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SPACING AND ORIENTATION OF THE SIPHONS OF THE TUNICATE, *STYELA PLICATA*

BY

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SPACING AND ORIENTATION OF THE SIPHONS OF THE TUNICATE, <u>STYELA PLICATA</u>

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ABSTRACT

The spacing and orientation of sipons of Styela plicata (Lesueur) in swift and slow currents were examined. Within aggregates, the siphons were spaced farther apart in slow currents than in swift currents. The siphons were not horizontally oriented but were vertically oriented in slow currents. The adaptive values of spacing and orientation under different currents are discussed.

INTRODUCTION

Many sessile marine organisms extract food from currents of water that they generate themselves and from currents imposed upon them. Consequently any mechanism which would maximize the use of water currents would give these animals an advantage.

One mechanism that has been found to serve this purpose is spacing. Knight-Jones and Moyse (1961) have demonstrated spacing among large associations of barnacles. Some form of species recognition enables individuals to space themselves and reduce intraspecific competition. Orientation is another factor that has been examined by several workers. Wainwright and Dillon (1969) found that as sea fans (*Gorgonia ventalina* and *G. flabellum*) developed they exposed maximum surface area to water currents. Moore (1933, 1935) reported that barnacles (*Balanus balanoides*) also changed orientation and maximized food gathering potential in the direction of water movement. McDougall (1943) observed that barnacles were more strongly oriented to light than to currents; however, the current velocity was much lower than that reported by Moore. Other organisms, such as the polychaetes, position food-catching tubes to obtain maximum advantage from water currents (Mangum *et al.*, 1968). Biological aspects of water movement in the micro-region adjacent to marine organisms have been studied by Riedl (1968, 1969, 1971) and Vogel and Bretz (1972).

An organism well suited to the study of spacing and orientation is the tunicate, *Styela plicata* (Lesueur). It removes suspended food from the water by creating a current into the oral siphon, retaining food particles in the branchial sac, and passing the filtered water out of the atrial siphon. *Styela plicata* grows singly or in aggregates ranging from two to over twenty individuals. The spacing of siphons, and their orientation could determine overlap of feeding currents or interactions between oral and atrial currents. In addition, spacing would reflect the influence of major water currents. Potential interactions existing in slow currents would be masked in swift currents. It is the purpose of this paper to examine the spatial relationships of *S. plicata* under conditions of swift and slow current.

MATERIALS AND METHODS

Styela plicata was collected on October 17, 1971 from two locations in Bogue Sound, Morehead City, North Carolina. One location was Sunset Shore's jetty which extends approximately 100 feet into the sound, close to the main channel. Animals attached to the jetty were exposed to strong tidal currents. The other location was Spooner's Creek, a boat marina, where tunicates were attached to the bulwarks and subjected to slight tidal currents.

The tunicates near the surface were collected by hand from a boat. Scuba gear was used to collect at depths ranging from 40 to 170 cm below the high tide mark. Each aggregate was numbered and labeled to indicate its orientation in relation to the direction of the water surface and a compass direction with respect to the substrate. The organisms were returned to the laboratory in plastic bags and placed in 15 % formalin.

In the laboratory, the number of organisms in the aggregate was determined and the length and width of each aggregate was measured. The distance between oral and atrial siphons within the same organism was measured to the nearest millimeter. Similarly, the distances separating oral-oral and oral-atrial siphons between organisms were measured.

The difficulty in examining orientation of three-dimensional aggregates was simplified through the use of diagrams. One diagram of the surface view, perpendicular to the substrate, and one of the side view, parallel with the substrate, were made. These diagrams were made by projecting the three-dimensional aggregates onto a plane, i.e. glass plate, above the aggregate (Figure 1). The horizontal and vertical orientations of each individual were then established respectively from the surface view and side view diagrams. Lines were drawn between the oral and atrial siphons of each individual. The compass directions of the oral siphon from the atrial siphon were determined.

