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# OYSTER REEF BROODSTOCK ENHANCEMENT IN THE GREAT WICOMICO RIVER, VIRGINIA

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***ABSTRACT*** The Great Wicomico River is a small, trap-type estuary on the western shore of the Chesapeake Bay that once supported substantial oyster populations. These populations were essentially eliminated by the combined effects of Tropical Storm Agnes in 1972, and subsequent disease mortalities related to *Perkinsus marinus* and *Haplosporidium nelsoni.* Oyster broodstock enhancement was initiated in June 1996 by the construction of a three-dimensional intertidal reef with oyster shell, followed by the "seeding," in December 1996, of that reef with high densities of large oysters from disease-challenged populations in Pocomoke and Tangier Sound. Calculations of estimated fecundity of the reef population suggest that oyster egg production from this source is within an order of magnitude of total egg production in the Great Wicomico River prior to Tropical Storm Agnes. Field studies in 1997 indicate spawning by reef oysters from July through September. *P. marinus* prevalence increased from 32% in June to 100% in July, whereas intensity increased from June to September; *H. nelsoni* was absent. Plankton tows recorded oyster larval concentrations as high of 37,362 ± 4,380 $m^{-3}$ on June 23. Such values are orders of magnitude higher than those typically recorded in Virginia subestuaries of the Chesapeake Bay in the past three decades, and lend support to a premise that aggregating large oysters may increase fertilization efficiency. Drifter studies suggest strong local retention of larvae, a suggestion reinforced by marked increases in local oyster spatfall on both shellstring collectors and bottom substrate compared with years prior to 1997. In locations where local circulation promotes larval retention, the combination of reef construction with broodstock enhancement may provide an accelerated method for oyster population restoration.

***KEY WORDS:*** Oyster, *Crassostrea virginica,* Great Wicomico River, reefs, fecundity, larvae, oyster settlement

## INTRODUCTION

The Eastern oyster, *Crassostrea virginica* (Gmelin), plays an important ecological role in the Chesapeake Bay and its tributaries as well as being the focus of a substantial commercial fishery. Oyster reefs developed in recent geological time as the current Chesapeake Bay was inundated by rising sea level. By early Colonial times, oyster reefs had become significant geological and biological features of the Bay. Intensive exploitation since Colonial times, combined with more recent impacts of two protistan parasites, *Perkinsus marinus* ("Dermo") and *Haplosporidium nelsoni* ("MSX"), have lead to the degradation of these reefs such that only two-dimensional "footprints" of these former reefs remain. Today, these "footprints" maintain drastically reduced oyster populations. The Virginia Marine Resources Commission (VMRC) supports an extensive replenishment program throughout most of its portion of the Bay. Traditional replenishment programs have focused on spreading thin veneers of shell substrate for larval settlement over coastal and estuarine bottoms. The purpose of this practice is to provide a suitable substrate for settlement at minimum cost. Ideally, the end product is the retrieval of seed or market-size oysters from these shell "plants"; however, these thin, two-dimensional carpets bear little resemblance to the intricate, three-dimensional reefs that once supported a large oyster population.

More recent replenishment programs have focused on the construction of three-dimensional reefs that resemble more closely what was found in Colonial times. Since 1993, reefs have been constructed in the Piankatank, Great Wicomico, Coan, Yeocomico, and James Rivers in the Virginia portion of the Chesapeake Bay, and Lynnhaven Bay on the southern side of the Bay mouth. These reefs are built on the "footprints" of former reefs and consist of several mounds of shell that protrude out of the water at low tide (e.g., Bartol and Mann, 1997). Reef communities have been allowed to mature naturally with no addition of broodstock oysters to the reef based on the premise that oysters would recruit to the reef from the plankton, and because there was no resident population of disease-infected oysters, would develop as a predominantly disease-free population. This was not found to be the case on an artificial reef built in the Piankatank River, Virginia (Mann et al. 1996, Mann and Wesson 1996). Endemic diseases did become established in the reef populations; however, the vertical relief of the reefs enhanced growth to such an extent that the oysters grew larger and faster than on adjacent "flat" oyster reefs. From 1993 to 1996 oyster populations on the Piankatank River reef developed to densities of 50–70 oysters $m^{-2}$ (Mann and Wesson unpublished data). This compares with densities of 200–350 $m^{-2}$ on the most commercially productive reefs (flat) in the James River system (Mann and Wesson unpublished data, Mann and Evans 1998). The disparity in these values suggests that development of very dense and stable oyster communities on constructed reefs is a long-term event that is delayed in regions that suffer poor natural recruitment, and may be accelerated with an initial stocking of broodstock.

The current study describes the impact on local plankton communities and oyster settlement of an artificial reef, constructed in 1996–1997 in the Great Wicomico River (Fig. 1) that was initially "seeded" with reproductively capable oyster populations. Specifically, the study sought to determine temporal spawning patterns of "seeded" oysters, estimate the fecundity and larval production from oysters on the reef, and examine subsequent larval abundance, distribution, and settlement in relation to local circulation patterns.

## METHODS AND MATERIALS

### *Data Collected as Part of Long-Term Monitoring*

In order to describe the ecological impact of the reef, it is necessary to present 1997 data in the context of a brief historical description of oyster populations. Original surveys of the limits of oyster distribution in the Great Wicomico River were provided by Baylor (1896) and subsequently revised by Haven et al. (1981). Temporal (intra- and inter-annual) description of oyster settlement (spatfall), population density, and demographics are available from continuing Virginia Institute of Marine Science (VIMS) stock monitoring programs.

