1994

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Recommended Citation

REPRODUCTIVE ACTIVITY OF OYSTERS, *CRASSOSTREA VIRGINICA* (GMELIN, 1791) IN THE JAMES RIVER, VIRGINIA, DURING 1987–1988

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ABSTRACT Reproductive activity in oysters, *Crassostrea virginica* Gmelin, in the James River, Virginia, was examined for 1987 from weekly estimates for fecundity and egg viability in oysters collected from Wreck Shoal, and for 1988 from weekly estimates of fecundity, egg viability, gonad volume fraction, gonad thickness, and mean egg size in oysters collected from Horsehead Reef. Maximum and mean fecundity values from Wreck Shoal oysters were higher than from Horsehead oysters. No relationship was evident between fecundity and egg viability at Horsehead Reef. A strong temporal relationship was observed between egg viability and peak oyster settlement in the James in both years of the study as estimated by off-bottom settlement substrates. In 1987 highest viability occurred from late June through mid August with peak settlement occurring from mid June through late August. In 1988 viable eggs were recorded from late July through the end of August; major settlement occurred from early August through mid September. Fishery independent estimates of oyster population abundance on Horsehead Reef, when combined with concurrent egg production and viability data, illustrate the losses that occur during the early life history stages of oysters in this location.

KEY WORDS: Oyster, *Crassostrea virginica*, James River, fecundity, gonad volume fraction, eggs, oyster settlement

INTRODUCTION

The James River, Virginia has served as the focal point for the Virginia oyster industry for over a century, being the source of the majority of seed oysters that were transplanted for grow-out to locations within the Virginia portion of the Chesapeake Bay and much further afield in the Middle Atlantic states. It has been the site of continuing investigations of oyster distribution in relation to bottom type (Baylor 1894, Moore 1911, Loosanoff 1931, Haven et al. 1981a, Andrews 1982, Haven and Whitcomb 1983), larval biology and settlement (Loosanoff 1931, Andrews 1951, 1954, Wood and Hargis 1971, Andrews 1979, Haven and Fritz 1985, Andrews 1983, Mann 1988), larval dispersal in relation to circulation (Pritchard 1953, Ruzecki and Moncure 1968, Ruzecki and Hargis 1988), disease impact (Andrews 1954, 1962, 1968, Burreson 1986, 1990) and a series of unpublished qualitative annual surveys of oyster resources by location (Virginia Institute of Marine Science Library Archive). Given the ecological importance (see Mann et al. 1991) and commercial value (see Haven et al. 1981b) of oysters originating from the James River it is surprising that comparatively little effort has been devoted to quantitative examination of the relationship between spawning and recruitment in the James River. Previously, Cox and Mann (1992) compared temporal and spatial variation in oyster fecundity in the James River; however, these were not coupled with estimates of egg viability or standing stock in an attempt to further estimate long term changes in egg production by James River oyster populations. This relationship becomes increasingly critical as both disease and commercial exploitation maintain significant pressures on the resource.

As part of a continuing effort to develop improved quantitative descriptions of the relationships between oyster standing stock, egg production, and recruitment in the James River this report describes studies to estimate temporal changes in fecundity and egg viability in oysters, *Crassostrea virginica* Gmelin, in surviving populations in the lower salinity regions of the river as described by Haven and Fritz (1985). Our initial plan was to focus a long term effort on Wreck Shoal, (Fig. 1, see also Haven and Whitcomb 1983), a large and commercially important oyster reef that has been implicated as an important broodstock area in the James River ecosystem by the works of Ruzecki and Hargis (1989) and Cox and Mann (1992); however, severe losses of oysters to disease (*Haplosporidium nelsoni* and *Perkinsus marinus*) at the Wreck Shoal site prevented further sampling at this site in 1988. Therefore, 1988 samples were collected from Horsehead Reef. Horsehead is one of the few reefs in the James River to have suffered minimal losses to disease, although low levels of *P. marinus* have been recorded in recent years (Burreson 1990). Horsehead reef is currently the site of intense fishing activity during the commercial season, from October 1 through May 31. The boundaries of this reef are described in Haven et al. (1981a), and Haven and Whitcomb (1983). In 1988 reproductive studies were supplemented by fishery independent estimates of population size to facilitate calculation of total egg production from oysters on Horsehead reef. Thus, Horsehead offers a suitable site for examination of seasonal reproductive activity in a defined oyster population as described here.