In order to estimate the effect of preservation, several live and then preserved specimens were analyzed as above except that the horizontal and vertical orientations of individuals were not determined. The mean distance separating oral and atrial siphons within the same individual after preservations was 76 % that before preservation, a difference that was statistically significant ($t = 8.52$, $df = 40$, $p < 0.05$). The mean distances separating siphons between individuals before and after preservation did not differ significantly.

A few individuals were used to measure the extent of the oral current. They were placed in a mixed suspension of carmine and methyl blue and observed with the unaided eye.

RESULTS

Observations with methyl blue and carmine particles indicated that particles were drawn into the oral siphon from a maximun distance of 20 - 30 mm. Food particles of equivalent size or less would be subject to the feeding currents within this distance.

The distribution and spacing of siphons were used as rough indices of the interactions of currents. Clark and Evans (1954) devised the nearest neighbor analysis to demonstrate the distribution of organisms within a given surface area. This analysis was used to determine the distribution of oral siphons by comparing distances separating nearest neighbors, i.e. an oral siphon and the next nearest oral siphon, with the hypothetical distances that would be expected for randomly distributed siphons. The deviation from randomness could be toward clumping or homogeneous spacing.

Several modifications of the nearest neighbor analysis were made because of the three-dimensional nature of the aggregate. First, illustrations made by projecting the oral siphons onto a plane above the aggregate were used to obtain the density of oral siphons within the area defined by the aggregate. The distances separating oral siphons and their nearest neighbors were measured on the two-dimensional illustrations. Siphons that were oriented differently from those used in the measurements were not included. Finally, to obtain a valid sample size, the data from each sample area (Sunset Shores and Spooner's Creek) were pooled. The results indicated that the oral siphon distribution did not deviate from a random distribution within aggregates from either locality.

Oral siphons of nearest neighbors from Sunset Shore and Spooner's Creek were separated by distances of 23 mm and 26 mm respectively (Table 1). The distances between oral siphons from Spooner's Creek were significantly greater than those from Sunset Shores ($t = 2.11$, $df = 45$, $p < 0.05$).

The percentages of nearest neighbor oral siphons that were within 20 mm, 30 mm, and 60 mm were estimated from a normal curve (Table 2). The mean distances separating oral siphons of nearest neighbors were used as the means for each normal curve. At Sunset Shores, 23 % of the nearest neighbor oral siphons were within 20 mm and 96 % were within 30 mm compared with 12 % within 20 mm and 79 % within 30 mm at Spooner's Creek. In both cases, Spooner's Creek had fewer nearest neighbor oral siphons within the stated distances.

The total number of pairs of oral siphons within the above mentioned distances could be related to aggregate size. This relationship was established through the use of linear regression, equation 1, where Y is the number of siphon pairs within a given distance, X is the number of individuals per aggregate, and A and B are constants.

$$Y = A + BX \qquad \text{(Equation 1)}$$

The least squares method yielded various parameters of the regression equation (Table 3). Values predicted from the regression equations indicated that for equal sized aggregates from the two sample areas, there were no significant differences in the number of siphon pairs within each of the three distances (Table 4).

Oral and atrial siphons on the same organism were separated by 14 mm at both collecting areas. The oral-atrial siphon distances between nearest neighbors from Sunset Shores and Spooner's Creek were 19 mm and 22 mm respectively, a significant difference ($t = 3.08$, $df = 46$, $p < 0.05$). The distances separating oral-atrial siphons between nearest neighbors were significantly greater than those within the same individual for both areas: Sunset Shores ($t = 6.78$, $df = 36$, $p < 0.05$) and Spooner's Creek ($t = 9.16$, $df = 56$, $p < 0.05$).

The extent of horizontal and vertical orientations of oral and atrial siphons for aggregates was determined according to the procedure of Batschelet (1965). Each directional point was assigned an equal mass value, and the center of mass determined the corresponding directional tendency. The center of mass was obtained through vector components which were the sine and cosine values for each angle. The method yielded the mean angular direction, standard deviation, and determined if a directional tendency was significant.

