VIMS Articles

1993

Estimation Of Oyster Shell Surface-Area Using Regression Equations Derived From Aluminum Foil Molds

Reinaldo Morales-Alamo
Virginia Institute of Marine Science

Recommended Citation

Morales-Alamo, Reinaldo, "Estimation Of Oyster Shell Surface-Area Using Regression Equations Derived From Aluminum Foil Molds" (1993). *VIMS Articles*. 520.
https://scholarworks.wm.edu/vimsarticles/520

Journal of Shellfish Research, Vol. 12, No. 1, 15–19, 1993.

ESTIMATION OF OYSTER SHELL SURFACE AREA USING REGRESSION EQUATIONS DERIVED FROM ALUMINUM FOIL MOLDS[1]

REINALDO MORALES-ALAMO
The College of William and Mary
Virginia Institute of Marine Science
School of Marine Science
Gloucester Point, Virginia 23062

ABSTRACT A method is described for estimation of surface area of shells of the American oyster, *Crassostrea virginica* (Gmelin 1791), as an alternative to direct measurement of surface area with aluminum foil molds. It is based on computation, from a small sample of shells, of the equation for regression of area of aluminum foil molds of shells on area enclosed within tracings of the shell outline. Area of other shells is then predicted from their shell outline area using the equation. Accuracy of the regression method in spatfall studies was established using data from shellstring collectors suspended in the Piankatank River, Virginia. For the most part, differences between foil mold area of individual shellstring shells and the area predicted from regression equations were small, and spat densities on individual shells, as computed from foil mold area and from regression-predicted area, were almost identical.

KEY WORDS: *Crassostrea virginica*, larval settlement, spatfall, oyster shells, surface area, aluminum foil

INTRODUCTION

Quantitative field studies of settlement of oyster larvae (spatfall) on shell cultch of the same species is hampered by difficulty in measurement of shell surface area (Butler 1954). For that reason settlement data have been presented most frequently as number of spat per shell or per oyster (e.g., Singarajah 1980, Haven and Fritz 1985, Morales-Alamo and Mann 1990, Adams et al. 1991); those data, however, cannot be compared with each other or with other data because they lack shell dimensions.

Some investigators have estimated shell surface area from the dimensions of the longer and shorter axes of the shell (Lunz 1954, Carreon 1973), from the weight of paper cutouts of shells (McNulty 1953) or from shell height (Galtsoff 1964, Marcus et al. 1989). Those methods, however, failed to account for shell shape and texture. Other investigators avoided the problem by using alternate materials with flat surfaces and square corners (e.g., Kennedy 1980, Osman et al. 1989, Kenny et al. 1990).

Healy (1991) made direct surface area measurements of oysters using aluminum foil molds that accounted for shell shape and surface texture. Foil had been previously used to measure surface area of corals (Marsh 1970), stones (Shelly 1979), and the bivalve mollusc *Donax serra* (Donn 1990). Whereas Donn (1990) and Healy (1991) prepared foil molds of each animal in their studies, a technique is presented here for estimation of the surface area of shells of *Crassostrea virginica* (Gmelin 1791) that reduces time and tediousness because it does not require a foil mold of every shell examined. Shell surface area is predicted from the equation for regression of actual (foil mold) area on the area enclosed by a tracing of the shell perimeter outline; Marcus et al. (1989) measured the area within the shell perimeter outline to validate their area estimates but apparently did not consider shell shape and texture.

MATERIALS AND METHODS

Source of Oyster Shells

Area measurements using aluminum foil were made on random samples of *C. virginica* shells from a natural oyster reef in the James River, Virginia (referred to as reef shells), and from refuse piles at local oyster-packing houses (house shells). Regression equations were computed for three samples: a 1983 sample of 48 mixed reef and house shells, a 1983 sample of 68 reef shells, and a 1990 sample of 80 house shells. Attached organisms were scraped off reef shell surfaces before foil molds were made.

The 1990 sample of house shells came from stock used to construct shellstrings deployed in the Piankatank River, Virginia, as part of a spatfall monitoring program (Barber 1990), and the equation derived from those shells was used to predict surface area of shellstring shells. Shellstring collectors were described by Haven and Fritz (1985).

