Chromatophore Arrangement and Distribution in the Gulf Squid *Lolliguncula brevis*

by

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ABSTRACT

Fifteen Lolliguncula brevis were divided into three groups based on body size (mantle length). The dorsal mantle, ventral mantle and fins of those squids were observed and the arrangements of chromatophores in those areas were reproduced onto clear acetate. The anatomically fixed arrangements of chromatophores into morphological units and the distribution of those units on the body of the animal were described. There was a significant difference ($P \leq .01$) between the number of chromatophores on the dorsal mantle vs the ventral mantle vs the fins within each of the three groups of animals. There was no significant difference ($P \leq .01$) between the number of chromatophores per mm^2 for a given body region across the size range encompassed by the three groups (40 mm to 70 mm in mantle length).

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INTRODUCTION

Cephalopod molluscs are the most highly developed of the invertebrates with respect to the complexity of their morphological and physiological adaptations to the environment. The class of Cephalopoda includes squid, octopus, cuttlefish and Nautilus. In general, the cephalopods are larger and have a higher metabolic rate than other molluscs (Wilbur and Yonge, 1964). They are also unique among the molluscs in that all forms except Nautilus exhibit a gradual reduction or absence (as in the octopods) of the shell (Wilbur and Yonge, 1964). Although all of the cephalopods are marine, their habitats within the marine environment are varied. Many species are pelagic, while others inhabit the shallow waters of the continental shelf and still others are bottom dwellers. Most cephalopods are fast moving predators. They receive a wider range of sensory information and show more precise motor control than other molluscs (Wilbur and Yonge, 1964).

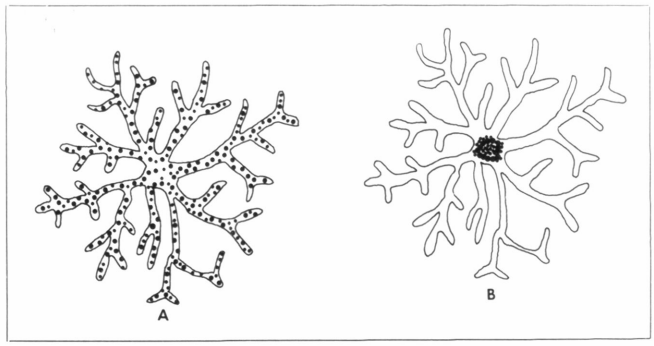
The adaptation of cephalopods to a free swimming, predacious existence resulted in a high degree of cephalization of the nervous system and a more efficient means of coordinating body activities (Purchon, 1968). Cephalopods have large brains in terms of the number of nerve cells. More than 168 x 10^6 cells comprise the brain of Octopus (Russel-Hunter, 1968). In contrast, roughly 100,000 neurons comprise the brains of larger crustaceans and only a few thousand cells comprise the brains smaller insects (Bullock, 1977). As a result, the cephalopods are able to exhibit relatively complex behaviors. One such complex behavioral repertoire involves conspecific communication by visual cues, e.g., through changes in body patterning.