Only 38 % of the aggregates from Sunset Shores showed significant horizontal orientation, and only 14 % of the aggregates from Spooner's Creek showed significant horizontal orientation. There was no consistency in the direction of orientation from a given substrate (Table 5).

Twenty-five percent of the aggregates from Sunset Shores showed significant vertical orientation while 61 % of the aggregates from Spooner's Creek were vertically oriented. There were a greater number of aggregates showing significant vertical orientation in slow currents than in swift currents (Table 6).

DISCUSSION

The aggregate growth form of _S. plicata_, together with the oral and atrial currents produced by each individual, results in multiple interactions of these currents. The addition of extrinsic currents may negate or exaggerate the individual siphon currents and their interactions. Swift waters may tend to mask the interactions which would exist in slow currents. Spacing and orientation of individuals within aggregates may be a response to these extrinsic currents.

Measurements of currents were only roughly determined in the present study. Extrinsic currents were designated as swift or slow and oral currents were estimated to extend 20 - 30 mm from the siphon. Hecht (1918) stated that oral currents exert influence over several millimeters and that the atrial current is expelled ten times the distance influenced by oral currents. Although the maximum extent of the oral current in _S. plicata_ is greater than those measured by Hecht, it appears that a distance of several millimeters may be important for current interactions.

The oral siphons were randomly distributed at both sample areas, but were farther apart in slow currents, e.g. Spooner's Creek - 26 mm, Sunset Shores - 23 mm. These distances are greater than those given by Hecht as being influenced by the inhalent current, yet they fall within the distances determined in this paper. It is possible that the greatest influence of oral currents is less than 20 - 30 mm, and that a separation distance increased by three millimeters is sufficient to reduce competition in slow water. If so, the tunicates in slow water appear to be under strong influence to reduce overlap of oral currents. Such spacing within slow currents could minimize interference and thus competition of oral currents. Overlapping currents could have detrimental effects by reducing the total amount of water filtered by the aggregate. This was found to be true for colonies of the bryozoan, Lophopodella carteri (Bishop and Bahr, 1971).

The number of oral siphon nearest neighbor pairs within 20 and 30 mm adds further support to the spacing of oral siphons in slow water. Spooner's Creek had 49 % and 18 % fewer siphon pairs within the 20 and 30 mm distances respectively than did Sunset Shores. However, when the total number of siphon pairs within 20 and 30 mm were examined there was no difference between the two sample areas. Since the distances represented maximal oral siphon influence, these results may indicate that maximal distances are not as important as nearest neighbor distances in the determination of current interactions.

The oral siphons were closer together in swift currents. This lack of spacing may reflect a tendency for swift currents to negate the tunicate's currents. The proximity of oral siphons in swift water also could be beneficial. Pooling of oral currents could increase the distance from which food particles may be attracted or increase the rate of water exchange. Overlapping oral currents also may be advantageous as the proximity of siphons could increase the turbulence close to the oral siphon. This turbulence would slow down food particles and facilitate their collecting (Mackie, 1963).

Oral and atrial siphons were spaced farther apart between nearest neighbors than within the same individual and were farther apart in Spooner's Creek than Sunset Shores. This spacing may minimize interactions between oral and atrial currents, especially in slow moving water.

Spacing of the tunicates is the result of a number of interdependent processes. These include settling, attachment, growth, and competition. Larvae hatch during early morning hours and can select the substrate before metamorphosis (Dybern, 1963 and Yamaguchi, 1970). Carlisle (1961) has observed limited movement over the substrate following metamorphosis. After attachment, the tunicates may alter their original settling pattern through growth responses toward or away from a particular stimuli (Knight-Jones, personal communication). It is also possible that spacing is the result of competition from which only individuals that were properly spaced were able to survive. These processes for spacing are related to water currents as well as to a number of other factors including physical stimuli, e.g. light (Yamaguchi, 1970), and those produced by the species, e.g. vibratory patterns (Vilenkin, 1971) and pheromones (Knight-Jones, 1963). Aspects of larval behavior have been little studied, thus spacing and orientation beginning with the larval stages present many possibilities for further examination.