MATERIALS AND METHODS

Oysters were collected in both years of the study with a 60 cm-wide dredge having 7.5-cm teeth. In 1987 oysters were collected from Wreck Shoal at weekly intervals from June 25 to October 8. Dredging continued until 80 oysters with heights (maximum dimension from the hinge to the opposite margin) greater than 76 mm, the effective minimum size for commercial exploitation at that time, were collected. In the laboratory, oysters were opened and sex determined by microscopic examination of gonad smears. Sex ratio was compared to unity using chi-squared analysis. The first 10 females opened were used for fecundity and egg viability determination as described below. On all sampling occasions water temperature was recorded at the surface and bottom using a mercury thermometer, and water samples were collected
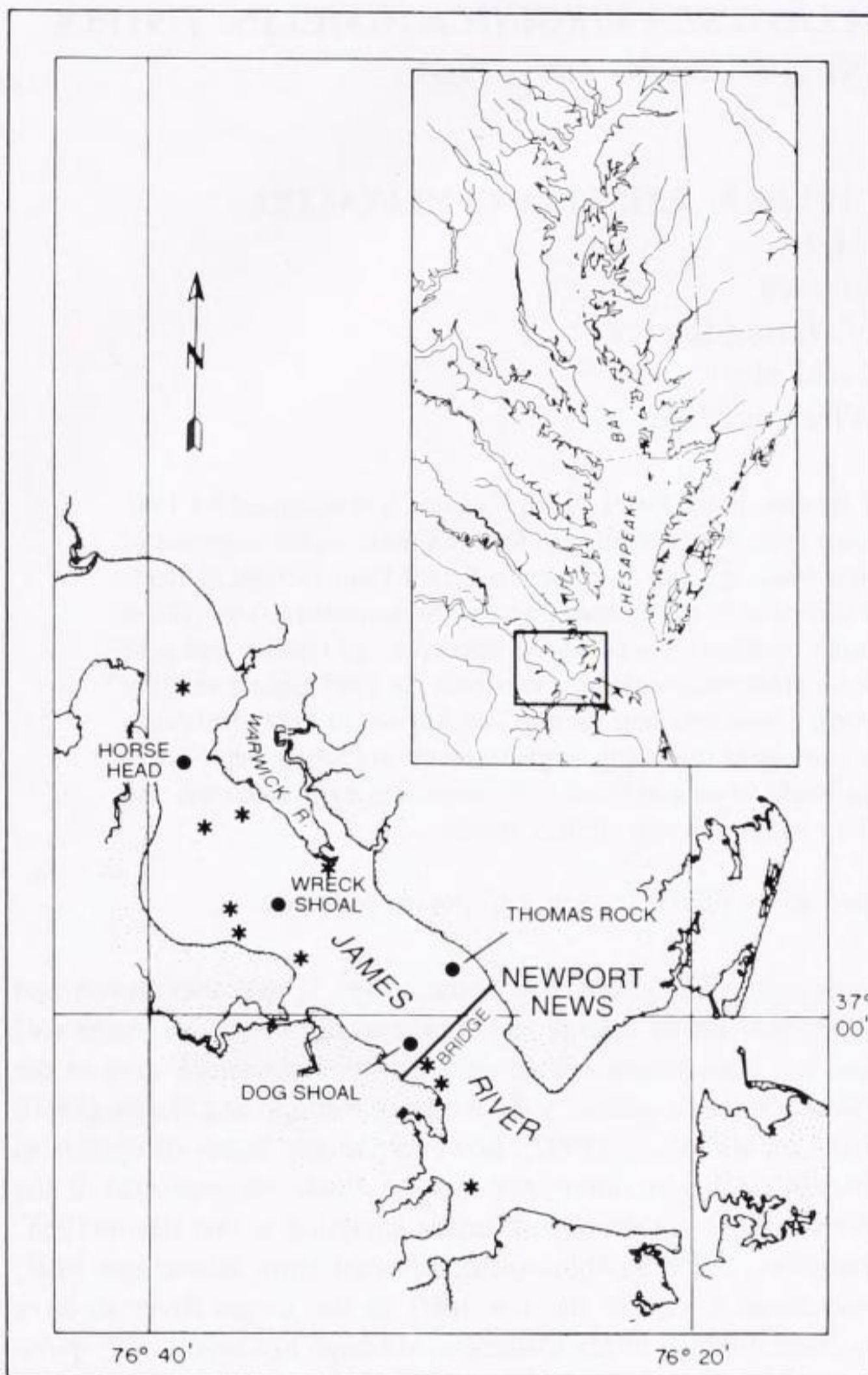
Figure 1. Location of collection sites in the James River, Virginia. Wreck Shoal and Horsehead are used in the present study. In addition, Dog Shoal and Thomas Rock were used by Cox and Mann (1992). These four, plus remaining sites market with * were used for monitoring spatfall.

using a Niskin bottle for subsequent estimation of salinity using a Beckman RS-10 induction salinometer.

Oysters were collected from Horsehead in 1988 at weekly intervals from June 15 through September 28. Dredging continued until 100 oysters were collected. Temperature and salinity measurements were also made in 1988. In the laboratory the oysters were opened, sex ratio determined, and that ratio compared to unity using chi-squared analysis. The first ten females were used to determine fecundity and viability, the second ten were fixed for subsequent histology and determination of gonad volume fraction (GVF), gonad thickness, and egg volume.

Fecundity, for the current study, is defined as the number of mature eggs contained in a single oyster. Separation of the gonadal mass from the visceral mass is impractical in oysters complicating direct estimation of gonad size for indirect estimation of fecundity. Histological procedures, such as employed by Morales-Alamo and Mann (1990), provide details concerning both stage of development and gonad volume fraction (GVF) but require multiple sections per specimen and are similarly impractical in rapid processing of a large number of specimens. Immunological techniques

(Choi et al. 1993) provide direct estimates of egg protein and have promise for future use; however, they were not available at the time of this study. Fecundity estimates were made using the method of Cox and Mann (1992). Whole, wet tissue of individual oysters was homogenized using a commercial blender. The homogenate was washed through a 500-μm sieve to remove large debris, and eggs were retained on further washing through a 25-μm sieve. Eggs were washed into a calibrated glass cylinder and the cylinder contents made up to a known volume with 1 μm filtered sea water. The cylinder contents were thoroughly mixed and subsamples removed for counting of eggs using a Sedgewick Rafter cell under low power magnification on a compound microscope. Triplicate counts were made. Fecundity estimates were obtained from proportional volumes of cylinder contents and subsamples. Such estimates suffer the limitation of the assumption that all eggs retained on the sieve are amenable to fertilization. Prudent choice of sieve size is important, but clearly a compromise. Retained material may include immature eggs causing an overestimate of fecundity. Alternatively, eggs damaged during homogenization may pass through the sieve resulting in underestimation of fecundity. The relative magnitude of these errors was not assessed.