Foil Mold Preparation and Area Measurements

Molds were made by pressing aluminum foil over the shell surface and molding it over mounds and ridges and into depressions and crevices. The mold of the inner surface included the ligament furrow in the left valve and the buttress and umbonal cavity in the right valve. The foil was smoothed out continuously during the molding process to avoid pleating. Excess foil extending over the shell edge was trimmed and the mold removed from the shell without distorting mold shape. Slits were cut into the mold from the margin inward and carefully flattened out, concave surface down. The outline of the flattened mold was traced on paper and area of the tracing measured with an electronic digitizing planimeter; this area will be referred to as the foil mold area (FMA). Shell outline area (SOA) was also measured with the planimeter from a tracing on paper of the perimeter outline of each shell.

Accuracy of FMA Measurements

The accuracy of FMA measurements was evaluated by comparison with another measure of true surface area based on division of the shell surface into 1-cm segments across the long axis of the shell and addition of the segment areas. Length of the lines between segments was measured with a cotton string following shell contours and surface area computed using the equation for the Trapezoidal Rule (Britton et al. 1965). Lohse (1990) also measured the area of *Mytilus edulis* valves directly by adding segmental areas.

---

[1] Contribution No. 1788, Virginia Institute of Marine Science, School of Marine Science, The College of William and Mary.