The spatfall survey has been completed annually from 1965 to the present. The collectors used to monitor spatfall were oyster-shellstrings, which consist of 12 oyster shells of similar size (about 76 mm, max. dimension) drilled through the center and strung (inside of shell down) on heavy gauge wire. Shellstrings were hung 0.5 m off the bottom at each station. Up to 16 stations have been used at various times throughout the history of the spatfall surveys; however, for consistency between years, this study reports only the six stations (see Fig. 1) that have been used yearly since 1965. Shellstrings were replaced after a 1-week exposure (with occasional deviations) from June through September, and the number of spat that attached to the smooth underside of the middle 10 shells were counted with the aid of a dissecting microscope.

The fall dredge survey provides information about spatfall and recruitment, summer mortality, and inter-annual changes in abundance of seed and market-size oysters. This survey has been completed yearly from 1971 to the present, excluding 1974–1976. Figure 1 shows the geographical locations of the bars sampled in the Great Wicomico River during this time. As with the shellstring data, only the most consistently sampled stations were used in the analysis. Three stations (Fleet Point, Whaley East, and Haynie Point) have been sampled since 1986. Analysis was limited to these three stations. Three to four 0.5 bushel samples of bottom material were taken at each bar using a 24-inch dredge having 4-inch teeth. For each sample the following were determined: number of market-size oysters (>76 mm, max. dimension), number of small oysters (below market size and yearlings), and the number of spat (young of the year oysters). In the case where only 0.5 bushels were counted, they were standardized to one bushel by doubling the counts. In the fall of 1995 and 1997, a collaborative survey effort between VMRC and VIMS resulted in a formal stock assessment on the oysters in the Great Wicomico River using patent tongs (Chai et al. 1992). The five oyster reefs that were sampled in the Great Wicomico River in 1995 and 1997 are shown in Figure 1. For each reef a uniform grid was generated over a current reef boundary map. Each grid location had a reference that could be located electronically by LORAN from the research vessel. Grid references were assigned a sampling order from a random number table to generate a randomized sampling grid. Samples were collected using hydraulic patent tongs with an opening of 1 $m^2$. All of the retained material was washed, and counts of live oysters as spat (young of the year), small oysters (<76 mm, max. dimension), and market oysters (>76 mm, max. dimension) were taken. Adequacy of sampling was assured using guidelines of Bros and Cowell (1987), as described in Mann and Evans (1998).

### *Estimation of Historical Egg Production, Fertilization and Embryo Production*

To place in context the impact of adding broodstock, estimates of historical egg production when adult oysters were still abundant