Egg viability was determined as follows. Three aliquots, each of 5,000 eggs, were removed from eggs isolated in the fecundity estimation procedure. Each aliquot was transferred to a 100-ml beaker and made up to 50-ml volume, resulting in a egg concentration of 100 eggs/ml. Sperm were isolated after homogenization of male oysters from the same source and repeated passage of homogenate through a 20-μm sieve. Sperm was added to the egg suspension to give a final concentration of 3×10^5 sperm/ml. All fertilizations were generally effected within one hour of isolation of gametes. Although this procedure is typical of research hatchery operations for naturally spawned eggs (see Castagna, Gibbons and Kurkowski, in press) the current method uses effectively stripped eggs. Stephano and Gould (1988) reported that stripped eggs of *Crassostrea gigas* are more susceptible to polyspermy than naturally spawned eggs, especially when fertilization is attempted within one hour after isolation suggesting that the current method may underestimate viability because of losses to polyspermy; however, we have observed no such polyspermy effects with *C. virginica* in pilot hatchery systems (Shaffer and Mann unpublished observations). Forty five minutes after addition of sperm, the sperm and egg mixture, now containing fertilized and developed eggs, was gently sieved through a 20-μm sieve to remove excess sperm and the filtrate returned to 100-ml beakers containing 50 ml of filtered sea water. The eggs were left to develop overnight, and three subsamples were subsequently removed to count the first-shelled veliger larvae present. In 1987, data were recorded only as percentage of cultures containing active veliger larvae 24 hours after the addition of sperm; the number of verligers per culture was not recorded. In 1988, the procedure was improved and egg viability was estimated as the number of active verligers per 1000 eggs. Losses of eggs and larvae due to handling during the washing procedure in 1988 were assumed to be negligible.

Oysters used for histological examination were fixed in Davidson's AFA fixative for 24h; at the end of that period transverse cuts were made through the visceral region, anterior and posterior to the junction of the gills and palps, to obtain a body segment approximately 6 mm thick. This segment was held in Davidson's fixative until it was embedded in paraffin after being dehydrated and cleared in an alcohol:xylene series. The time sequence for this

procedure was closely controlled to insure that any changes in egg size caused by fixation and dehydration were consistent throughout the study. Sections of 6μm thickness were subsequently cut, mounted on glass slides, stained in Harris' hematoxylin and eosin Y, and examined microscopically. Ten oysters were processed from each sampling date. On seven dates, however, measurements were made on only 8 or 9 oysters because the others were found to be either males or hermaphrodites or were heavily infected with the trematode *Bucephalus* sp., which compromised gonadal development.

Gonad volume fraction was measured by point-count volumetry using a square grid on a reticule mounted in the microscope eyepiece (Chalkey 1943, Weibel et al. 1966, Bayne et al. 1978). Four GVF measurements were made on each oyster at gonad locations selected at random from among eight regions around the circumference of the section. Up to eight counts were made in instances when the number of eggs in an oyster was extremely low. Estimates of statistical parameters were made after arcsine transformations to normalize data.

Gonadal thickness is described in terms of the total number of grid points included in computation of GVF as an estimate of gonad width in the section, and will be referred to as gonadal width throughout the text. Where gonad width exceeded maximum grid size (121, the maximum number of intersection points in the grid) width was measured by moving the grid toward the center of the section and adding to the count the number of points needed to reach the inside margin of the gonad. This procedure was required on most of the oysters collected on July 6 and August 3, and in one or two of the oysters on each of five other dates. Gonad width measurements were weighted for differences in total oyster cross-sectional area, by dividing the number of grid points by the cross-sectional area. Statistical comparisons of GVF and gonad width between sampling dates were made using the non-parametric Mann-Whitney test (Olson 1988) because variances were heterogeneous.

Egg size was measured for 4–8 animals from each date of collection as the cross sectional area of individuals egg, in histological preparations used for determination of GVF. Preparations were examined using a digitizing video imaging system (International Imaging Systems model 75) attached to a compound microscope (Olympus model BH-2). Five eggs were measured per field of view in 10 randomly chosen fields (total of 50 measurements) for each oyster section examined. The only criterion for selection of eggs to be measured was the presence of a large, distinct nucleus. Areas were converted to nominal diameter values (the diameter of a circle with the same area), and comparisons between sampling dates effected using the non-parametric Mann-Whitney test. Nominal diameters were used for comparison purposes only and are not to be interpreted as representing the actual diameter of the eggs.

Temporal and spatial changes in settlement of oysters throughout the James River were monitored in both 1987 and 1988 as part of a long term, continuing program. Methods were similar to those described by Haven and Fritz (1985). Shellstrings were deployed above the bottom at weekly intervals between June and October at the 14 stations (Fig. 1). A shellstring consisted of 12 oyster shells of similar size (about 76 mm in height) drilled through the center and strung on a piece of heavy gauge wire. After a one-week exposure, the number of spat attached to the smooth surface (underside) of the center ten shells was counted with the aid of a dissecting microscope. This number was then divided by 10 to

obtain the number of spat per shell for that time interval. Weekly sampling allowed examination of settlement trends over the course of the summer at the various locations. Comparison between years were made by adding weekly values of spat per shell for the entire settlement period.