In addition to spacing, other mechanisms play an important role in interactions between siphon currents. The extent and velocity of oral and atrial currents reduce interaction (Knight-Jones, 1963). Variations in velocity may be due to varying degrees of siphon constriction as demonstrated in the bivalve, Scrobicularia plana (Green, 1966). The orientation of siphons on the organism and the capability for movement of those siphons also may minimize interferences. Casual underwater observations noted that the siphons of S. plicata are capable of constriction and movement.

Orientation as well as spacing may be related to water currents. There was no tendency toward a common horizontal orientation from any given substrate. These results agree with the findings of Riedl (1971), who states that organisms are dependent on water movements to varying degrees as a result of their method of feeding. Organisms that are external and passive filter feeders, e.g. sea fans and barnacles in swift currents, appear to be subject to orientation, while organisms which are internal and active filter feeders, e.g. tunicates, would be less likely to show orientation.

The tunicates were oriented vertically toward the surface in slow currents. Similar orientation was seen in Ciona intestinalis by Millar (1953), who said that this position was most likely an adaptation to catch falling food. If such is the case, vertical orientation would be best utilized in slow currents where gravity would affect the distribution of food particles; whereas in swift waters, mixing would cause food to remain suspended. The results for vertical orientation tend to support this concept, as 61 % of the tunicate aggregations at Spooner's Creek showed vertical orientation, whereas only 25 % were oriented toward the surface at Sunset Shores.

In addition to the aggregates taken from the vertical jetty walls of Sunset Shores, two large aggregates were from a horizontal substrate near the jetty. Individuals within these horizontal aggregates were distributed in a circular pattern with the siphons located around the periphery. This deviation in the aggregate growth form may be related to water currents as well as the substrate and merits further examination.

In order to determine the amount of interaction between individuals, observations should be made _in situ_ of the aggregates noting the amount of movement for the siphons and the currents within the aggregates. Measurements also should be made of the current velocities to which the aggregates are subjected and of those created by the organisms. There are a number of thermistors which could measure currents; however, they are expensive and the thermistor itself tends to affect the currents being measured (Riedl, 1968).

To determine the effect of currents on the spacing of individuals, further laboratory and field studies should be made. In the laboratory, tunicates could be grown under controlled conditions of swift and slow currents. In the field, tunicates could be encouraged to settle on fouling blocks under a given current velocity and then moved to a different velocity. In each case the effect of current could be determined.

LITERATURE CITED

Batschelet, Edward. 1965. Statistical methods for the analysis of problems in animal orientation and certain biological rhythms. Amer. Inst. Biol. Sci. Monogr. Washington, D.C. 57 p.

Berrill, N. J. 1950. The Tunicata with an account of the British species. Ray Society, London. 354 p.

Bishop, John W. and Leonard M. Bahr. 1971. Interactions in feeding among zooids of the bryozoan, Lophopodella carteri. Abstr. Progr. (Geol. Soc. Amer.), 3: 506 (Abstr.).

Carlisle, D. B. 1961. Locomotory powers of adult ascidians. Proc. Zool. Soc. Lond. 136: 141 - 146. Cited in H. R. Millar. The biology of Ascidians. In Sir Frederick S. Russell and Sir Maurice Yonge (ed.). Advances in marine biology. vol. 9. Academic Press. New York.

Clark, Phillip J., and Francis C. Evans. 1954. Distance to nearest neighbor as a measure of spatial relationships in populations. Ecology 35: 445 - 453.

Dybern, Bernt I. 1963. Biotope choice in Ciona intestinalis (L.). Influence of light. Zool. Bidrag. Uppsala, 35: 589 - 601. (Abstr.).

Green, J. 1967. Activities of the siphons of Scrobicularia plana (da Costa). Malacol. Soc. Lond. Proc. 375: 239 - 241.