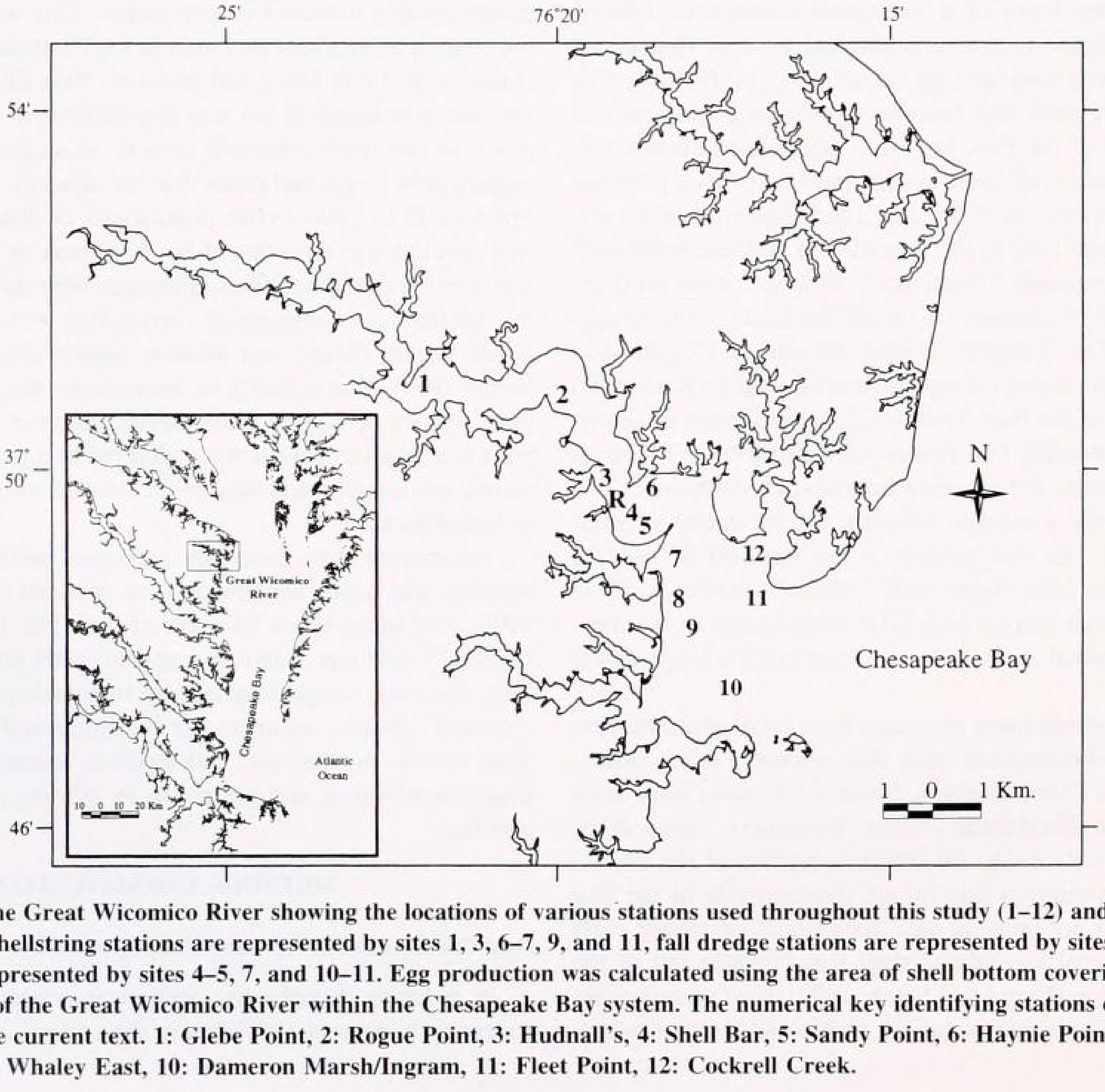

**Figure 1. Map of the Great Wicomico River showing the locations of various stations used throughout this study (1–12) and the location of the artificial reef (R). Shellstring stations are represented by sites 1, 3, 6–7, 9, and 11, fall dredge stations are represented by sites 6, 7, and 9, patent tong stations are represented by sites 4–5, 7, and 10–11. Egg production was calculated using the area of shell bottom covering sites 2–12. Inset shows the location of the Great Wicomico River within the Chesapeake Bay system. The numerical key identifying stations corresponds to that used throughout the current text. 1: Glebe Point, 2: Rogue Point, 3: Hudnall's, 4: Shell Bar, 5: Sandy Point, 6: Haynie Point, 7: Cranes Creek, 8: Whaley West, 9: Whaley East, 10: Dameron Marsh/Ingram, 11: Fleet Point, 12: Cockrell Creek.**

in the river are required. Quantitative historical stock assessment data for the Great Wicomico are lacking, but there are current data for extant reefs in the James River in similar salinity regimes which have similar qualitative (based on dredge survey data) population demographics. A combination of revised Baylor survey data (Haven et al. 1981) of reef area in the Great Wicomico (Fig. 1) and current James River quantitative stock assessment data (Mann and Wesson unpublished, Mann and Evans 1998) were used to estimate historical oyster demographics in the Great Wicomico (see Table 1). Since salinity plays a role in reproductive success, it was necessary for the reefs being compared to have similar salinity regimes. Reefs in three salinity regimes (8.5, 10.5, and 13.5 ppt, see Mann and Evans 1998) in the James River were used in the calculation. Egg production per unit area for each reef in the James River, based on the appropriate size frequency distribution, was estimated by methods described in detail in Mann and Evans (1998) using size-specific fecundity taken from Thompson et al. (1996), parity in sex ratio as suggested by Cox and Mann (1992), and density-dependent fertilization efficiency as described by Levitan (1991).

### *1997 Field Studies*

The location selected for the study was Shell Bar Reef in the Great Wicomico River, Virginia (location R in Fig. 1). The reef was constructed in June of 1996 by deploying old oyster shells from a barge with a crane into a series of intertidal structures approximately 215 m long and 18 m wide. Broodstock oysters from the Tangier and Pocomoke Sound regions were planted on the reef in December of 1996. Oyster standing stock and density was obtained from VMRC records (Olsen and Wesson 1997). According to these records, 2,281 bushels of oysters were planted on the 3,900 $m^2$ reef in December of 1996. Estimating 500 oysters per bushel (Wesson, personal communication), density of broodstock oysters on the reef was approximately 300 $m^{-2}$. Oysters surviving as sparsely distributed individuals in many regions of the Bay are continually exposed to intense disease challenge and selection pressure. Consequently, they would be expected to have higher resistance to disease than low salinity populations where intermittent disease pressure fails to eradicate genetically susceptible individuals, which then continue to breed with more resistant individuals and thus fail to promote the process of developing uniformly high resistance. Tangier and Pocomoke Sounds are locations where higher salinities (25–30 ppt) occur, but oyster densities are low ($<1$ $m^{-2}$), thus failing to maximize the fertilization efficiency. The intent of aggregating the few remaining oysters from disease endemic areas was to increase fertilization efficiency of freely released gametes.

Field studies were conducted biweekly from the 23rd of June through the 22nd of September 1997 (total of 8 field days). This time frame was chosen based on the historical timing of spat settlement in the Great Wicomico River system. To obtain a description of tidal patterns of circulation and larval abundance in the system, all sampling was effected over one complete tidal cycle (approximately 12 h). Surface temperature for all field studies was measured near the reef (Station N1; in Fig. 2) throughout the duration of the study. Temperature and salinity at the surface and bottom of the water column were obtained at three sites (Fig. 2) starting on July 28th (dates of collection coincided with the circulation study). Bottom water was collected using a Niskin bottle. Temperature was measured with an alcohol thermometer and salinity was measured with a refractometer.

### *Oyster Reproductive Biology and Disease Status*

Initial broodstock oyster size frequency on the reef was obtained by measuring 150 oysters collected with the aid of hand tongs. Total egg production on Shell Bar reef, after broodstock enhancement, was calculated using density and size frequency data, as described in detail in Mann and Evans (1998).

Temporal patterns of gametogenic development of the broodstock oysters on the reef was examined by collection, with hand

TABLE 1.