Fishery independent estimates of oyster populations on Horsehead were made using divers and quadrat collections from preselected sites. Ideally, samples would be collected from sites randomly selected from a uniform grid overlaying the reef as defined by the boundaries illustrated in Haven and Whitcomb (1983). Development of such a grid was practically unreasonable using LORAN given the temporal drift in LORAN signals and the requirement to repeatedly locate grid coordinates with accuracy over extended time periods. Marking a large number of grid coordinates with buoys was complicated by extensive boat traffic in the study area. As an alternative the latitude and longitude of a large number of sites were identified that could be approximately located by LORAN then fixed with accuracy by triangulation on known landmarks, these being either navigational buoys, prominent coastal features or both. Sampling stations were then chosen by designating site numbers and choosing stations for sampling from random number tables. Collections were made on September 22, 1988 by divers from quadrats (1 m²). All material within the quadrat was retained. Samples were examined for total volume of oyster and shell collected, total numbers of oysters and size-frequency distribution, by 10 mm height intervals starting at 10 mm, of those oysters. Biomass estimates (wet tissue weight) were made from these values using mean individual data for oysters collected from Horsehead in July–August, 1979 (Haven and Morales-Alamo, unpublished). The number of quadrats required to provide estimates of oyster density with the smallest variation was determined by plotting the number of collections examined versus the standard error of the mean of the number of oysters found. Little decrease in standard error was observed when more than ten samples were included. Therefore twelve quadrat samples were collected on each date.

RESULTS

Data for oyster reproductive activity at Wreck Shoal (1987) and Horsehead (1988) are summarized in Table 1 and Table 2, respectively. In both years temperature gradually increased to a maximum in excess of 29.0°C in late July through mid August, after which temperature decreased to approximately 22.0°C by the end of the annual study period. Salinity was consistently higher at Wreck Shoal in 1987 than at Horsehead in 1988. Wreck Shoal values were generally between 16 and 20 ppt, with lower values on June 25, July 16 and September 24. Horsehead values were lower, generally increasing from below 7 ppt in June 1988 to above 15 ppt by mid August 1988. A value of 21.30 ppt was recorded during a period of consistently low precipitation in mid September 1988.

Percentage of female oysters in each sample was highly variable throughout the study period in both years, varying from 3% to 73% at Wreck Shoal in 1987 and 19% to 62% at Horsehead in 1988. All 1987 samples except July 2 were significantly different (P < 0.01) from 50%, a value corresponding to a sex ratio of 1:1. On three dates in 1988 the percentage of females in the sample was significantly higher (P < 0.05) than 50%; however, on seven dates the percentage was significantly lower (P < 0.05 or 0.01, see Table 2) than 50% indicating a preponderance of male oysters.

TABLE 1.

Summary of 1987 field studies at Wreck Shoal.

A	B	°C	ppt	F ± s.e. (×10⁶)	V′	R%	Spat
6/25	6/20–26	26.2	14.0	0.64 ± 0.13	33	26**	0.3
7/2	6/27–7/3	25.5	19.3	1.25 ± 0.25	10	40	1.0
7/9	7/4–10	27.0	15.2	2.28 ± 0.39	70	29**	17.9
7/16	7/11–17	28.5	10.7	1.05 ± 0.09	60	65**	379.7
7/23	7/18–24	28.5	17.5	0.85 ± 0.18	100	73**	706.7
7/30	7/25–31	29.0	18.9	2.76 ± 1.10	30	73**	611.9
8/6	8/1–7	29.5	19.2	0.13 ± 0.04	80	29**	261.1
8/13	8/8–14	29.5	18.1	0.51 ± 0.28	20	23**	88.6
8/20	8/15–21	28.0	20.9	5.97*	0	21**	44.3
8/27	8/22–28	27.5	18.3	0.54 ± 0.20	0	32**	13.9
9/3	8/29–9/4	25.8	19.8	0.15 ± 0.05	0	29**	16.9
9/10	9/5–11	25.5	17.5	1.20 ± 0.43	40	23**	10.5
9/17	9/12–18	25.8	19.9	0.66 ± 0.44	17	19**	27.8
9/24	9/19–25	26.5	7.2	0.28 ± 0.12	0	15**	14.4
10/1	9/26–10/2	24.0	16.2	0.79*	0	3**	2.3
10/8	n.d.	22.5	13.9	1.77*	0	5**	n.d.

Date A is collection of dredge samples for fecundity and sex ratio estimation.

Date B is exposure period for shellstrings to estimate spat settlement.

Temperature (°C) and salinity (ppt) values are for bottom water collected on date A at site of dredge collections.

Mean fecundity (F ± s.e., × 10⁶, n = 10 except * when n = 1).

Viability estimates (V′) are percentage of individual oysters having eggs that produce veliger larvae.

Percentage female (R = [f/f + m] × 100%, n = 80, ** indicates significantly different from 50%, P < 0.01). Spatfall values are totals of data from fourteen stations (see Fig. 1), each station being included as spat per shell for the given time interval.

n.d. indicates no data collected.

Low percentage female values were consistently observed at the beginning and end of the study periods each year. Percentage of females at Wreck Shoal in 1987 was low, between 19% and 32%, on most dates. High percentages were found only between July 16 and July 30; however, no consistent pattern of variation was observed during the July-September period of either year. During the early July–mid September period in 1987, the percentage of female oysters increased gradually to 73% (July 23–30), but subsequently decreased to consistently <32% (August 6 onwards). In contrast, percentage of females demonstrated greater temporal stability at Horsehead in 1988, varying in the range 36–62% during the period June 22–September 14.

Mean fecundity values for the 1987 collections from Wreck Shoal were consistently higher by approximately one order of magnitude than the 1988 collections from Horsehead. 1987 values varied in the range 0.13–2.76 × 10⁶ (excluding the single value for August 20, 1987) with individual values as high as 12.04 × 10⁶ being observed on July 30, 1987. 1988 mean fecundity values were in the range 0.14–2.91 × 10⁵ with a maximum individual value of 8.1 × 10⁵. High variability in fecundity values within a sample of oysters of comparable size was observed at both sites suggesting asynchrony in gametogenesis within a population. Large temporal changes in mean fecundity, especially decreases over short time intervals suggesting spawning activity, were also observed with minimum individual fecundity values of <10⁴ being recorded in both years.