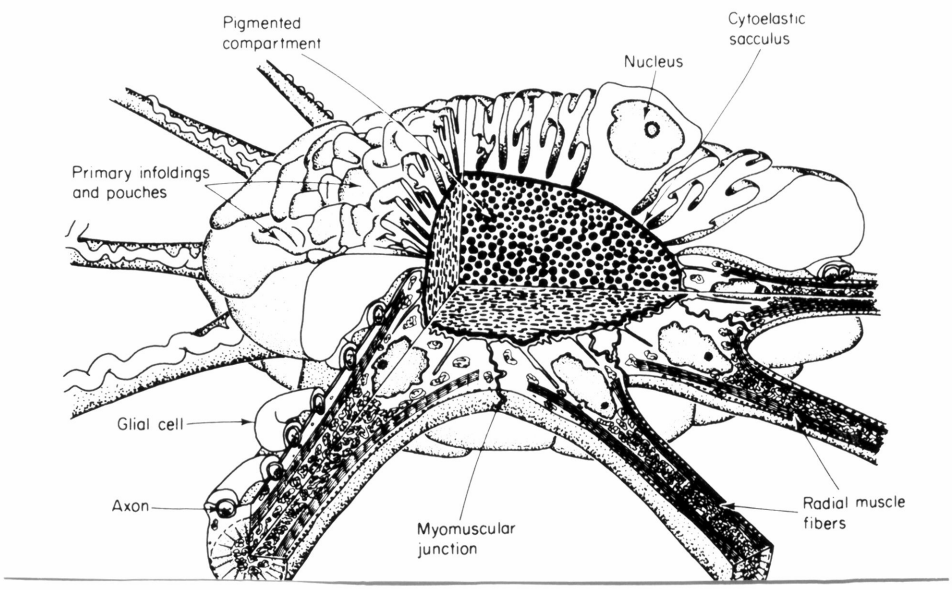
Cephalopods are capable of producing a wide range of body patterns which are used for camoflage as well as visual communication. These body patterns are produced by rapid changes in body coloration and alterations in posture (Moynihan and Rodaniche, 1977). A given body posture coupled with a particular color pattern produces a stereotyped display. The chromatic or color components of patterning are made up of morphological units which in turn are comprised of chromatophores and irridescent cells in the dermis of the skin. The chromatophores and irridescent cells are the basic elements of cephalopod skin patterning (Packard and Hochberg, 1977).

The chromatophores of cephalopods are unique in the Animal Kingdom both in their structure and function. In chromatophores of other invertebrates, the pigment moves within a fixed, highly branched cell (Fig. 1). In such cases, pigment movement is usually under hormonal control. Cephalopod chromatophores are small, sac-like cells. Each cell is surrounded by a series of radiating muscle fibers (Fig. 2). The muscle fibers are innervated by motoneurons whose cell bodies reside in the chromatophore lobes of the brain. Each chromatophore cell consists of an elastic sacculus which contains the pigment. The sacculus is attached along the equator of the cell to the plasma membrane. The plasma membrane is in turn anchored to the radiating muscle fibers (Florey, 1969). In the retracted state, with the pigment concentrated into a small area, the plasma membrane is intricately folded (Florey, 1969). A darkening of the skin is produced by the contraction of the chromatophore muscles, which stretches and thus expands the cell, spreading the sacculus thinly over a larger area. When the chromato-

Figure 1. Crustacean chromatophores in which pigment is dispersed (A) and concentrated (B). C, Fiddler crabs in pale (nighttime) and dark (daytime) phases. (From Prosser, A. L., 1973: Comparative Animal Physiology. W. B. Saunders Co., Philadelphia, p. 924.)

Figure 2. The unretracted chromatophore organ of the squid Loligo opalescens. (From Cloney and Florey, 1968: Z. Zellforsch., 89:254.)


A
B


Pigmented compartment
Cytoelastic sacculus
Nucleus
Primary infoldings and pouches
Glial cell
Axon
Myomuscular junction
Radial muscle fibers

phore muscles relax, there is an elastic recoil of the sacculus which again concentrates the pigment into a small sphere and causes the dermal area surrounding the cell to appear lighter or clear.

The position of individual chromatophores is apparently anatomically fixed in the cephalopod skin and the various color patterns they produce result from selective nervous excitation of chromatophore cells and groups of cells (Packard, 1982). Packard and Hochberg (1977) have suggested that the hierarchial arrangement of patterning (chromatophores grouped into morphological units, and the morphological units into the chromatic components of patterning) reflects the organization of the cephalopod nervous system.

While the arrangement, organization, and distribution of chromatophores in the squid Loligo plei have been carefully described (Hanlon, 1982), no similar study has been conducted on the common Gulf squid, Lolliguncula brevis. This animal has a limited repertoire of body patterns and less complex behavior than that exhibited by Loligo plei. These simpler features may facilitate the determination of chromatophore arrangements and the correlation of those arrangements with particular color patterns and behavioral responses.

