillae contain blood vessels, blood cells, and other loosely organized connective tissue.

Melanin pigmentation is restricted to the epidermis and is consistently more concentrated at the edges of the epidermis than elsewhere. It is usually most concentrated around the bases of the dermal papillae (at the apices of the epidermal rete pegs) and extending in bands from the apices of the dermal papillae. The pigment has been classified here into three groups for purposes of quantification. "Diffuse" pigment appeared simply as an area which stained darker green than the background and in which pigment grains could not be discerned even at 1,250 diameters magnification. In addition to diffuse pigment, there were granules ranging from less than 0.1 μm to over 5 μm in diameter. Most were spherical or ellipsoid. Granules less than 5 μm in greatest diameter were termed "small grains." These seemed to be actually aggregations of even smaller granules. Those termed "large grains" were larger than 5 μm in diameter and were so dense as to appear as single entities even at high power. Diffuse pigment was characteristically situated peripheral to the nuclei of the prickle cells. This was particularly evident in the central areas of the epidermis. Pigment granules were in general most concentrated at the edges of the epidermis and appeared to extend toward the surface in diffuse bands. In the more lightly colored skin specimens these bands extended only from the apices of the dermal papillae, but in more densely pigmented skin they were more numerous and tended to blend together, resulting in uniform density of pigment throughout the epidermis.

The density of pigment was noted in three regions in each sample: around the bases and edges of the dermal papillae and in the bands described above (Table 1). The observations were each coded from 0 to 4 as follows: for diffuse pigment, 0 = none, 1 = very small amount, 2 = small amount, 3 = medium amount, 4 = large amount; for small grains, 0 = $<$ 1 grain per mm^2, 1 = 1-3 grains per mm^2, 2 = 4-7 grains per mm^2, 3 = 8-11 grains per mm^2, 4 = $>$ 12 grains per mm^2; for large grains, 0 = 0 grains per 4 mm^2, 1 = 1 grain per 4 mm^2, 2 = 2-3 grains per 4 mm^2, 3 = 3-6 grains per 4 mm^2, 4 = $>$ 6 grains per 4 mm^2. Skin samples which appeared white were completely unpigmented or showed very small amounts of diffuse and/or small grains. The buff color characteristic of the thoracic patch (terminology of Mitchell 1970) was associated with either equal prominence of diffuse and granular pigment or

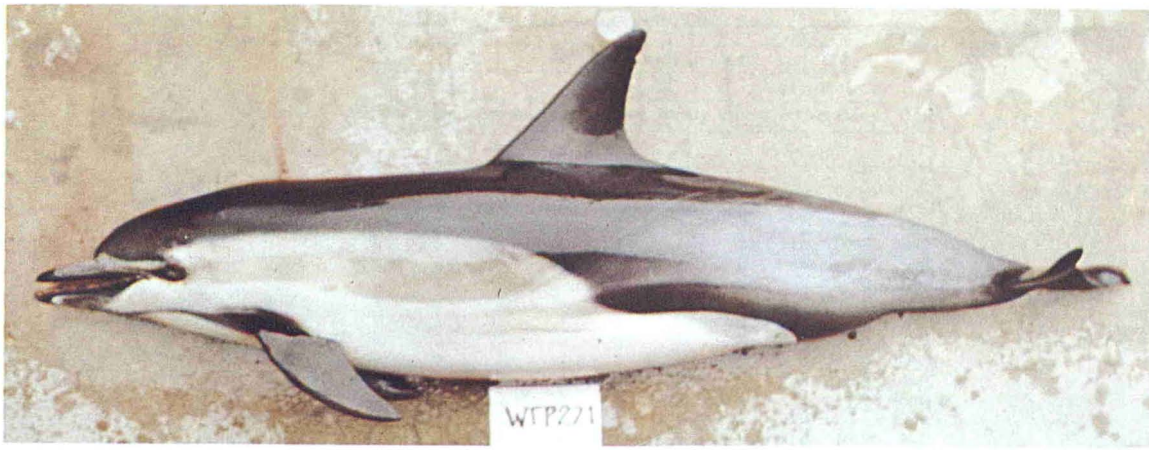

FIGURE 2.—*Delphinus delphis*: Specimen no. WFP 221.