Viable eggs were consistently found at Wreck Shoal from June 25, 1987 through August 13, 1987, with all individuals producing viable eggs on July 23, 1987. Viable eggs were again observed on September 10 and 24, 1987. In 1988, viable eggs were only observed at Horsehead during the period July 20–August 31. Viability was generally low with population means in the range 1.3–

8.7 larvae/1000 eggs and a maximum individual value of 33.3 larvae/1000 eggs. Individual oysters devoid of viable eggs were observed on all sampling dates in 1988.

Oysters used for GVF estimates of 1988 Horsehead collections had shell heights ranging from 55.5 to 88.5 mm. Although this did not include representation from small size classes present in abundance in June and early July collections (see Table 3) it adequately represented the larger animals present throughout the June through September study period. Trends in fecundity, egg size, GVF, and gonad width did not match date for date through the season (Table 2). There was, however, a general trend in which peaks or valleys in means of the individual parameters deviated from each other by only one week. The common tread was characterized by statistically significant (P ≤ 0.05) decreases in mean values with respect to the prior values on June 22, July 13, July 27 (except for gonad width), and August 17. Statistically significant increases with respect to the prior values (P ≤ 0.05) occurred on June 29, July 6 (except for gonad width), and August 3; no detectable change was evident in gonad width between June 29, when a high value was attained, and July 27, because of high variability in the data.

These common trends suggest at least two separate major spawning periods at Horsehead in 1988; July 13 through 27, and August 3 through 24. A spawning event in late June is also suggested by statistically significant reductions in GVF and egg size on June 22 and June 29, respectively, in comparison to prior values. Decreasing mean values were also recorded for gonad width and fecundity in that period but associated variance values were high. September data were characterized in most instances by high variations which obscured any possible differences between dates for mean fecundity and GVF values. Egg size decreased significantly between August 10 and 24, and between August 31 and September 14, but increased again by September 28. A sig-

TABLE 2.

Summary of 1988 field studies at Horsehead Reef.

A	B	°C	ppt	F ± s.e. (×10⁵)	V'' ± s.e.	GVF ± s.e. (n)	GW ± s.e.	D ± s.e. (μm, n)	R%	spat
6/15	6/13–6/19	24.5	6.65	1.11 ± 0.41	0	0.62 ± 0.05 (8)	1.61 ± 0.18	34.2 ± 0.2 (5)	18**	0.0
6/22	6/20–26	26.9	6.55	0.30 ± 0.09	0	0.48 ± 0.07 (10)*	1.29 ± 0.20*	34.0 ± 0.3 (5)	41*	0.0
6/29	6/27–7/3	n.d.	n.d.	0.14 ± 0.03	0	0.64 ± 0.03 (8)*	2.33 ± 0.38	31.4 ± 0.2 (8)*	40*	0.2
7/6	7/4–10	25.0	8.93	0.85 ± 0.19	0	0.75 ± 0.02 (8)*	2.08 ± 0.16	35.2 ± 0.2 (5)*	60*	5.9
7/13	7/11–17	27.1	10.48	1.48 ± 0.79	0	0.68 ± 0.03 (10)*	2.52 ± 0.20	33.2 ± 0.2 (6)*	41*	1.8
7/20	7/18–24	27.9	8.46	0.81 ± 0.20	7.3 ± 3.4	0.69 ± 0.03 (9)	2.15 ± 0.26	33.1 ± 0.2 (5)	62*	9.2
7/27	7/25–31	28.6	14.03	0.10 ± 0.02	8.7 ± 4.3	0.61 ± 0.04 (10)*	2.02 ± 0.25	30.8 ± 0.2 (5)*	52	5.8
8/3	8/1–7	29.0	9.75	2.91 ± 0.48	1.3 ± 1.0	0.76 ± 0.03 (9)*	3.42 ± 0.43*	33.5 ± 0.2 (5)*	55	38.2
8/10	8/8–14	29.5	12.58	0.86 ± 0.24	6.3 ± 2.7	0.54 ± 0.02 (10)*	2.42 ± 0.42*	34.8 ± 0.2 (7)*	51	53.7
8/17	8/15–21	29.4	12.02	1.10 ± 0.49	4.7 ± 1.7	0.44 ± 0.03 (9)*	2.27 ± 0.28	33.8 ± 0.2 (6)*	60*	4.7
8/24	8/22–28	26.4	15.47	1.85 ± 0.83	0	0.49 ± 0.6 (10)	1.53 ± 0.20*	31.4 ± 0.2 (5)*	49	10.7
8/31	8/29–9/4	25.7	14.22	1.53 ± 0.51	2.3 ± 1.6	0.44 ± 0.06 (10)	1.96 ± 0.20	34.5 ± 0.1 (5)*	45	21.6
9/7	9/5–11	23.5	16.18	0.97 ± 0.41	0	0.33 ± 0.09 (10)	1.82 ± 0.40	33.6 ± 0.2 (5)*	36**	18.3
9/14	9/12–18	23.7	21.30	2.47 ± 0.82	0	0.29 ± 0.10 (10)	1.07 ± 0.39	33.3 ± 0.1 (5)	45	11.0
9/21	9/19–25	24.0	14.80	0.96 ± 1.26	0	0.23 ± 0.08 (10)	1.24 ± 0.27	34.3 ± 0.2 (4)*	19**	2.1
9/28	9/26–10/2	22.0	n.d.	2.09 ± 0.20	0	0.30 ± 0.12 (9)	0.66 ± 0.23	34.5 ± 0.2 (5)	20**	0.6

Date A is collection of dredge samples for fecundity and sex ratio estimation.