In the present study, close observations of the skin of twenty L. brevis made it possible to describe 1) specific arrangements of chromatophores into morphological units and 2) the distribution of those units on the animals body. The number of chromatophores per mm^2 was used as a unit of measure in determining the over-all distribution of chromatophores on the animal and in determining the relationship between animal size and chromatophore number.

MATERIALS AND METHODS

Animals

The Gulf squid, Lolliguncula brevis was used for this study. These animals were provided by the Marine Biomedical Institute (MBI) of the University of Texas Medical Branch at Galveston. The squids were trawl-caught in Galveston Bay and transported in shipboard sea water tanks to the MBI. In the laboratory, they were maintained in round 2 meter diameter tanks of recirculating artificial sea water as described by Hanlon (1978).

Data Collection

All close-up observations were made with a Wild Steromicroscope on animals narcotized in 1% Ethanol in sea water. Color Video tapes were made by adapting the videocamera to the third viewing eyepiece of the stereomicroscope. Fiber optics were used to illuminate the subject. Only chromatophores on the dorsal mantle, ventral mantle, and fins were considered for this study. The arrangments or groupings of chromatophores within these three areas were observed in 18 animals. Chromatophore arrangements in each body area were reproduced directly onto acetate overlays by placing clear acetate over the videoscreen and tracing the viewing area. Measurements of the number of chromatophore per mm^2 were obtained from these overlays. Fifteen squids were divided according to their size (i.e., mantle length, ML) into 3 groups. Group 1 consisted of 5 animals ranging in size from 40 to 50 mm ML. Group 2 was comprised of 5 animals between 51 and 60 mm ML and Group 3 consisted of 5 animals ranging from 61 to 70 mm ML.

Data Analysis

Single factor analysis of variance (Sokal and Rohlf, 1969) tests (two-tailed, confidence level of $P<.01$) were performed to determine if there was a significant difference between (1) the chromatophore density (number per mm^2) on the dorsal mantle vs the ventral mantle vs the fins within each of the size groups of squid and (2) the chromatophore densities for similar body regions but across the size range of animals encompassed by the three groups.

RESULTS

Hanlon (1982) described and named the morphological units found in Loligo plei. His terminology was used in the present study to designate particular morphological units seen in Lolliguncula brevis.

The squid, L. brevis was seen to have basically two types of chromatophores: yellow and brown. In general, the yellow chromatophores were smaller in size than the browns. Gradations in color occur in both types, often as a result of varying degrees of chromatophore expansion or retraction. For example, a retracted yellow chromatophore, i.e., one in which the pigment was concentrated into a small sphere, may have appeared brown in color. The exact molecular nature of the pgiments found in cephalopod chromatophores has not been determined.