**Estimates of historical egg production in the Great Wicomico River using demographics obtained from analogous reefs in the James River in 1993.**

| Reef # | Reef Area $m^2$ | Egg Production $10^6$ $m^{-2}$ | Total # of Eggs * $10^{12}$ | Salinity to Estimate Fs | Fs | Ff | Corrected Production $10^6$ $m^{-2}$ | Corrected Total # of Eggs * $10^{12}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 29355 | 1160 | 34 | 8.5 | 0.09 | 0.16 | 16.8 | 0.49 |
| 2 | 23475 | 565 | 13 | 10.8 | 0.51 | 0.13 | 38.5 | 0.9 |
| 3 | 3636 | 565 | 2 | 10.8 | 0.51 | 0.13 | 38.5 | 0.14 |
| 4 | 29462 | 565 | 17 | 10.8 | 0.51 | 0.13 | 38.5 | 1.1 |
| 5 | 33261 | 565 | 19 | 10.8 | 0.51 | 0.13 | 38.5 | 1.3 |
| 6 | 83452 | 136.2 | 11 | 13.5 | 1 | 0.04 | 4.9 | 0.41 |
| 7 | 137735 | 136.2 | 19 | 13.5 | 1 | 0.04 | 4.9 | 0.68 |
| 8 | 251573 | 136.2 | 34 | 13.5 | 1 | 0.04 | 4.9 | 1.2 |
| 9 | 82723 | 136.2 | 11 | 13.5 | 1 | 0.04 | 4.9 | 0.41 |
| 10 | 22047 | 136.2 | 3 | 13.5 | 1 | 0.04 | 4.9 | 0.11 |
| 11 | 71929 | 136.2 | 10 | 13.5 | 1 | 0.04 | 4.9 | 0.35 |
| TOTAL | | | 173 | | | | | 7.1 |

See Mann and Evans (1998) for explanation of calculations.

Reef area—taken from the Great Wicomico Baylor survey data (Haven et al., 1978).

Egg production—calculated from size-specific fecundity of oysters in the James River (average of all reefs in the James River with the same salinity).

Total # of eggs—reef area in the Great Wicomico multiplied by egg production (based on James River demographics).

Fs—correction for salinity, based on reefs sharing similar salinities in both rivers.

Ff—correction for fertilization efficiency, based on densities found in the James River (also used for egg production calculations).

Corrected production—egg production/$m^2$ corrected for salinity, fertilization efficiency, and disease (production * Fd * Fs * Ff).

Corrected total # of eggs—corrected egg production multiplied by reef area (in the Great Wicomico).