Date B is exposure period for shellstrings to estimate spat settlement.

Temperature (°C) and salinity (ppt) values are for bottom water collected on date A at site of dredge collections. Adult fecundity (F ± s.e., ×10⁵, n = 10), and Viability (V) values are from live dredge collections. Viability (V'') values are numbers of veliger larvae obtained per 1000 eggs in 50 ml cultures (see Methods).

Gonad Volume Fraction (GVF ± s.e., n in parentheses).

GW is Point Count Data (Gonad Width, GW ± s.e., n as for GVF).

Nominal Egg Size (D ± s.e. μm, n in parentheses is number of oysters examined, see Methods).

Percentage Female Oysters (R = [f/f + m] × 100%, n = 100) values are from histological preparations.

* Adjacent to a GVF, GW or D value indicates a significant difference ($P < 0.05$) from the preceeding value.

* or ** adjacent to a R% value indicates a significant difference from 50% at either the $P < 0.05$ or $P < 0.01$ level, respectively.

Spatfall values are totals of data from fourteen stations (see Fig. 1), each station being included as spat per shell for the given time interval.

n.d. indicates no data collected.

nificant decrease in gonad width was observed between August 3 and 24, and August 31 and September 28.

Distinct spat settlement events in July and August were observed at Horsehead, but not at Wreck Shoal in 1987 (unpublished V.I.M.S. monitoring reports and Tables 1 and 2). By contrast, both sites exhibited July and August settlements in 1988 with the earlier event being atypical of the river-wide mean value for the James River. Cumulative values of all stations (see Fig. 1) at each sampling period indicate that overall settlement was higher throughout 1987 than 1988, with highest values occurring between July 11 and August 21, 1987 and settlement continuing at decreased levels through September 25, 1987. Horsehead and Wreck Shoal were the only stations in the James River that showed major spatfall peaks in July 1988 although high values were observed at most of the stations in August 1988. Settlement events were observed both upstream and downstream of the sampling sites in September 1988 resulting in modest river-wide values despite low values at Horsehead and Wreck Shoal.

Diver collections in September 1988 (Table 3) contained oysters of all size classes between 10 and 100 mm height with largest numbers in the 30–39.9 and 50–69.9 size classes; however, individuals of size less than 50 mm constituted only 11.3% of the biomass estimated as wet tissue.

DISCUSSION

The James River has long been a focal point of the Virginia oyster industry, providing settlements of oysters on an annual basis (Haven and Fritz 1985). Small "seed" oysters were removed from the James in vast quantities for transplant and growth to market size (now >63 mm in height) at locations throughout the Virginia portion of the Chesapeake Bay. Continuing encroachment of P. marinus and H. nelsoni into the James in the past three decades has decimated oyster reefs below Wreck Shoal (Fig. 1) with associated loss of spawning oysters (see comments in Ruzecki and Hargis 1989). This encroachment, together with a gyre-like circulation in the lower James (Ruzecki and Hargis 1989, Mann 1988), and a lack of significant oyster resources in the lower Chesapeake Bay adjacent to the James, suggests that the James River oyster resource is now an isolated, self-sustaining population and that the brood stock for this population probably resides upriver of Wreck Shoal in a salinity environment not conducive to high prevalence of P. marinus and H. nelsoni. Oyster fecundity data from our stations in the James River, including Wreck Shoal and Horsehead, during the summer of 1986 illustrate a decrease in mean fecundity in upstream collections with lowest values at Horsehead (Cox and Mann, 1992). Mean weekly values extracted from Cox and Mann (1992) and presented in Figure 2 illustrate a trend of decreasing fecundity in oysters in the Wreck Shoal-Horsehead region between 1986 and 1988.

There are marked quantitative differences in fecundity between 1987 collections from Wreck Shoal and 1988 collections from Horsehead. These are single year and site observations, therefore we cannot definitively state whether the differences are site related, reflect interannual variation, or a progressive detrimental effect of H. nelsoni and P. marinus. Settlement data (Tables 1 and

TABLE 3.

Cumulative size class distribution data of Horsehead oysters from twelve quadrat collections made on September 22, 1988.

Height (mm)	n	w (gm)	W (gm)	%
<9.9	0			0
10–19.9	10	0.53	5.3	0.1
20–29.9	104	0.77	80.1	1.3
30–39.9	246	1.29	317.3	5.2
40–49.9	129	2.20	283.8	4.7
50–59.9	233	4.08	950.6	15.6
60–69.9	320	6.36	2035.2	33.4
70–79.9	171	9.29	1588.6	26.0
80–89.9	58	12.02	697.2	11.4
90–99.9	12	12.02	144.2	2.4
TOTAL	1283		6102.3	
Mean m^{-2}	107		508.5	
Total	6.45×10^7		3.07×10^5	