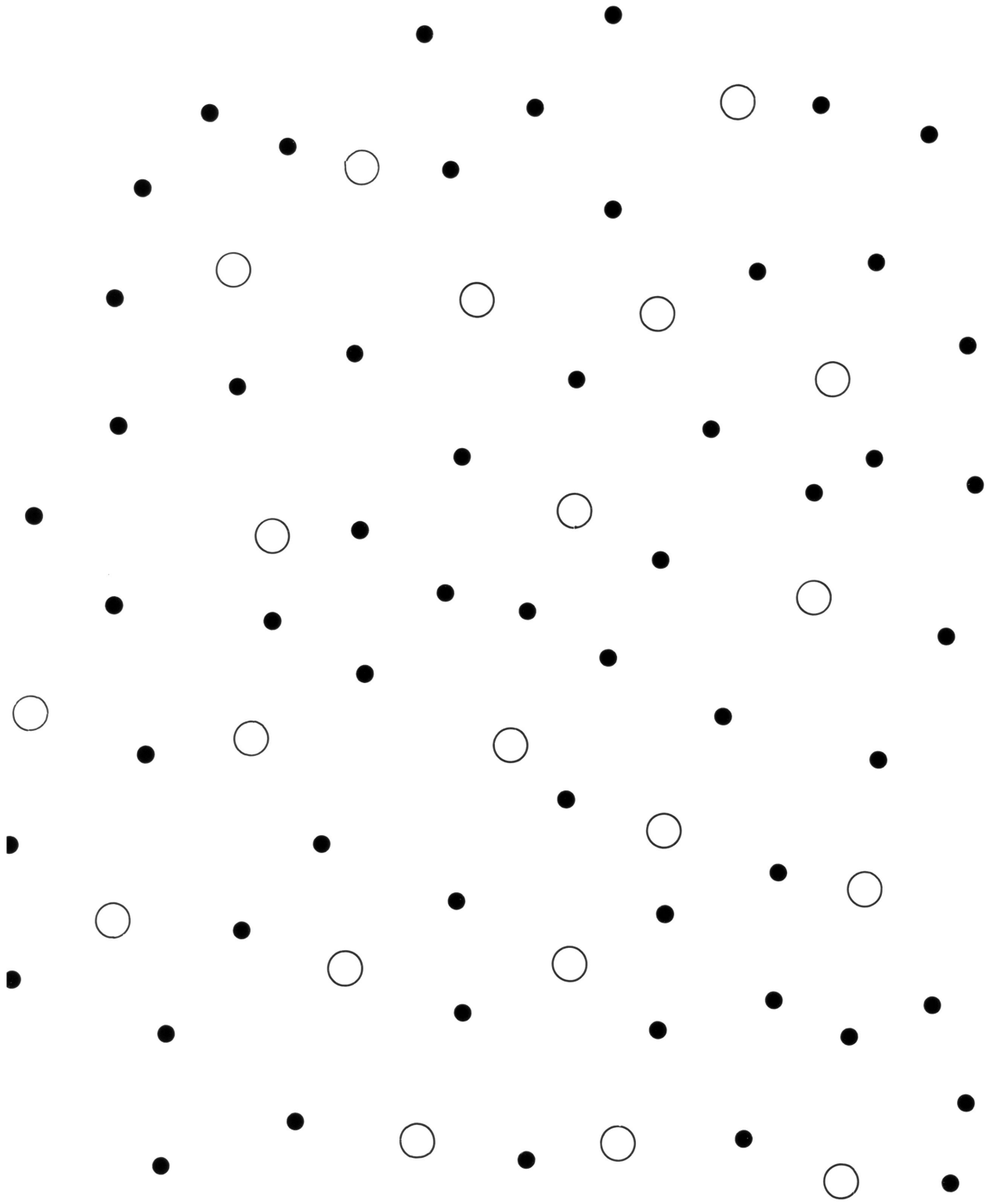
Chromatophore arrangement vs body region

One chromatophore arrangement consistently seen on the dorsal mantle and the central region of the fin was composed of one large brown chromatophore surrounded by a ring of approximately 5 to 7 smaller yellows (Fig. 3). This arrangement has been described by Hanlon (1982) for Loligo plei as a "Yellow-Brown Discoid Unit" because the general dimensions of the unit are disc-like.

Only brown chromatophores were seen in the ventral mantle of L. brevis. These appeared to be evenly spaced and of a similar size (Fig. 4).

The chromatophore arrangement on the fins of L. brevis differed slightly from the arrangements found on either the dorsal or ventral mantle. The fin edge was defined by a narrow band of small brown

Figure 3. Yellow-Brown Discoid Units as they occur on the dorsal mantal of L. brevis.