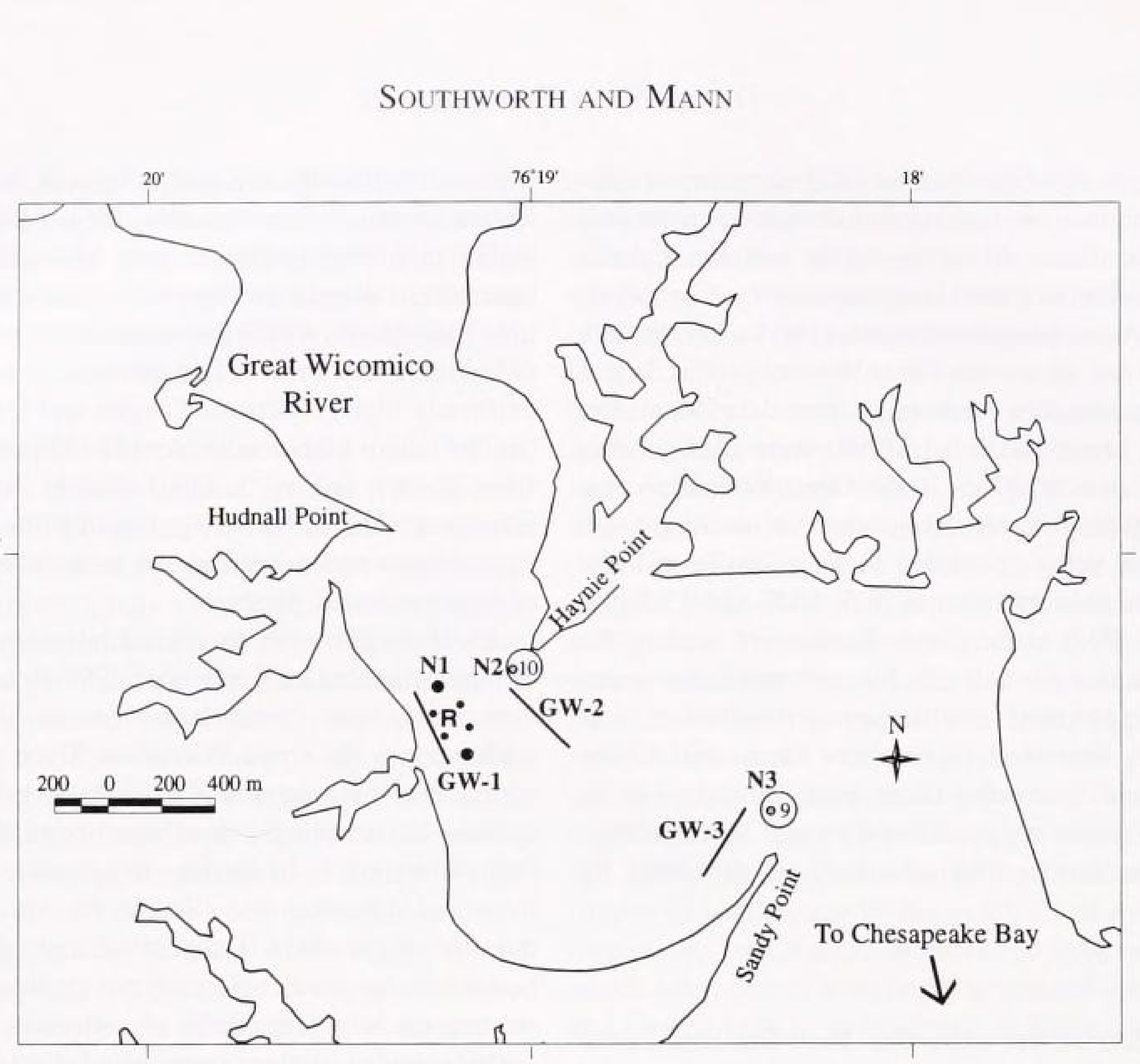

**Figure 2. Location of zooplankton (GW 1–3) samples and water samples (N 1–3) taken in the Great Wicomico River. R denotes the location of the reef; 9 & 10 mark the main channel in the river.**

tongs, of 25 oysters per sampling day for a total of 200 oysters. Sections of the gonad and visceral mass were removed and fixed in Bouin's solution. Following fixation, specimens were dehydrated in alcohol, cleared in xylene, and embedded in paraffin wax. Histological sections were cut at 7–10 μm, stained in Delafield's hematoxylin, and counterstained in eosin Y following the methodology of Humason (1962). Developmental stages were identified based on those originally described for *C. virginica* by Kennedy and Battle (1964) and for *C. gigas* by Mann (1979). Stages of gonadal development were defined as follows:

(1) *Inactive:* No evidence of the presence of follicles peripheral to the digestive gland. Sex is essentially indeterminable.

(2) *Early active:* **Male.** Many follicles filled primarily with spermatogonia and spermatocytes. No spermatozoa. **Female.** Eggs not well developed. A few nuclei in oocytes, but no nucleoli. Oocytes are still attached to the follicle wall.

(3) *Late active:* **Male.** Follicles predominately filled with spermatids. Characteristic swirling pattern of spermatozoa with tails oriented toward the center beginning to be evident, but follicle is not completely filled. **Female.** Some free oocytes. Most have distinct nuclei, with fewer than 50% having distinct nucleoli.

(4) *Ripe:* **Male.** Swirling of tails in the middle of the follicle. **Female.** Primarily free oocytes. Greater than 50% have a distinct nuclei and nucleoli. All of the oocytes are about the same size.

(5) *Spawning or spent:* **Male.** Most follicles are empty or partially so. Some phagocytes present. **Female.** Granular looking eggs (ameobocyte activity). Eggs of varying sizes that appear to be breaking down. Follicles are empty or partially so.

Monthly assays to determine *Perkinsus marinus* and *Haplosporidium nelsoni* (MSX) infections were effected using oysters collected for the reproductive development portion of the study. *Perkinsus* infection and prevalence were measured by Fluid Thioglycollate assay (Ray 1963). MSX infections were detected using paraffin histology, as in Burreson et al. (1988).

### *Plankton Studies*

A series of 36 zooplankton samples were taken on each sampling day (three replicates per site, per tidal stage). Samples were collected at three stations in the river (Fig. 2). Plankton samples at GW-1 describe larval abundance near the reef, GW-2 describes abundance in the main channel of the river, and GW-3 describes abundance near the sand spit at Sandy Point, a feature that affects and effects some local retention in the system. Samples were collected using a 0.3 m diameter, 3:1 aspect ratio zooplankton net (Sea Gear Corporation, Melbourne, FL). The filtering surface consisted of an 80 μm Nytex mesh cone attached to a PVC collection bucket lined with 80 μm mesh. The net was attached to a metal ring and towed by a three-point bridal system attached to the ring. The net was towed 0.05–0.10 m below the water surface at approximately 1.5 m $sec^{-1}$ for 3.25 min. The nets used were calibrated in a separate study following the same protocol (Harding and Mann, in review). Samples were taken over a full tidal cycle. All samples were immediately preserved in 95% ethanol.

Samples were split using a 0.5 L Folsom plankton splitter (Wilco Supply Company, Cass, MI). Final splits were filtered through a 400 μm Nytex mesh filter to remove large zooplankton that interfered with the counting. To ensure that no oyster larvae were lost in this process, samples were randomly chosen and counts were made before and after filtering. The difference between these counts was less than 1%. Non-enumerated splits, as well as the filtrate from the final splits, were archived. Counts of umbo stage oyster veligers (larvae) in each subsample were made with the aid of a dissecting scope. To verify adequate mixing (i.e., a homogenous mixture of larvae within the sample), both halves of the final split were counted, and coefficients of variation (CV) were calculated following Van Guelpin et al. (1982). Acceptable CVs for invertebrate samples range from 5 to 20%. Counting error of the total abundance of organisms within a sample was kept to 10% or less by ensuring (when possible) that at least 100 veligers were counted from each subsample. Total number of larvae per sample was obtained by multiplying the number of veligers in the split by the split number. The number of larvae per $m^3$ was then obtained by dividing the total number per sample by the volume of water filtered. The mean volume of water filtered, was determined to be 1.054 $m^3$ in a separate net calibration study (Harding and Mann, in review).