No animals <9.9 mm were recorded. n is the cumulative number of oysters in each size class within all 12 quadrats of 1 m^2 each. w is the mean wet tissue weight per individual (data from Haven and Morales-Alamo, unpublished studies of oysters collected from Horsehead in July–August, 1979). Size classes from >80 mm use the same value of w. W = n × w. % is the percentage of the total biomass (as wet tissue weight) in the size class. Total values are based on the area estimate of 603,062 m (Haven and Whitcomb 1973).
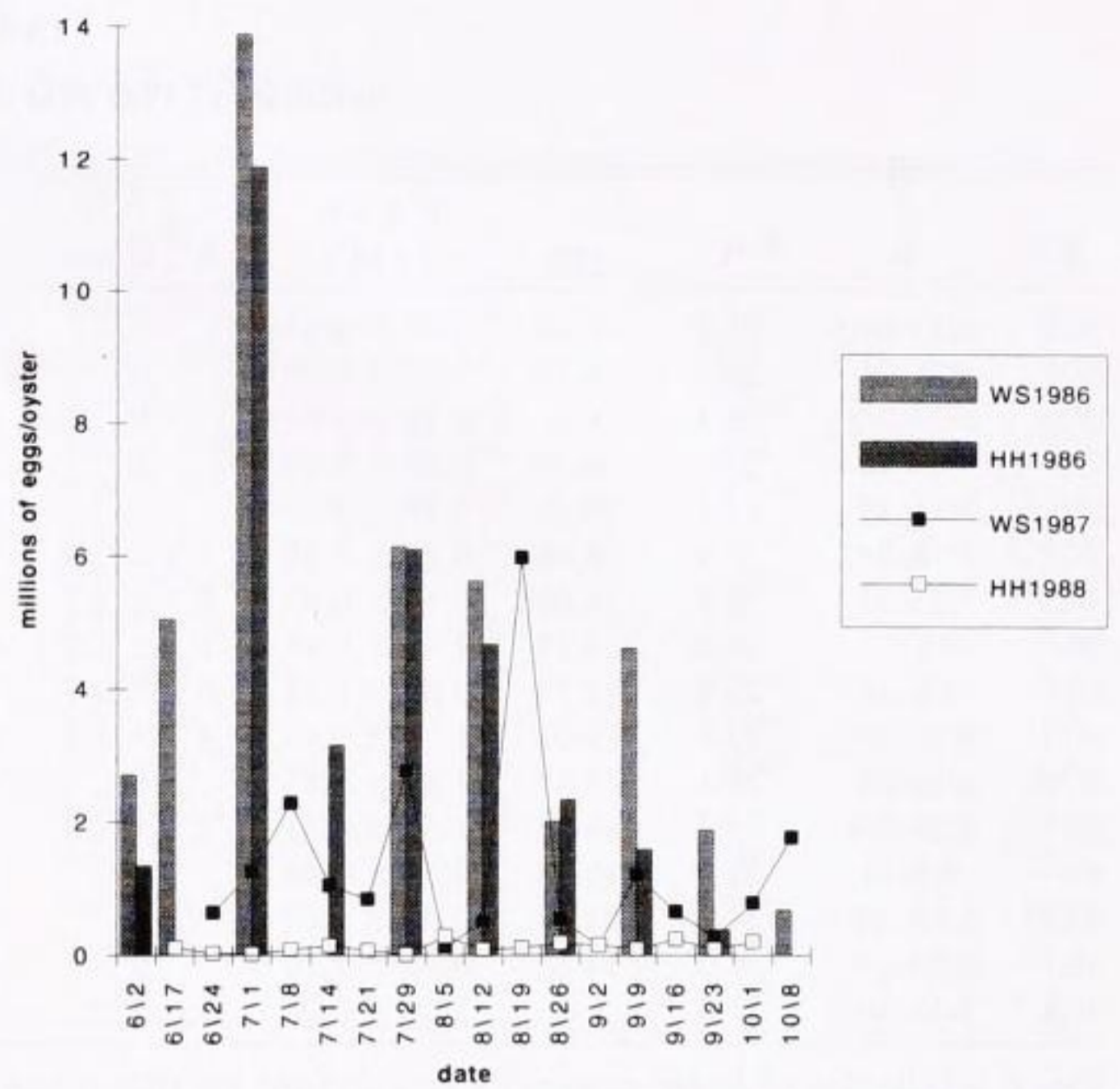

Figure 2. Fecundity of oysters at Wreck Shoal and Horsehead, James River, 1986–1988. 1986 data (bars) are recalculated from Cox and Mann (1992). 1987 and 1988 data (connected symbols) are from Tables 1 and 2 of this study.

2) show a marked decrease in settlement in 1988 coincident with low river flow, unusually high mid-summer salinities in the upper river, and a general upstream movement of the boundary of infective activity (Dr. E. M. Burreson, Virginia Institute of Marine Science, unpublished data). *H. nelsoni* has been shown to significantly reduce fecundity in *Crassostrea virginica* (Barber et al. 1988), and while it was recorded at Wreck Shoal it was very infrequent at Horsehead, typically less than 5% of animals examined, and then at low intensity. *P. marinus* was both more abundant and intense than *H. nelsoni* at Horsehead. The quantitative impact of *P. marinus* on fecundity is poorly understood, but the current observations suggest this as a subject worthy of further examination.

There was no consistent relationship between egg viability and fecundity estimates in 1988 collections from Horsehead (Table 2). The observed coincidence of high fecundity and low viability may be caused by overestimate of the former through counting of immature eggs, that is a methodological error, or production of uniformly inadequate eggs caused by physiological or nutritional stress of the adults (Gallager and Mann 1986, Gallager et al. 1986). Fecunity values alone may have limited value as indicators of reproductive potential in a location of consistently low salinity, such as Horsehead. Concurrent direct examination of other indicators, such as egg viability, would appear advisable. Cox and Mann (1992) present estimates of temporal variation in fecundity at four stations in the James River, including Wreck Shoal and Horsehead, during the summer of 1986 for oysters in the size range of 22 to 122 mm, with the majority between 30 and 105 mm. Oysters in 1986 Horsehead collections were more abundant but also smaller than at other stations. They also included individuals that were smaller than the size range of 55.5 to 88.5 mm used in the present study for GVF estimates for 1988 Horsehead collections. Observed differences in percentage of female oysters present may have been related to this size difference. In 1986

collections, male oysters were consistently more abundant than females at all stations. With the exception of July 16–30 collections in 1987, Wreck Shoal oysters also exhibited a preponderance of males (Table 1). By contrast, 1988 collections at Horsehead approached sexual parity throughout most of the study period.