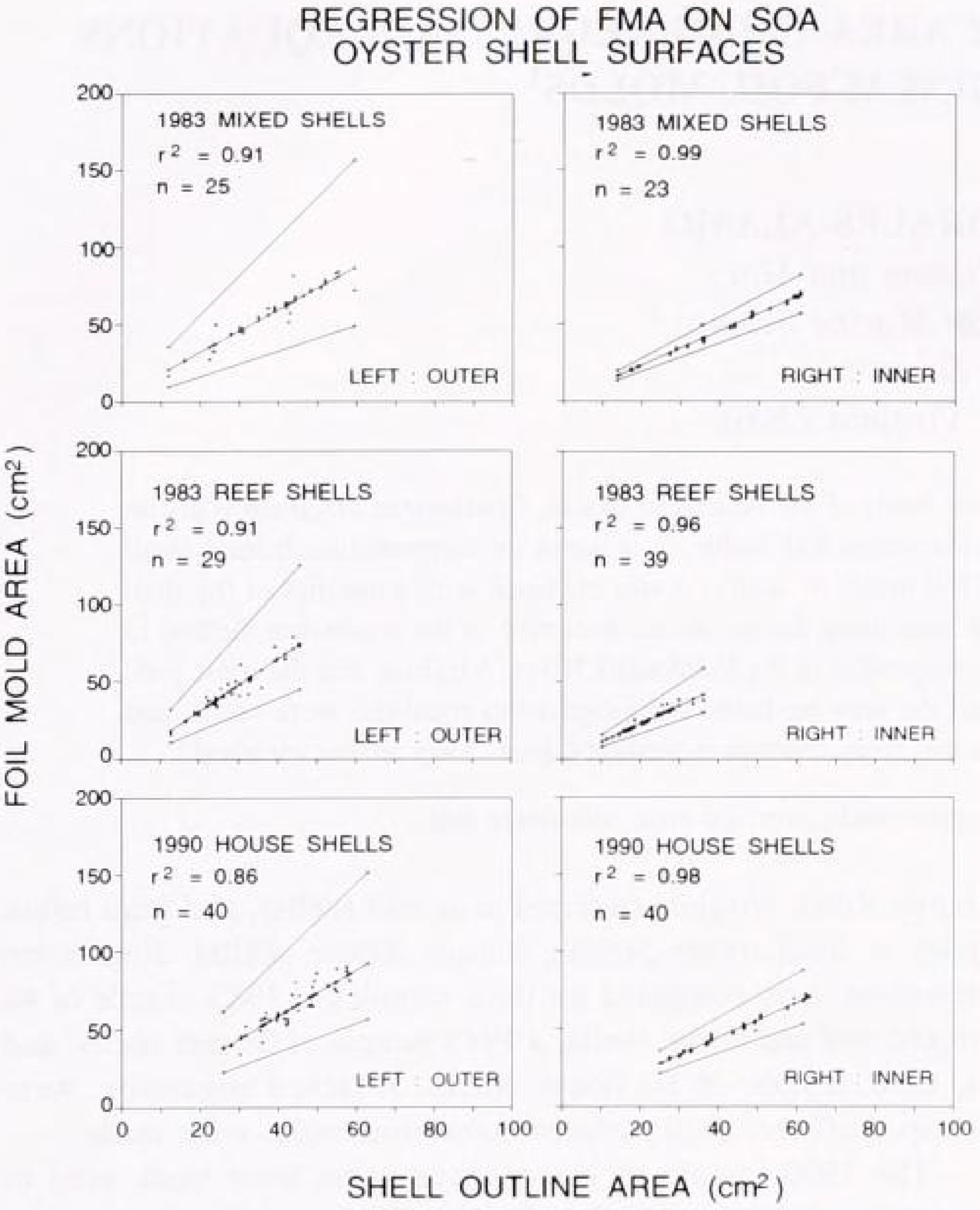

Figure 1. Line and 95% prediction interval for the regression of FMA (foil mold area) on SOA (shell outline area) in three different samples of oyster shells. Lack of symmetry of prediction intervals is due to conversion of computed values from logarithms to original form. Mixed shells were a mixture of reef and house shells. Left: outer = outer surface of left valve; Right: inner = inner surface of right valve.

Reproducibility of FMA Measurements

Reproducibility of FMA measurements was tested by replicating the process 10 times for the outer surface of each of two shells and computing the coefficient of variation (CV). The outer surface was selected for this test because it is more uneven and complex than that of the inner surface, thus providing a more rigorous test. One of the shells was a very convex left valve with outer surface deformations originating from another oyster previously attached to it; the other shell was a relatively flat right valve.

Regression Equations

Equations for regression of FMA on SOA were computed by the least-squares method after logarithmic transformation of the data to correct for heterogeneity of variance. Shell surface area was then predicted from those equations for a multiple number of SOA measurements. Use of the same regression equation to make multiple predictions precludes application of the usual prediction interval (Tiede and Pagano 1979, Snedecor and Cochran 1980). In its place, a prediction interval given by Snedecor and Cochran (1980) was computed.

Accuracy of Predicted Surface Areas

Accuracy of surface area predictions was tested by comparing FMA of shellstring shells with the area predicted from the regression of FMA on SOA (the regression-predicted area, or RPA). Spat densities on the shellstring shells as derived from FMA and as obtained from RPA were also compared.

RESULTS

Accuracy of the Aluminum Foil Mold Measurement

There was a high correlation between FMA and the area obtained from the sum of the segmental areas of the shell; the coefficients of determination (r^2) for the outer and inner surfaces were 0.99 and 0.98 in a mixed sample of 20 reef and 20 house shells which ranged from 10.27 to 70.64 cm^2 in SOA. The absolute percent difference between the two types of measurements for individual shells ranged between 0.1 and 9.6 (mean = 3.0; standard deviation (SD) = 2.4) for the outer surface and between 0.1 and 13.6 (mean = 4.8; SD = 3.8) for the inner surface.

Mean surface areas obtained by the two methods were almost identical: for the outer surface, 45.2 cm^2 (SD = 20.8) by the foil mold method and 45.5 cm^2 (SD = 21.2) by the sum of segmental areas; for the inner surface they were 36.9 cm^2 (SD = 16.9) and 36.6 cm^2 (SD = 16.8), respectively. The coefficient of variation for ten FMA replications of the outer surface of each of two individual house shells was very low (1.2 and 1.4), indicating that this technique is highly reproducible.

Regression of FMA on SOA

There was a strong correlation between FMA and SOA in each of three shell samples analyzed (Fig. 1, Table 1). All coefficients

TABLE 1.

Equations for the regression of foil mold area (Y) on shell outline area (X) in shells of *Crassostrea virginica* from three sources.