### *Circulation Studies*

Simple surface drogues (drifters) were constructed after the method of Davis et al. (1982) (Fig. 3). This design was used to ensure that the drifter was moved by the currents in the system with little input from the wind. The drifters were released at various sites around the reef and in the main channel of the river. The drifter locations were recorded approximately every hour using a hand held GPS system. The drifters were followed over one full tidal cycle. In the event that a drifter ran aground, it was repositioned to another location, with exact location depending on the stage of the tide. Throughout the course of the sampling season, a total of 23 drifter paths were obtained on 5 separate days. Of the 23 drifter tracks obtained, 6 were discarded because of multiple lost GPS points and 3 were discarded because of excessive stoppage (they ran aground at least three times). This left a total of 14 drifter tracks to be analyzed. Drifter time and location information was loaded into the Geographical Information System/ArcView computer program in the Coastal Inventory Program at VIMS. The drifter paths were then plotted in ArcView software and mean current speeds were estimated for each series of drifter recordings. These were then compared with predicted tidal flow for Sandy Point in the Great Wicomico River system (Tides and Currents for Windows, version 2.2, Nautical Software Inc.).

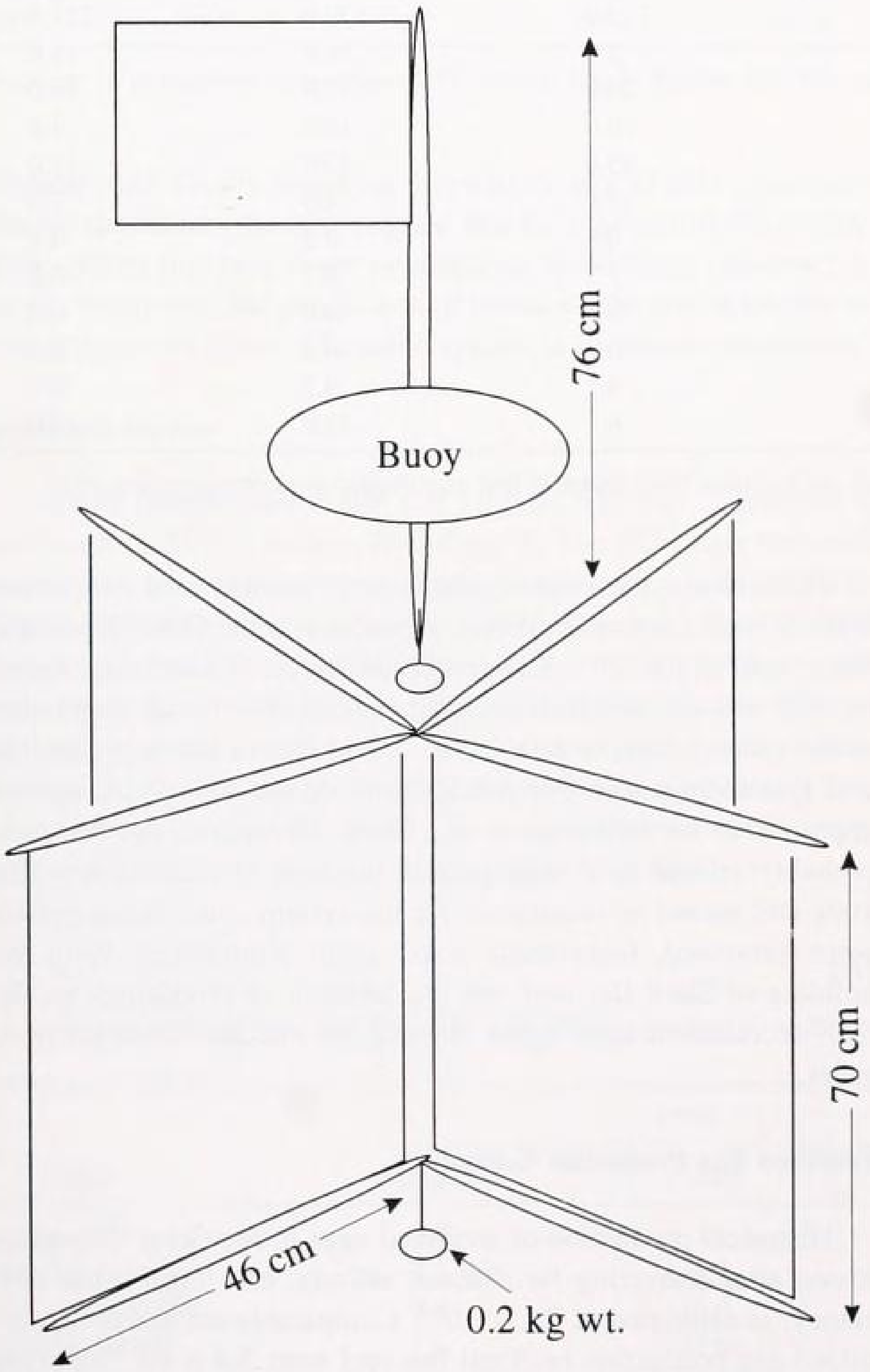

**Figure 3. Design of surface drogue (drifter) used in the circulation studies.**

## RESULTS

### *Data Collected as Part of Long-Term Monitoring*

Spatfall estimates obtained from the historical shellstring data are summarized in Table 2. From 1965 through 1971 spatfall in the Great Wicomico River was relatively high with mean weekly values (number of larval oysters physically adhering to the substrate) ranging from 1 to 494 spat/shellstring per week. In 1970 nearly all stations received a moderate (20–50 spat/shellstring) to heavy (>50 spat/shellstring) peak value and the settlement period extended over most of the summer–fall season. In 1971, no significant settlement occurred until late fall. In 1972, due to Tropical Storm Agnes, oyster settlement was at or near zero at all stations. This year marked the beginning of a major decline in spatfall in the river. The years 1973 through 1979 were characterized by a very light settlement, usually less than one spat/shellstring per week. Starting in 1980, settlement events again became more consistent (lasting throughout most of the season) and heavier (2–38 spat/shellstring per week). This increase in the number of spat in the 1980s coincided with a heavy private ''planting'' of a large number of small (seed) oysters on private lease grounds in the Great Wicomico; however, as these were harvested in the late 1980s and early 1990s, a further decline in the number of spat was observed. The latest signal in the river occurred during the 1997 setting season, after the artificial reef was built and stocked with broodstock oysters. In 1997, spatfall was recorded between the end of June and the beginning of September, with a peak set occurring in mid- to late July. During this peak settlement period, spatfall ranged from 0 to 29.3 spat/shellstring per week, with the most intense sets occurring upstream or immediately adjacent to the reef (Glebe Point, Hudnall, Haynie Point, and on the reef itself; Fig. 1). Mean spatfall estimates for 1997 from shellstring data ranged from 1.4 to 43 spat/shellstring per week.