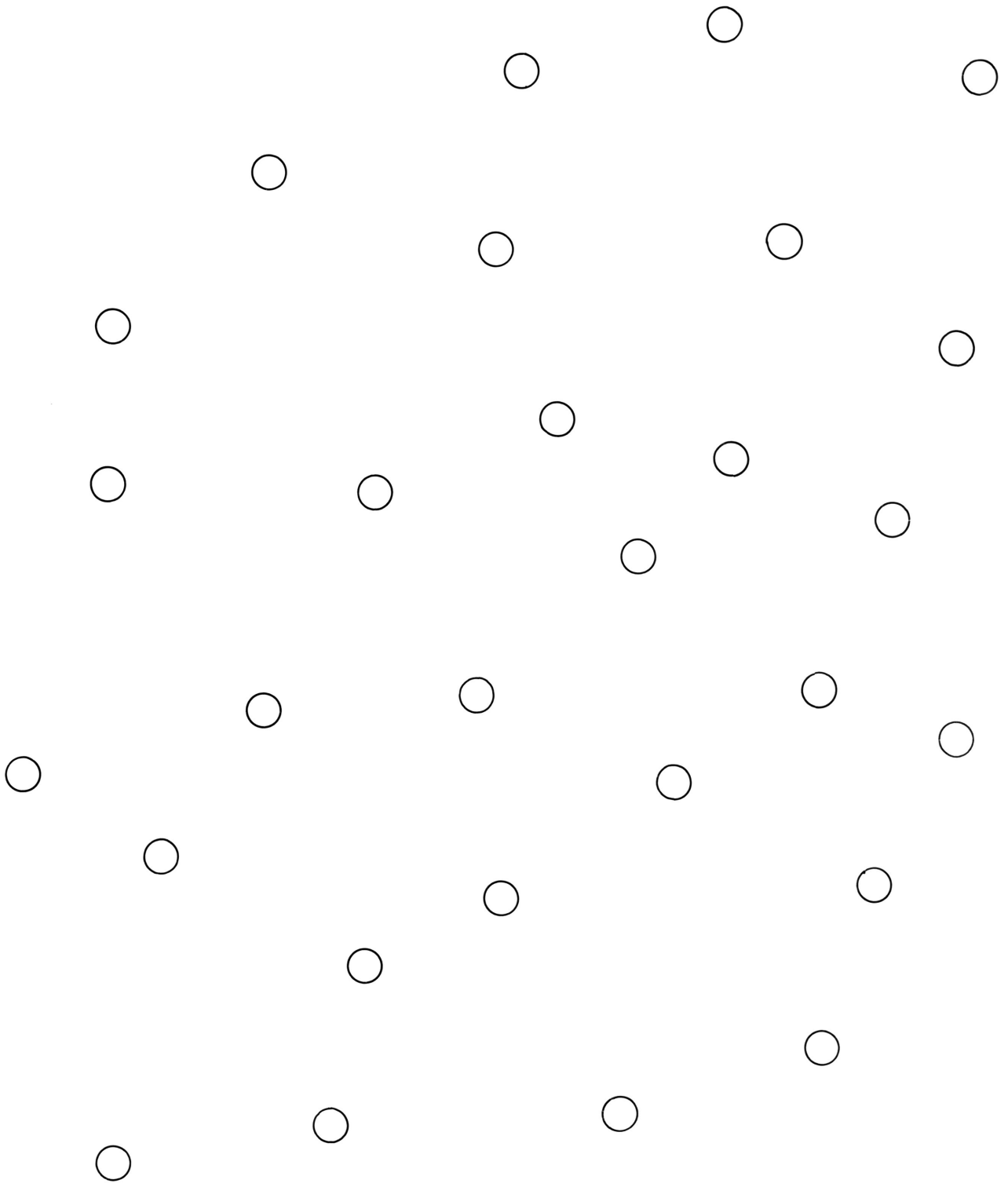


○ brown chromatophores

● yellow chromatophores

DORSAL MANTLE **Discoid Units**

Figure 4. Arrangement of brown chromatophores on the ventral mantle of L. brevis.