Source of Shells Valve and Surface	Regression Equation ($\log\hat{Y} = a + \log X$)	r^2
1983 Mixed Reef & House Shells:		
Left Valve (n = 25)		
Outer Surface:	$\log\hat{Y} = 0.249 + 0.954 \log X$	0.91
Inner Surface:	$\log\hat{Y} = 0.171 + 0.949 \log X$	0.96
Right Valve (n = 23)		
Outer Surface:	$\log\hat{Y} = 0.072 + 1.039 \log X$	0.97
Inner Surface:	$\log\hat{Y} = 0.115 + 0.964 \log X$	0.99
1983 Reef Shells:		
Left Valve (n = 29)		
Outer Surface:	$\log\hat{Y} = 0.057 + 1.094 \log X$	0.91
Inner Surface:	$\log\hat{Y} = 0.038 + 1.047 \log X$	0.96
Right Valve (n = 39)		
Outer Surface:	$\log\hat{Y} = 0.051 + 1.047 \log X$	0.91
Inner Surface:	$\log\hat{Y} = 0.006 + 1.038 \log X$	0.96
1990 House Shells:		
Left Valve (n = 40)		
Outer Surface:	$\log\hat{Y} = 0.153 + 1.004 \log X$	0.86
Inner Surface:	$\log\hat{Y} = 0.085 + 0.994 \log X$	0.95
Right Valve (n = 40)		
Outer Surface:	$\log\hat{Y} = -0.012 + 1.086 \log X$	0.95
Inner Surface:	$\log\hat{Y} = 0.093 + 0.967 \log X$	0.98

Reef shells collected from Wreck Shoal in the James River Virginia; house shells, origin unknown, were obtained from shucking-house refuse piles in Virginia. Logarithms to the base 10. $\hat{Y}$ = fitted Y, i.e., estimated Y (RPA in text).

TABLE 2.

Cumulative percent frequency distribution of the difference (in percentages, sign ignored) between foil mold area (FMA) and regression-predicted area (RPA) for individual oyster shells from shellstrings suspended in the Piankatank River in 1990 (predictions based on 1990 house shells).

	Left Valves				Right Valves			
	Outer Surf.		Inner Surf.		Outer Surf.		Inner Surf.	
Pct. Difference	n	Pct.	n	Pct.	n	Pct.	n	Pct.
<5.00	15	53.6	18	64.3	4	25.0	12	75.0
5.01–10.00	6	75.0	9	96.4	5	56.3	3	93.8
10.01–15.00	5	92.9	1	100.0	5	87.5	0	93.8
15.01–20.00	1	96.4			2	100.0	1	100.0
20.01–25.00	1	100.0						
n	28		28		16		16	
Mean	6.5		4.2		9.1		4.1	
SD	5.3		2.8		5.1		4.7	

SD = standard deviation.

of determination were higher than 0.86. Prediction intervals for the regression lines were very wide because a value of 500 was used for the number of future predictions in the equation from Snedecor and Cochran (1980). Figure 1 only includes the data for inner surface of right valves and outer surface of left valves because they represent extremes of shell flatness and concaveness (or convexity), respectively; regression data for the other two valve-surface combinations were intermediate in prediction interval width.

Comparison of Area and Spat Density Estimates

Differences between FMA and RPA were either all or mostly all under 15% for both surfaces of left and right valves in individual shells from Piankatank River shellstrings (Table 2). The same was true for shellstring shells used in the James River in 1983 (R. Morales-Alamo and D. S. Haven, unpublished data). Means for FMA and RPA were very close in each of the four groups of Piankatank River shellstring shells (Table 3).

Spat densities computed for individual shellstring shells using the two area estimates were identical or nearly identical in most shells (Table 3). Mean spat densities for each shellstring were identical in 6 of the 8 surface comparisons and very similar in the other two. The large variation around these means is associated with variations in larval settlement between shells in the same shellstring and not with variation in area estimates.

DISCUSSION

Surface area measurements of oyster shells using aluminum foil molds provide the closest approximation to true surface area of any technique proposed to date because they are the only ones that account for variations in shell shape and texture among individual shells. Their accuracy was demonstrated here by comparison with the sum of shell segmental areas.