Hecht, S. 1918. The water current caused by Ascidia atra (Lesueur). Jour. Exp. Zool. 20: 429 - 434. Cited in N. J. Berrill. The Tunicates with an account of the British species. Ray Society, London.

Knight-Jones, E. W. and J. Moyse. 1961. Intraspecific competition in sedentary marine animals. Symp. Soc. Exp. Biol. 15: 72 - 95.

McDougall, Kenneth D. 1943. Sessile marine invertebrates of Beaufort, N. C. Ecol. Monogr. 13: 321 - 374.

Mackie, G. O. 1963. Siphonophores, bud colonies and superorganisms, p. 329 - 337. In E. C. Doughery (ed.). The lower metazoa comparative biology and phylogeny. Univ. California Press. Berkeley.

Mangum, C. P., S. L. Santos, and W. R. Rhodes, Jr. 1968. Distribution and feeding in the onuphid polychaete, *Diopatra cuprea* (Bosc.) Mar. Biol. (Berlin). 2: 33 - 40.

Millar, R. H. 1953. *Ciona* L. M. B. C. Mem. Typ. Br. Mar. Pl. Anim. 35: 1 - 123. Cited in R. H. Millar. The biology of Ascidians. In Sir Frederick S. Russell and Sir Maurice Yonge (ed.). Advances in marine biology. vol. 9. Academic Press. New York.

Millar, R. H. 1971. The biology of Ascidians, p. 1 - 100. In Sir Frederick S. Russell and Sir Maurice Yonge (ed.) Advances in marine biology. vol. 9. Academic Press. New York.

Moore, H. B. 1933. Changes of orientation of a barnacle after metamorphosis. Nature. 132: 969 - 970.

Moore, H. B. 1935. The biology of *Balanus balanoides*, IV. Relation to environmental factors. J. Mar. Biol. Assoc. 20: 279 - 307.

Riedl, Rupert, and Helmut Forstner. 1968. The microstructure of water movement in the benthos. (in German, English summary). Sarsia 34: 163 - 188.

Riedl, Rupert. 1969. Marine biological aspects of water movement. (in German, English summary) Mar. Biol. (Berlin) 4: 62 - 78.

Riedl, Rupert. 1971. Water movement - Animals, p. 1123 - 1156. In Otto Kinne (ed.) Marine ecology. vol. I part 2. Wiley-Interscience, New York.

Wainwright, Stephen A., and John R. Dillon. 1969. On the orientation of sea fans (genus Gorgonia). Biol. Bull. 136: 130 - 139.

Vilenkin, B. Ya. and M. N. Vilenkina. 1971. Vibrations in water produced by the activities of benthic marine invertebrates. Proc. Acad. Sci. U.S.S.R. Biol. Sci. Section. Dokl. Akad. Nauk U.S.S.R. 196: 5 - 7.

Vogel, Steven, and William L. Bretz. 1972. Interfacial organisms: passive ventilation in the velocity gradients near surfaces. Science. 175: 210 - 211.

Yamaguchi, Masashi. 1970. Spawning periodicity and settling time in Ascidians, Ciona intestinalis and Styela plicata. Rec. Oceanogr. Works. Jap. 10: 147 - 155. (Abstr.).

Table 1. Distances separating oral (O) and atrial (A) siphons of _Styela_ _plicata_ within individuals and between nearest neighbors (in millimeters).

mean distance ± standard deviation (number of observations)

Sample area	Within individuals O-A	Between nearest neighbors O-O	O-A
Sunset Shores (swift currents)	14 ± 2 (184)	23 ± 4 (208)	19 ± 2 (184)
Spooner's Creek (slow currents)	14 ± 2 (223)	26 ± 4 (274)	22 ± 5 (223)

Table 2. Percent of nearest neighbor oral siphons of *Stycla plicata* that were within the stated distances. Estimates obtained from the normal curve with mean distances separating oral siphons used as the mean of the normal curve.