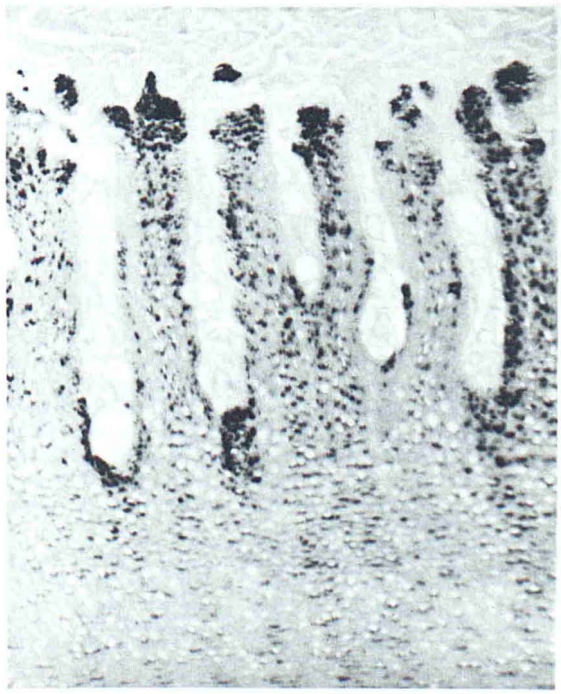

FIGURE 3.—Typical appearance of dermal-epidermal boundary of skin of *Delphinus delphis* (WFP 221 sample no. 10 in Table 1). Note concentration of pigment granules around bases of dermal papillae and in bands extending from ends of papillae. Magnification 1,250 diameters.

greater prominence of diffuse pigment. In gray samples from the flank patch, large grains were present, but the small grains were most prominent. Samples of black skin from flippers and flukes showed the highest densities of large grains, which were more prominent than the small grains.

The histological evidence (summarized in Table 2) supports the concept of interacting color pattern components. As indicated previously, the spinal field may be described as a result of the interaction of the pigmentation of the cape and the dorsal field. The buff colored thoracic patch (i.e., the cape) contains relatively high concentrations of diffuse pigment, while in the grey flank patch (i.e., the dorsal overlay) granular pigment is more prominent. The spinal field is characterized by a larger amount of diffuse pigment than is present in the flank patch, and a larger amount of granular pigment than is present in the thoracic patch. The high concentration of grains in the spinal field indicates a possible synergistic effect of the two component systems.

Perrin (1972) suggested that the flukes are pigmented only as part of the cape system. The skin of the flukes (sample no. 13) contained large amounts of all three types of pigment, indicating that pigmentation of the flukes involves both the cape system and the dorsal overlay system. The skin of the flipper (sample no. 8) also contained large amounts of all three types of pigment.

The major difference between samples taken from WFP 125 (female) and those taken from WFP 221 (male) was that the epidermis was thicker (0.75-1.70 mm as opposed to 0.50-0.95 mm) in the latter animal. Thus a lower density of pigment was required to produce the same color. Also, the samples taken from the buff-colored area of WFP 221 showed conspicuously less diffuse pigment.

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TABLE 1.—Results of microscopic examination of pigment distribution in skin of two *Delphinus delphis*. Codes explained in text.

	Pigment density (coded)									
	Diffuse			Small grains			Large grains			
Sample no.	Bases	Edges	Bands	Bases	Edges	Bands	Bases	Edges	Bands	Color
WFP 125♀										
1	4	3	2	2	2	2	0	0	0	buff
2	4	3	1	4	4	2	4	3	3	black
3	3	3	1	4	3	2	4	3	2	black
4	3	3	2	2	2	1	1	0	0	buff
5	3	3	2	2	2	1	1	0	0	buff
6	3	3	1	2	2	1	0	0	0	buff
7	2	2	1	3	2	1	0	0	0	white
8	3	2	1	4	3	3	4	3	2	black
9	2	2	0	1	1	0	0	0	0	white
10	4	3	0	3	3	2	2	1	0	gray
11	2	2	1	1	1	0	0	0	0	white
12	2	1	0	3	2	2	1	0	0	gray
13	4	3	2	4	3	2	4	3	2	black
14	3	3	1	4	2	2	3	2	1	black
15	3	3	2	2	2	1	0	0	0	buff
16	2	2	0	3	2	2	1	0	0	gray
17	2	2	1	1	1	0	0	0	0	white
WFP 221♂										
1	2	2	1	2	2	1	0	0	0	buff
2	2	2	2	4	4	3	3	3	3	black
3	3	2	2	4	4	2	3	2	2	black
4	1	1	1	2	2	2	0	0	0	buff
5	2	2	1	2	2	1	0	0	0	buff
6	2	2	1	2	2	0	0	0	0	buff
7	0	0	0	1	1	0	0	0	0	white
8	3	2	1	4	4	3	3	2	2	black
9	0	0	0	0	0	0	0	0	0	white
10	3	2	1	3	3	2	2	2	2	gray
11	1	1	0	0	0	0	0	0	0	white
12	0	0	0	2	2	1	0	0	0	gray
13	3	2	2	4	4	2	3	2	2	black
14	3	3	2	3	3	2	3	2	2	black
15	2	2	1	1	1	0	0	0	0	buff
16	2	1	1	2	2	1	1	1	1	gray
17	1	1	0	1	1	0	0	0	0	white

TABLE 2.—Average pigment density values for four major features of the color pattern of *Delphinus delphis*. Coded values in Table 1 are averaged over bases, edges, and bands, over all relevant samples, and over both animals. Number of samples included for each animal is in parentheses.