The fall dredge data taken from the VIMS database (VIMS archive) can be summarized as follows (Table 3): Between the years 1971 to 1987, the number of small oysters ranged from 90 to over 600 per bushel for all three stations (Fig. 1). During this time the number of spat per bushel ranged from a low of 0 in 1972 (the year of Hurricane Agnes) to a high of 1,900 per bushel in 1987. This year marked the beginning of a slow decrease in the number of oysters in the system. For the past 3 ys, numbers of small oysters have ranged from 31 to 126 per bushel. 1987 also marked the beginning of essentially the absence of market-size oysters in the system. Before 1987 there were comparatively more market oysters (0–128) per bushel than after (0–22 oysters per bushel).

TABLE 2.

Spatfall survey data from shellstrings reported as average spat/shellstring per week.

| Year | Dameron Marsh (10) | Fleet Point (11) | Cranes Creek (7) | Haynie Point (6) | Hudnall (3) | Glebe Point (1) |
|---|---|---|---|---|---|---|
| 1965 | 75.0 | NS | 524.0 | 253.0 | 300.0 | 98.0 |
| 1966 | NS | NS | NS | NS | 155.0 | 127.0 |
| 1967 | 8.5 | NS | 20.0 | 61.0 | 86.0 | 174.0 |
| 1968 | 49.0 | NS | 257.0 | 61.0 | 227.0 | 182.0 |
| 1969 | 8.7 | 24.0 | 45.0 | 112.0 | 89.0 | 36.0 |
| 1970 | 36.0 | 21.0 | 100.0 | 306.0 | 302.0 | 494.0 |
| 1971 | 2.6 | 0.9 | 2.4 | 4.4 | 9.6 | 24.0 |
| 1972 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 1.8 |
| 1973 | 2.2 | 1.7 | 0.5 | 1.9 | 0.1 | 0.7 |
| 1974 | 0.1 | 4.1 | 0.8 | 1.0 | 1.9 | 7.3 |
| 1975 | 0.1 | 1.0 | 0.4 | 1.1 | 0.1 | 0.2 |
| 1976 | 0.1 | 0.0 | 0.1 | 0.1 | 0.6 | 0.8 |
| 1977 | 0.6 | 1.4 | 1.6 | 0.8 | 2.7 | 0.8 |
| 1978 | 0.3 | 1.7 | 0.1 | 0.7 | 0.9 | 1.4 |
| 1979 | 0.6 | 0.9 | 0.1 | 1.3 | 8.3 | 4.1 |
| 1980 | 9.1 | 7.8 | 9.9 | 8.5 | 38.0 | 7.0 |
| 1981 | 2.3 | 1.5 | 3.8 | 17.3 | 21.0 | 83.0 |
| 1982 | 23.0 | 36.0 | 46.0 | 54.0 | 89.0 | 228.0 |
| 1983 | 6.8 | 23.0 | 3.8 | 6.9 | 8.6 | 0.4 |
| 1984 | 0.5 | 1.0 | 0.7 | 0.4 | 1.9 | 1.3 |
| 1985 | 5.4 | 49.0 | 4.5 | 4.7 | 8.9 | 6.8 |
| 1986 | 25.0 | 24.0 | 71.0 | 113.0 | 139.0 | 227.0 |
| 1987 | 17.0 | 110.0 | 17.0 | 7.6 | 26.0 | 13.0 |
| 1988 | 22.0 | 5.1 | 8.6 | 24.0 | 32.0 | 16.0 |
| 1989 | 3.2 | 4.9 | 5.2 | 10.0 | 16.0 | 4.8 |
| 1990 | 18.0 | 10.0 | 23.0 | 43.0 | 59.0 | 12.0 |
| 1991 | 8.5 | 5.2 | 6.6 | 10.0 | 4.0 | 2.3 |
| 1992 | 0.5 | 4.2 | 0.2 | 0.8 | 0.8 | 0.8 |
| 1993 | 0.6 | 1.7 | 0.1 | 1.2 | 0.7 | 0.2 |
| 1994 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1995 | 0.0 | 1.3 | 0.2 | 0.3 | 0.1 | 0.9 |
| 1996 | 2.9 | 2.9 | 2.3 | 4.1 | 0.2 | 0.7 |
| 1997 | 1.9 | 4.9 | 1.4 | 6.1 | 43.0 | 24.0 |

Numbers in parenthesis correspond with the station IDs in Figure 1. NS means no samples were taken at that site during the corresponding year.