Sample area	Distances separating oral siphons		
	20 mm	30 mm	60 mm
Sunset Shores (swift current)	23	96	99
Spooner's Creek (slow current)	12	79	99

Table 3. Linear regression values for the number of siphon pairs (Y) of Styela plicata within a given distance as a function of the number of individuals per aggregate (X). (A is y - intercept, B is slope, S. E. is standard error of mean, r is correlation coefficient).

Sample area	Distance separating oral siphons (in mm)	A	B	S.E.	r
Sunset Shores (swift currents)	20	.72	.29	2.47	.75
	30	-1.51	1.03	3.28	.95
	60	-9.41	3.34	7.88	.97
Spooner's Creek (slow currents)	20	-1.96	.49	1.75	.82
	30	-4.18	1.15	2.48	.92
	60	-8.48	2.64	7.64	.87

Table 4. Total number of oral siphon pairs (Y) of <u>Styela plicata</u> within a given distance predicted for a given aggregate size (X is number of individuals per aggregate).

Sample area	Distance separating oral siphons (mm)	X	Y
Sunset Shores (swift currents)	20	4	2
	30	8	7
	60	14	37
Spooner's Creek (slow currents)	20	4	0
	30	8	5
	60	14	28

Table 5. Statistically significant mean horizontal orientation of individuals within aggregates of <u>Styela plicata</u>. Orientation is the mean angular direction ± standard deviation in degrees (number of individuals per aggregate).

(reference points: 0 - north, 90 - west)

Sample area	Substrate orientation	Number of aggregates examined	Orientation
Sunset Shores (swift currents)	West	7	111 ± 45 (11) 92 ± 41 (6) 2 ± 52 (9)
	South	4	246 ± 44 (7)
	East	4	231 ± 47 (14) 164 ± 28 (7)
	North	1	—
Spooner's Creek (slow currents)	South	3	325 ± 51 (8)
	East	25	292 ± 61 (26) 184 ± 48 (14) 145 ± 43 (8)

Table 6. Statistically significant mean vertical orientation of individuals within aggregates of <u>Styela plicata</u>. Orientation is the mean angular direction ± standard deviation in degrees (number of individuals per aggregate).
(reference points: 0 – north, 90 – surface).