GVF measurements were made on histological preparations of female oysters collected from Horsehead in 1965 and 1970 for disease diagnosis and stored in the VIMS histology archives. Measurements were also made on oysters collected in 1984 for evaluation of their gametogenic condition (Morales-Alamo and Mann 1989). Individual oyster size data were not available for these preparations; however, all oysters collected for these studies exceeded the effective market size of 75 mm height at the time of collection. Historical trends in gonad volume fraction values obtained from Horsehead female oysters collected in July and August of earlier years were significantly higher (P < 0.05) than those recorded in 1988. Mean GVF values in July and August 1988 were less than 0.60, compared to values of 0.80 (n = 13) and 0.76 (n = 15) for the same months in 1965, 0.77 (n = 9) for July 1970, and 0.73 (n = 29) and 0.88 (n = 30) for July and August of 1984.

A generalized trend with time was common to weekly 1988 values for fecundity, egg size, GVF and gonad width, and suggested a minor peak in gametogenic activity in June followed by two major peaks in the first half of both July and August. The major peaks were apparently followed by spawning events in the second half of July and August respectively. Settlement data (Table 2) illustrates settlement at all sites throughout the lower James River including Wreck Shoal and Horsehead. Asynchronous spatial settlement has been observed (Haven and Fritz, 1985); however, the synchrony of spawning and settlement events are unresolved. Settlement events exhibited two predominant peaks in mid July and August, and may be associated after a reasonable time interval to represent larval development, with the June and mid July spawnings. Likewise, minor settlement events at Horsehead between August 31 and September 14 could be associated with

August spawning. The quantitative and temporal contribution to the observed settlement of spawnings originating from oysters at locations in the James other than Horsehead Reef is unknown.

Spawning activity could not be related to specific conditions of temperature or salinity. Cox and Mann (1992) reported temporal changes in fecundity as indicators of spawning activity at four stations in 1986, using decreases in mean fecundity in successive samplings as indicators of spawning and variability about the mean as an indicator of synchrony within the population. Spawning at Wreck Shoal began July 1 and July 29 in 1986, with minor indication of a second spawning event in Wreck Shoal oysters at the beginning of September. Spawnings in 1986 occurred during periods of increasing temperature and salinity; however, this pattern was not consistent for 1987 spawnings where decreases in mean fecundity were recorded after collections on July 9, July 30, August 20 (only one individual), and September 10. A marked decrease in standard error values on July 16 and August 6 support spawnings immediately prior to these dates. There was a mass spawning at Horsehead at the beginning of July 1986 concurrent with a marked increase in both temperature and salinity. A second increase in fecundity in late July was followed by a protracted spawning which persisted into September as both temperature and salinity decreased from maximum summer values. All 1988 spawnings occurred during periods of increasing temperature, but were inconsistent with respect to salinity change (Table 2). There is an increasing volume of literature relating spawning activity of bivalves to food availability (Nelson 1955, 1957, Breese and Robinson 1981, Starr et al. 1990). We do not, however, have a detailed time course of data describing such changes in the James River and are therefore unable to investigate this possibility in the present study.

Fecundity estimates for 1988 Horsehead collections (Table 2) were made from animals in the size range 55.5 to 88.5 mm. Using size distribution data for September 1988 from Table 3 and including all individuals >60 mm and one half of the individuals in the 50–59.9 mm size class results in an estimated density of 56.5 oysters m^{-2}. This represents 80.1% of the estimated biomass. The product of areal density, reef area (603,062 m^2 from Haven and Whitcomb, 1983), and egg production corrected for viability and sex ratio (mean value of $[F \times V''.10^{-3} \times R.10^{-2}]$ from Table 2 for the six occasions when viable eggs were found), provides an estimate of first-shelled veliger larvae production from Horsehead, that is $0.98 \times 10^4 \, m^{-2}$ or 5.90×10^9 in total, during the summer of 1988. This value assumes a single spawning. In comparison it is notable that survival to **all** size classes >10 mm, representing a number of years of cumulative recruitment, is 107 m^{-2} (Table 3). The fate of larvae originating at Horsehead is unknown; they contribute to the pool which supplies the James River in total. While many in this pool are undoubtedly lost to tidal flushing and mortality, the remainder represent the primary larval supply for settlement in the James River. Cumulative estimates of seasonal settlement by station (data recalculated from Tables 1 and 2, not shown) indicate that Horsehead sustained lower settlement than other stations in the James, an observation consistent with previous observations for the period 1963–1980 (Haven and Fritz 1985, Table 4). Even when considering this situation a simple comparison of areal larval production with juvenile survival illustrates the enormous numerical losses that must occur during the early life history of oysters in the James. Roegner (1989) also observed high mortalities in early post-settlement stages of oysters which, after settling on prepared substrates in the laboratory, were immediately transferred to field locations at various intertidal exposures. In all subtidal exposures mortality approached or usually exceeded 90%, often much higher, within four weeks after settlement. Estimates of areal density of oysters on other reefs in the James, together with total reef area remaining unaffected by disease are not available; however, the present focus of the commercial fishing industry on Horsehead reef supports the argument that this location may represent a substantial proportion of the total broodstock remaining in the James at this time. Prudent conservation of this resource is warranted.

Estimation of fecundity and egg viability values have clear value in both management of commercial fisheries and ecological modelling. To date, efforts to construct quantitative life cycle budgets for oyster communities which include the larval phase have been limited for obvious methodological problems which, as illustrated here, have not yet been pursued to submission. These are, however, research areas that must be addressed if we are to use current modelling techniques in practical application.

ACKNOWLEDGMENTS

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