VENTRAL MANTLE

BROWN CHROMATOPHORES

chromatophores which formed a continuous ring around the margin of both fins. Medial to this marginal ring was a region of discoid units. This region was approximately crescent-shaped and followed the contour of the fin, being wider at its midpoint that it was anteriorly and posteriorly (Fig. 5). It did not form a continuous ring around both fins but appeared as a separate zone on each fin. The discoid units within this region differed from those on the dorsal mantle in that yellow chromatophores, approximately the same size as browns, occured in the centers of most units. Therefore, most discoid units within this particular region consisted of one large yellow chromatophore surrounded by a ring of smaller yellows. This region was most apparent when the chromatophores were expanded, giving a distinctive bright yellow color to that particular area of the fin.

Chromatophore Density (number of chronatophores per mm^2)

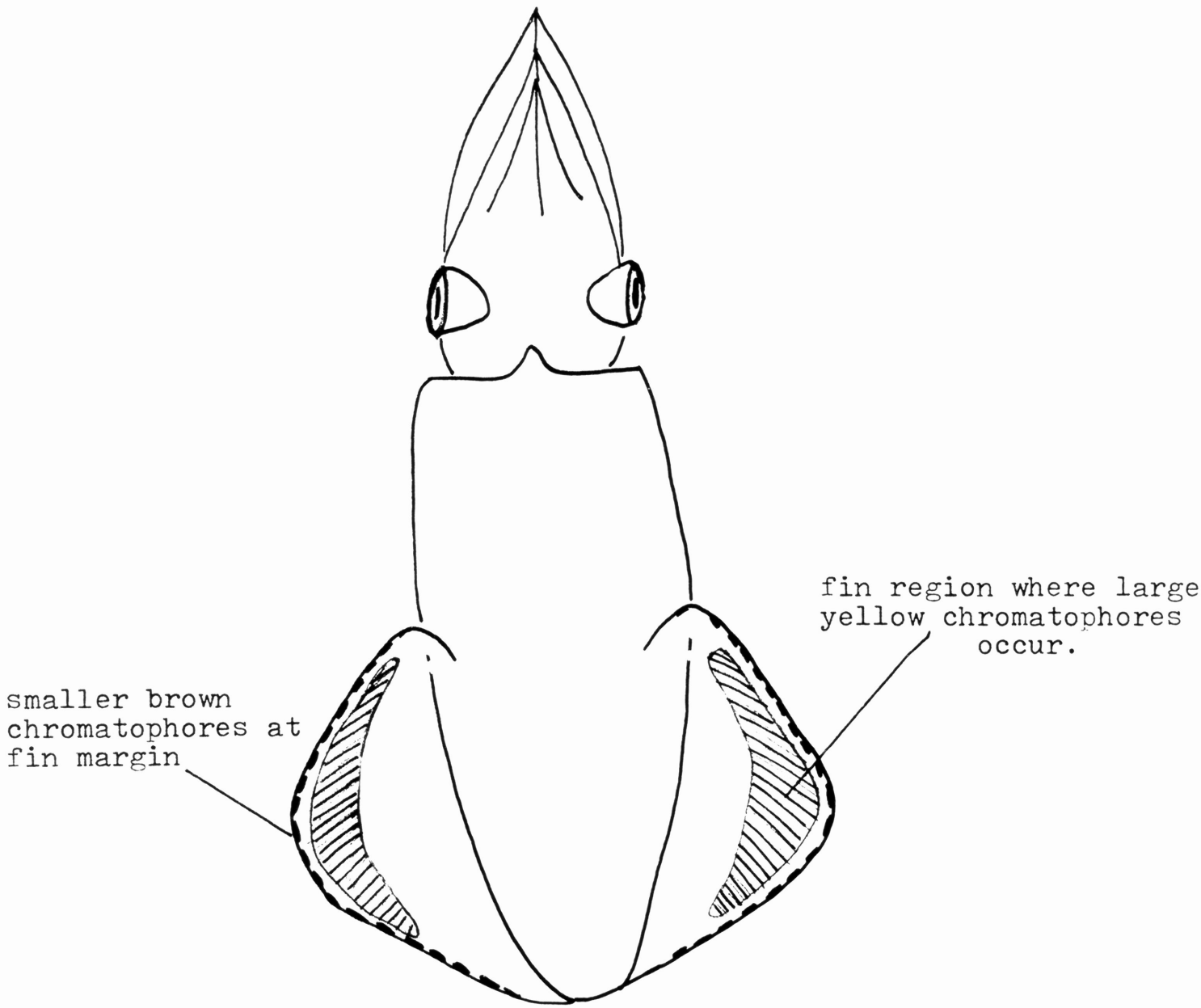
Within each group of 5 animals, there was a significant difference ($P<.01$) between the number of chromatophores per mm^2 on the dorsal mantle vs the ventral mantle vs the fins. The fins were found to have the largest number of chromatophores per mm^2, followed by the dorsal mantle and ventral mantle, respectively.