	Pigment density		
Feature	Diffuse	Small grains	Large grains
Ventral field: white (4)	0.96	0.67	0
Thoracic patch: buff (5)	2.37	1.60	0.07
Flank patch: gray (2)	0.92	2.00	0.42
Spinal field: black (4)	2.46	3.08	2.67

Discussion

The color pattern of *Delphinus* evidently consists of two overlapping and interacting pigmentation components that differ mainly in the density and size of pigment particles. The pigment exhibits a continuum of grain size, with the very smallest particles being associated with a buff color. It seems likely that the diffuse pigment is also composed of particles too small for resolution with the light microscope. This evidence suggests that there may be a developmental progression in grain size from the unpigmented or truly white condition through buff and gray to black. This result is consistent with Mitchell's (1970) proposal of the "saddled" pattern as most primitive in recent cetaceans. Inhibition of pigment aggregation could result in the high concentration of diffuse pigment which is typical of buff regions in *Delphinus*. Fox (1953) and others have stated that there are different types of melanins, possibly characterized by different chemical compositions or structures, although none have been adequately described chemically, due to their insolubility and their general lack of adaptability to most physicochemical methods. From the previously described evidence it may be suggested that the type of malanin which produces the buff color is of a composition which does not allow its further polymerization, but may well favor its combination with a protein, as phaeomelanin, instead of aggregation into granules. One might also hypothesize control by dispersing or concentrating

hormones, although this would be physiologically and developmentally more complex. In gray and black areas, aggregation of pigment seems to continue, and melanocytes migrate toward the surface from the base of the epidermis until diffuse pigment is largely replaced by granular pigment. The process is apparently stopped at some point, after which increase in thickness of the epidermis may result in a lower average density of pigment.

Acknowledgments

Benjamin Landing offered suggestions for histological treatment of samples, and Ernest Link did the actual histological preparation of sections. Photomicrography was done by Tedd Wells.

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OCCURRENCE OF TWO CONGRIDAE LEPTOCEPHALI IN AN ESTUARY

During the night of 23-24 August 1971, I caught two congrid leptocephali in Montsweag Bay, part of the Sheepscot River-Back River estuary, Wiscasset, on the southern Maine coast. These larvae were identified as conger eel, *Conger oceanicus* (D. G. Smith, pers. commun., 15 April 1974). The estuary was described by Stickney (1959). Recksiek and McCleave (1973) provide additional information about the estuary and Montsweag Bay. The leptocephali were collected near their sampling station G3 (lat. 43°56'N, long. 69°42'W). Briefly, Montsweag Bay is a shallow (1 m at mean low water) and wide (2.4 km) basin, but it has a narrow channel (9 m deep at mean low water) through most of its length. Narrow openings at its northern and southern ends allow tidal flow. Mean tidal difference is approximately 3 m. Seasonally, water temperature extremes in Montsweag Bay range from 0.0° to 18.5°C. Salinity ranges from 7 to 30‰. Gear used was essentially that described by Graham and Venno (1968).

One larva (98 mm TL) was captured during the flooding tide 1 m below the surface; the other (91 mm TL) during the ebbing tide within 3 m of the bottom. Water depth at this location was approximately 9 m at mean low water. During this period, the average salinity was 26.0‰ and the average water temperature was 17.7°C.

Conger eel adults and leptocephali have been reported from the Gulf of Maine (Bigelow and Schroeder 1953), but apparently most leptocephali are found in the western North Atlantic (Schmidt 1931). Conger eel leptocephali, however, have never been reported from such low-salinity water. Bigelow and Schroeder (1953) illustrated one 84 mm long from Chesapeake Bay, but they do not give the salinity at the collection site. They also state that conger eel leptocephali grow to 150-160 mm. Smith (pers. commun., 15 April 1974) commented that my specimens were beginning to metamorphose since the gut of each had shortened noticeably. Conger eel leptocephali apparently are able to tolerate this low-salinity water at least during metamorphosis.

If conger eel leptocephali typically grow to the size reported by Bigelow and Schroeder (1953), then they must shrink tremendously in length during metamorphosis. My specimens probably shrank during storage, but probably not enough to account for that much size difference.