Direct foil measurement of every shell examined (as done by Donn 1990 and Healy 1991) is the most desirable option. However, in studies that involve large numbers of shells, as in extensive spatfall monitoring programs, that method would require an inordinate amount of time and effort. The same would be true in studies involving natural reef shells because direct measurement with foil molds would require preliminary removal of fouling organisms. The regression method presented here is a suitable alternative that would substantially reduce time and effort investment because few actual foil mold measurements are required. A maximum of 40 each of the right and left valves would be satisfactory to derive a regression equation; tracing shell outline and measurement of the area enclosed for all other shells is done relatively quickly.

Use of direct foil mold measurements or predictions made from regression of FMA on SOA solves some of the problems associated with substrate suitability in larval settlement studies with oysters: (1) they provide dimensional measures of spat density, unlike data presented in terms of spat per shell, (2) they make it unnecessary to use, just for dimensional purposes, alternate materials that may be potentially less attractive than oyster shells to oyster larvae (Kennedy 1980), and (3) they permit comparison of settlement on oyster shells with settlement on alternate materials when such comparisons are required. They also offer the option of making counts on several measured small areas of the shell surface, instead of on the whole shell, when the number of spat is extremely large. The mean of those counts would be comparable to those made on whole shells.

Regression of FMA on SOA may be characterized by wide predictive intervals, depending on the valve and surface being analyzed, which would ordinarily handicap use of the regression for prediction purposes. In actual practice, however, percent differences between individual FMA and RPA were for the most part small and when tested in spatfall studies, their effect was inconsequential: spat density values for Piankatank River shellstring shells were almost identical regardless of whether the area measured with foil mold or the area predicted from regression was used. In that context, therefore, it is acceptable to ignore the wide predictive regression intervals.

A drawback of methods based on foil molds is that a foil mold of the outer surface of an oyster shell cannot account for surface areas inside very small depressions, crevices and pits on the shell surface. They may, thus, underestimate the total area available to settling oyster larvae in heavily-pitted shells. That, however, is not a serious problem when house shells are used because their outer surfaces are relatively unblemished. Old shells from natural reefs are usually heavily pitted and the problem created by that condition must be acknowledged when surface area estimates are made using foil molds.

Investigators using shellstrings in spatfall studies have often stated that they used shells of similar size (Lutz et al. 1970, Kennedy 1980, Singarajah 1980). Although those data may present an adequate picture of relative spatfall at different stations and in different years, absence of dimensional units considerably reduces confidence in comparisons with other data. Adoption of the technique presented here, as an alternative to direct foil mold measurements of all shells, would be advisable in spatfall studies that use whole oyster shells as collection substrate. Refinement of the method for improved accuracy is possible by using very flat right valves and examination of only the inner surface. Differences in size and shape of shells from different geographic locations and environments require computation of separate regression equations in subsamples from each of those populations to ensure the highest accuracy of predictions based on the equations.

TABLE 3.

Surface area of oyster shells and density of spat in shellstrings suspended in the Piankatank River, VA.