There was no significant difference ($P<.01$) between the number of chromatophores per mm^2 for a given body region across the size range encompassed by the three groups. This indicates that, at least for animals 40 to 70 mm ML, the chromatophore density on the three body areas remained constant as animal size increased.

The range, mean, and standard error of the mean for the number of chromatophores per mm^2 was determined for the three body areas within

each group and the data presented in Table 1. Figure 6 is a representation of that data.

Figure 5. Distribution of chromatophores on the fins of L. brevis.



Lolliguncula brevis - DORSAL VIEW

TABLE 1.

Group 1 (40-50 mm ML)

Dorsal mantle

Mean	9.64
Range	4-19
Standard error of the mean	± .49

Ventral mantle

Mean	3.8
Range	1-5
Standard error of the mean	± .16

Fin

Mean	13.92
Range	9-20
Standard error of the mean	± .43

Group 2 (51-60 mm ML)

Dorsal mantle

Mean	5.86
Range	2-11
Standard error of the mean	± .35

Ventral mantle

Mean	1.86
Range	0-4
Standard error of the mean	± .14

Fin

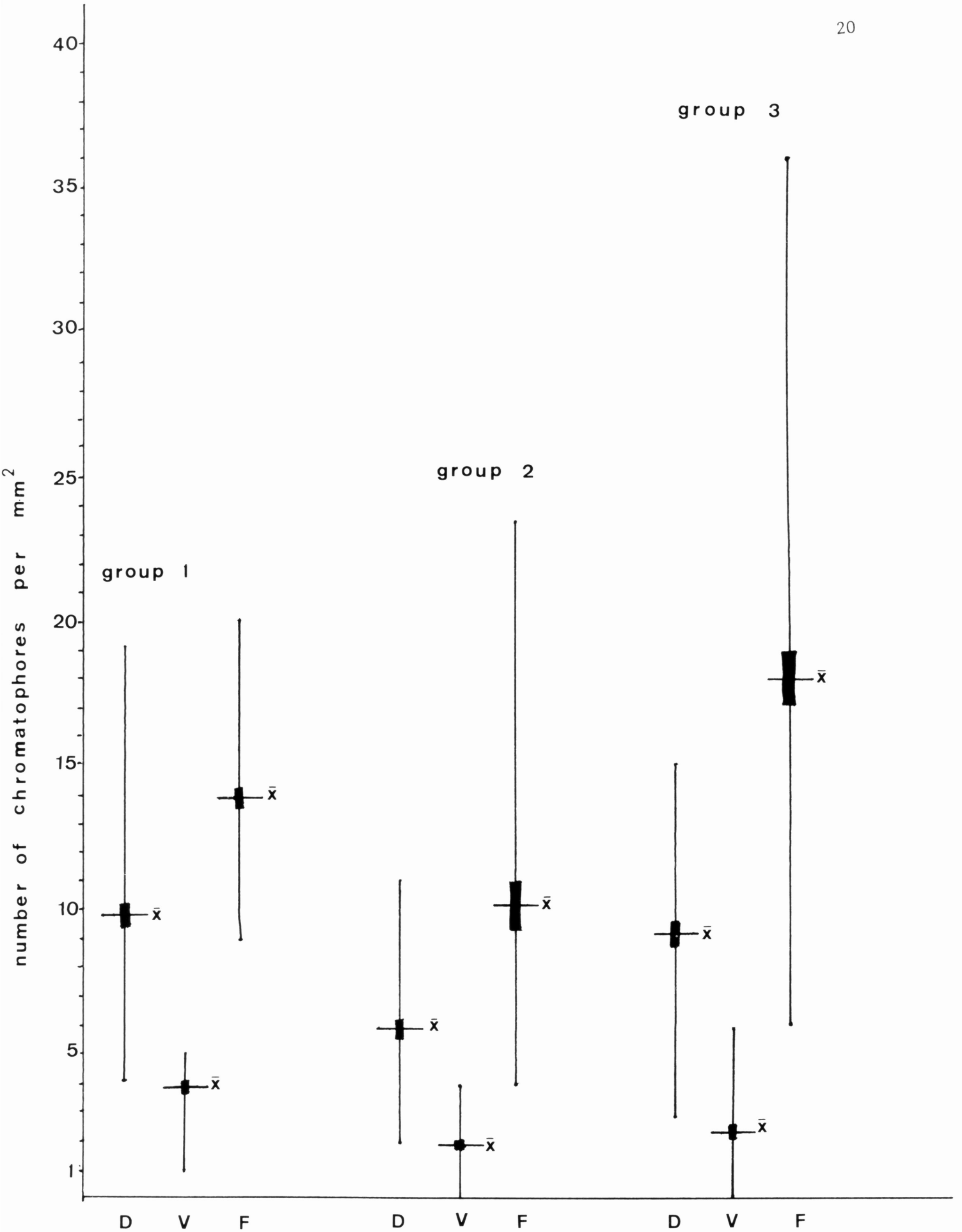
Mean	10.36
Range	4-23
Standard error of the mean	± .88