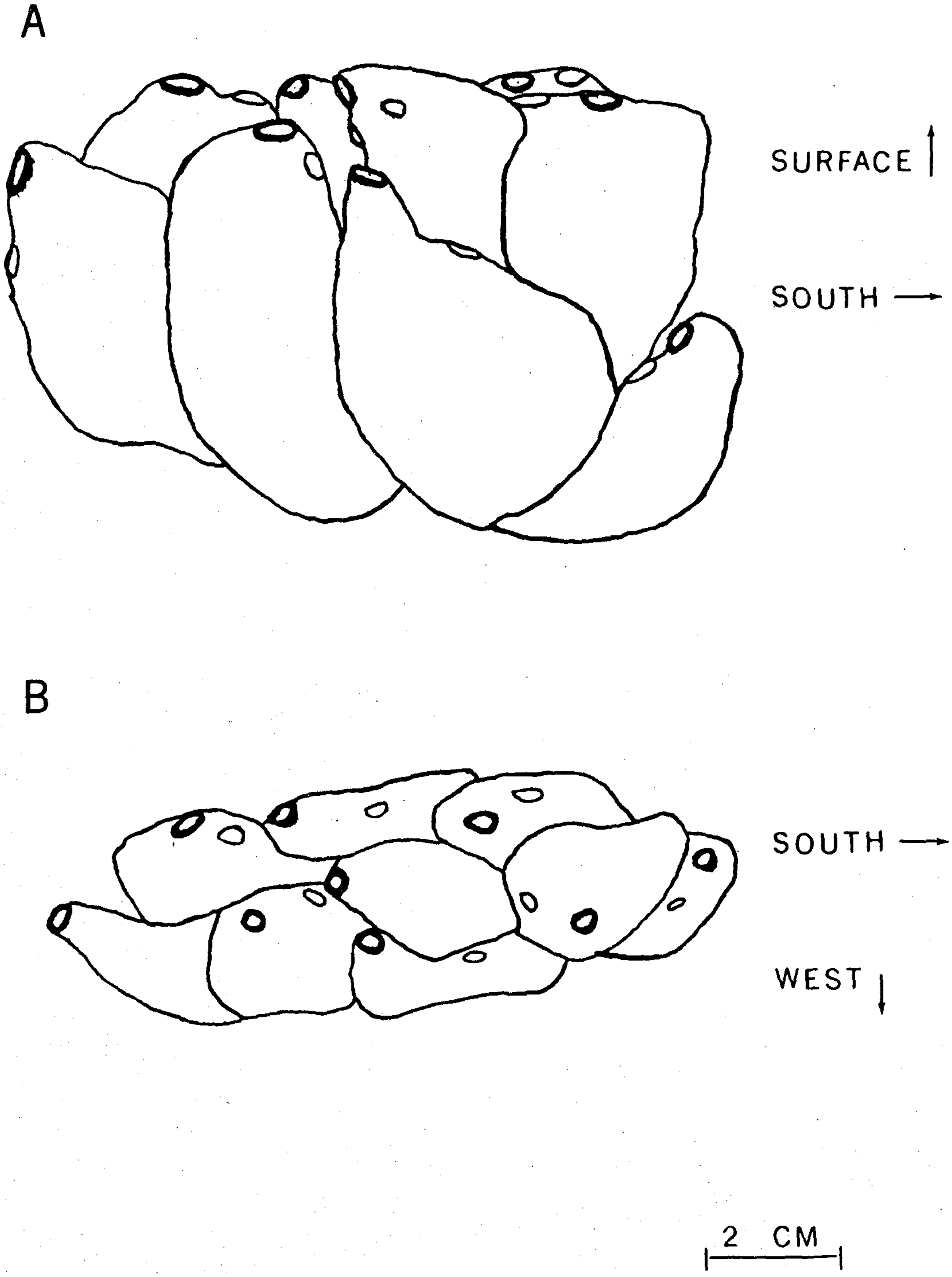
Sample area	Substrate orientation	Number of aggregates examined	Orientation
Sunset Shores (swift currents)	West	7	118 ± 30 (3) 57 ± 51 (8)
	South	4	—
	East	4	166 ± 51 (13) 31 ± 57 (12)
	North	1	—
Spooner's Creek (slow currents)	South	3	—
	East	25	52 ± 49 (9) 53 ± 54 (10) 69 ± 29 (5) 72 ± 11 (6) 75 ± 49 (10) 80 ± 44 (6) 82 ± 30 (5) 83 ± 36 (7) 89 ± 37 (7) 89 ± 51 (9) 93 ± 54 (10) 93 ± 54 (20) 100 ± 54 (14) 103 ± 54 (12) 118 ± 54 (12) 122 ± 56 (15) 130 ± 41 (14)

Figure 1. Illustrations of aggregates of *Styela plicata*.

A. West facing side view of aggregate, parallel with the substrate.

B. Surface view of aggregate, perpendicular to the substrate - attached to substrate on East side.

● - Oral Siphon
○ - Atrial Siphon


A
SURFACE
SOUTH
B
SOUTH
WEST
2 CM

VITAE

Alfred P. Chestnut was born September 24, 1946 in Port Norris, N. J. He graduated from Morehead City High School in 1964 and from Wake Forest University in June, 1968 with a B. S. in biology. He worked as a research assistant at the University of North Carolina Institute of Marine Sciences during the summer of 1968. From the fall of 1968 through January of 1970, he taught high school biology. The spring of 1970 was spent as a research assistant in the fish tagging program of the N. C. Department of Conservation and Development. He entered the graduate program at the University of Richmond in the fall of 1970. During the summer of 1971, he studied at the University of Virginia's Mountain Lake Biological Station.