		Shell Surface Area (cm^2)				No. Spat and Density (No./cm^2)					
		Outside Surface		Inside Surface		Outside Surface			Inside Surface		
V	Outline Area (cm^2)	FMA	RPA	FMA	RPA	No. Spat	Dens. (FMA)	Dens. (RPA)	No. Spat	Dens. (FMA)	Dens. (RPA)
Exposure Period: 16–23 Aug 1990											
Palace Bar (n = 12)											
L	36.33	54.62	52.42	43.03	43.24	8	0.15	0.15	8	0.19	0.19
L	41.99	67.88	60.62	46.10	49.94	14	0.21	0.23	7	0.15	0.14
R	45.57	64.74	61.56	49.02	49.77	2	0.03	0.03	9	0.18	0.18
R	30.67	40.73	40.05	32.91	33.94	7	0.17	0.17	10	0.30	0.29
L	31.22	50.95	45.02	37.51	37.19	12	0.24	0.27	10	0.27	0.27
R	48.86	61.67	66.41	52.59	53.24	16	0.26	0.24	13	0.25	0.24
R	60.15	79.67	83.22	64.77	65.09	3	0.04	0.04	6	0.09	0.09
R	47.50	57.94	64.40	51.31	51.80	9	0.16	0.14	6	0.12	0.12
R	40.27	58.78	53.83	42.88	44.16	5	0.09	0.09	11	0.26	0.25
L	55.41	82.18	80.09	66.63	65.78	13	0.16	0.16	3	0.05	0.05
L	61.06	85.66	88.29	71.26	72.45	7	0.08	0.08	4	0.06	0.06
L	52.77	78.93	76.26	65.41	62.67	10	0.13	0.13	9	0.14	0.14
Mean	45.98	65.31	64.35	51.95	52.44	8.8	0.14	0.14	8.0	0.17	0.17
SD	10.30	13.92	15.26	12.47	12.01	4.4	0.07	0.08	2.9	0.09	0.08
Burton Point (n = 12)											
Mean	39.17	59.22	56.20	47.04	46.38	5.0	0.09	0.09	3.0	0.07	0.07
SD	7.90	14.31	11.93	10.46	9.63	4.2	0.07	0.07	2.3	0.05	0.05
Exposure Period: 23–30 Aug 1990											
Palace Bar (n = 10)											
L	49.97	75.38	72.19	57.90	59.36	22	0.29	0.30	32	0.55	0.54
L	69.60	100.38	100.69	89.92	82.52	30	0.30	0.30	31	0.34	0.38
L	60.56	100.97	87.56	74.98	71.86	7	0.07	0.08	52	0.69	0.72
R	45.01	55.49	60.74	53.76	49.18	41	0.74	0.67	29	0.54	0.59
R	39.13	58.86	52.18	44.22	42.95	9	0.15	0.17	25	0.57	0.58
L	48.93	74.50	70.69	62.62	58.14	10	0.13	0.14	30	0.48	0.52
L	35.92	55.62	51.83	43.58	42.76	5	0.09	0.10	25	0.57	0.58
L	53.26	70.33	76.97	59.36	63.25	23	0.33	0.30	55	0.93	0.87
R	39.83	66.45	53.19	53.01	43.69	13	0.20	0.24	22	0.42	0.50
L	37.42	62.98	54.00	46.51	44.53	1	0.02	0.02	9	0.19	0.20
Mean	47.96	72.10	68.00	58.59	55.82	16.1	0.23	0.23	31.0	0.53	0.55
SD	10.90	16.63	16.79	14.53	13.74	12.6	0.21	0.19	13.6	0.20	0.19
Ginney Point (n = 10)											
Mean	47.89	66.09	66.73	56.01	54.03	3.5	0.06	0.06	18.8	0.34	0.35
SD	10.90	15.66	14.43	10.00	10.91	3.3	0.06	0.06	7.6	0.12	0.13

Key to abbreviations: n = Number, V = Valve, L = Left, R = Right, SD = Standard Deviation.

Individual data for Palace Bar strings and means only for two other stations. Areas given as measured from aluminum foil molds (FMA) and as obtained from the regression equation of foil mold area on shell outline area for a sample of the house shells used to construct the shellstrings (RPA). Spat density computed using both surface area values.

ACKNOWLEDGMENTS

The author is indebted to Dexter S. Haven and Roger Mann for their support, manuscript reviews, and many valuable suggestions. Comments by Patrick K. Baker, Bruce J. Barber, David P. Lohse and other anonymous reviewers contributed significantly to the final form of the manuscript. Responsibility for its shortcomings, however, rest exclusively with the author. The technical assistance of Catherine A. Lawrence and Mary Y. Munro is gratefully acknowledged.

LITERATURE CITED

Adams, M. P., R. L. Walker, P. B. Heffernan & R. E. Reinert. 1991. Eliminating spat settlement on oysters cultured in coastal Georgia: a feasibility study. *J. Shellfish Res.* 10:207–213.