Table 1. continued

Group 3 (61-70 mm ML)

Dorsal mantle	
Mean	9.04
Range	3-15
Standard error of the mean	± .46
Ventral mantle	
Mean	2.58
Range	0-6
Standard error of the mean	± .22
Fin	
Mean	17.76
Range	6-37
Standard error of the mean	± 1.31

Figure 6. Chromatophore densities (number per mm^2) on the dorsal mantle (D), ventral mantle (V) and fin (F) of L. brevis. Vertical lines represent the range, horizontal lines represent the mean, and dark thickened region represents the standard error of the mean.


group 1
group 2
group 3
number of chromatophores per mm²
40
35
30
25
20
15
10
5
1
D
V
F
x̄

DISCUSSION

Morphological units comparable to those found in Loligo plei were seen in Lolliguncula brevis. Specific unit arrangements were found on each of three body areas examined. A correlation of those units with particular color patterns or behavior has not been determined. However, since L. brevis has a relatively limited repertoire of body patterns, it should be possible to correlate specific behaviors with specific color patterns and in turn correlate the anatomically fixed unit arrangements with the color patterns they produce. The statistical tests performed suggested that there is a significant difference in chromatophore densities on the dorsal mantle is the ventral mantle vs the fins. Those results also suggested that the density of chromatophores within a given body area remains constant as animal size increases from 40 to 70 mm ML. The greater chromatophore density on the dorsal surface of this animal suggests that a dorso-ventral color gradient exists. This type of color gradient is characteristic of many pelagic marine organisms. Most pelagic fish are darker on the dorsal surface and lighter on the ventral surface. This coloration is refered to as countershading (Bond, 1979). It is interesting to note that hatchline L. brevis have more chromatophores ventrally and thus a ventro-dorsal color gradient exists (McConathy, 1980). The significance of this ventro-dorsal gradient is unknown and the gradient becomes dorso-ventral as the animal grows.

Future work on this project should emphasize two lines of research. First, the range of animal sizes should be expanded to include squids with mantle lengths less than 40 mm and greater than 70 mm. Secondly, a study of the behavioral responses and associated color patterns

produced by Lolliguncula brevis would aid in defining the role of the observed morphological units in producing stereotypical color patterns.

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