Barber, B. J. 1990. Oyster Spatfall in Virginia Waters: 1990 Annual Summary. Marine Resources Special Report, Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA. 7 pp. + 2 tables. (Available from the author).

Britton, J. R., R. Ben Kriegh & L. W. Rutland. 1965. *University Mathematics, I.* W. H. Freeman & Co., San Francisco, CA. 658 pp.

Butler, P. A. 1954. Selective setting of oyster larvae on artificial cultch. *Proc. Natl. Shellfish. Assoc.* 45:95–105.

Carreon, J. A. 1973. Ecomorphism and soft animal growth of *Crassostrea iredalei* (Faustino). *Proc. Natl. Shellfish. Assoc.* 63:12–19.

Donn, T. E., Jr. 1990. Morphometrics of *Donax serra* Roding (Bivalvia: Donacidae) populations with contrasting zonation patterns. *J. Coastal Res.* 6:893–901.

Galtsoff, P. S. 1964. *The American Oyster: Crassostrea virginica* Gmelin. *Fish. Bull.* U.S. Fish and Wildlife Service, 64:1–480.

Haven, D. S. & L. W. Fritz. 1985. Setting of the American oyster *Crassostrea virginica* in the James River, Virginia, USA: temporal and spatial distribution. *Mar. Biol.* 86:271–282.

Healy, D. K. 1991. The effect of physical disturbance on settlement and recruitment of the American oyster, *Crassostrea virginica* (Gmelin). Thesis. University of South Carolina. 60 pp.

Kennedy, V. S. 1980. Comparison of recent and past patterns of oyster settlement and seasonal fouling in Broad Creek and Tred Avon River, Maryland. *Proc. Natl. Shellfish. Assoc.* 70:36–46.

Kenny, P. D., W. K. Michener & D. M. Allen. 1990. Spatial and temporal patterns of oyster settlement in a high salinity estuary. *J. Shellfish Res.* 9:329–339.

Lohse, D. P. 1990. Interactions between a marine mussel and its epibionts: influence on population dynamics and community structure. Ph.D. Dissertation, University of California, Santa Barbara, CA. 174 pp.

Lunz, G. R. 1954. The general pattern of oyster setting in South Carolina. *Proc. Natl. Shellfish. Assoc.* 45:47–51.

Lutz, R. A., H. Hidu & K. G. Drobeck. 1970. Acute temperature increase as a stimulus to setting in the American oyster, *Crassostrea virginica* (Gmelin). *Proc. Natl. Shellfish. Assoc.* 60:68–71.

Marcus, J. M., G. I. Scott & D. D. Heizer. 1989. The use of oyster shell thickness and condition index measurements as physiological indicators of no heavy metal pollution around three coastal marinas. *J. Shellfish Res.* 8:87–94.

Marsh, J. A., Jr. 1970. Primary productivity of reef-building calcareous red algae. *Ecology* 51:255–263.

Morales-Alamo, R. & R. Mann. 1990. Recruitment and growth of oysters on shell planted at four monthly intervals in the lower Potomac River, Maryland. *J. Shellfish Res.* 9:165–172.

McNulty, J. K. 1953. Seasonal and vertical patterns of oyster setting off Wadmalaw Island, S.C. *Contrib. Bears Bluff Labs.* No. 15, 17 pp.

Osman, R. W., R. B. Whitlach & R. N. Zajac. 1989. Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. *Mar. Ecol. Progr. Ser.* 54:61–73.

Shelly, T. E. 1979. The effect of rock size upon the distribution of species of Orthocladiinae (Chironomidae: Diptera) and *Baetis intercalaris* McDunnough (Baetidae: Ephemeroptera). *Ecol. Entomol.* 4:95–100.

Singarajah, K. V. 1980. Some observations on spat settlement, growth rate, gonad development and spawning of a large Brazilian oyster. *Proc. Natl. Shellfish. Assoc.* 70:190–200.

Snedecor, G. W. & W. G. Cochran. 1980. *Statistical Methods*. The Iowa State University Press, Ames, IA. 507 pp.

Tiede, J. J. & M. Pagano. 1979. The application of robust calibration to radioimmunoassay. *Biometrics* 35:567–574.