The number of spat recorded per bushel also started to decrease in the late 1980s. For the 5 years prior to the building of the reef, mean spatfall values were about 55 spat per bushel, whereas a mean of 155 spat per bushel was recorded for the 1997 fall survey.

Patent tong surveys revealed that in 1995 the number of market oysters ranged from 0.3 $m^{-2}$ at Fleet Point to 1.6 $m^{-2}$ at Sandy Point (see Fig. 1 for location of patent tong sites and Table 4 for summary of survey data). The number of small oysters ranged from 4 to 22 oysters $m^{-2}$ with the lowest densities at Shell Bar and Fleet Point and the highest at Sandy Point. The number of spat $m^{-2}$ ranged from 6.5 at Cranes Creek to 13.4 at Fleet Point. The overall density for the five reefs combined was 0.7 market oysters $m^{-2}$, 10.2, small oysters $m^{-2}$, and 9.7 spat $m^{-2}$. Whereas the mean number of market (1–3 $m^{-2}$) and small (9–37 $m^{-2}$) oysters recorded in 1997 were similar to those recorded in 1995, the number of spat were considerably higher in 1997. Spatfall estimates ranged from 4.6 $m^{-2}$ at Ingram and Fleet Point to 103 $m^{-2}$ at Shell Bar. The spatial pattern reflected that observed in shellstring studies with intense settlement upstream of the sand spit, and near or adjacent to the artificial oyster reef, and a trend of decreasing settlement in a downstream direction. Overall values represent a threefold increase in density of spat from 1995 to 1997.

Collectively, these survey data can be summarized as follows: Oysters were present in relative abundance in the Great Wicomico River until about 1971. The combined effects of Hurricane Agnes in 1971 and disease decimated the natural broodstock population in the system. This in turn led to a decrease in larval production and spat recruitment. For a brief time during the 1980s, oysters appeared to be returning to the Great Wicomico, but this was probably related to a large private planting of seed oysters that grew and served as broodstock for the system. Once these oysters were harvested, recruitment once again plummeted. With the building of Shell Bar reef and the addition of broodstock on the reef, recruitment once again showed an increase from previous years.

### Fertilized Egg Production Estimates

Historical production of fertilized eggs in the Great Wicomico River, after correcting for disease, salinity, and fertilization efficiency, is estimated at $7.1 \times 10^{12}$. Comparable estimates for fertilized egg production on Shell Bar reef were $5.4 \times 10^{12}$ embryos using size frequency distribution data from Figure 4, a salinity value of 10.8 obtained from field observations, and a fertilization

TABLE 3.

Dredge survey data reported as number of market (>76 mm), small (<76 mm), and spat per bushel.

| Year | Fleet Point (7) | | | Whaley East (9) | | | Haynie Point (6) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Market | Small | Spat | Market | Small | Spat | Market | Small | Spat |
| 1971 | NS | NS | NS | NS | NS | NS | 0.0 | 648.0 | 68.0 |
| 1972 | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| 1973 | 128.0 | 260.0 | 0.0 | NS | NS | NS | 64.0 | 246.0 | 2.0 |
| 1977 | 60.0 | 88.0 | 82.0 | 48.0 | 182.0 | 24.0 | 38.0 | 112.0 | 156.0 |
| 1978 | 88.0 | 152.0 | 10.0 | 58.0 | 138.0 | 46.0 | 58.0 | 214.0 | 44.0 |
| 1979 | 32.0 | 138.0 | 430.0 | NS | NS | NS | 32.0 | 88.0 | 220.0 |
| 1980 | 80.0 | 344.0 | 448.0 | 72.0 | 368.0 | 72.0 | 64.0 | 180.0 | 98.0 |
| 1981 | 82.0 | 502.0 | 286.0 | 116.0 | 544.0 | 306.0 | 44.0 | 356.0 | 442.0 |
| 1982 | 30.0 | 414.0 | 1198.0 | 36.0 | 394.0 | 432.0 | 34.0 | 292.0 | 818.0 |
| 1983 | 28.0 | 476.0 | 124.0 | 32.0 | 188.0 | 74.0 | 10.0 | 208.0 | 78.0 |
| 1984 | 32.0 | 544.0 | 22.0 | 24.0 | 546.0 | 24.0 | 40.0 | 178.0 | 30.0 |
| 1985 | 76.0 | 366.0 | 1436.0 | 126.0 | 350.0 | 566.0 | 36.0 | 584.0 | 536.0 |
| 1986 | 9.5 | 154.0 | 1114.0 | 14.0 | 212.0 | 504.0 | 15.0 | 504.0 | 638.0 |
| 1987 | 0.0 | 107.0 | 1911.0 | 4.7 | 281.0 | 337.0 | 0.7 | 271.0 | 501.0 |
| 1988 | 0.0 | 145.0 | 134.0 | 0.0 | 3.0 | 179.0 | 1.3 | 228.0 | 467.0 |
| 1989 | 4.0 | 207.0 | 300.0 | 0.0 | 174.0 | 151.0 | 1.3 | 225.0 | 182.0 |
| 1990 | 11.0 | 297.0 | 473.0 | 0.7 | 275.0 | 44.0 | 0.7 | 141.0 | 397.0 |
| 1991 | 9.3 | 317.0 | 217.0 | 0.7 | 229.0 | 147.0 | 6.0 | 176.0 | 328.0 |
| 1992 | 0.0 | 33.0 | 51.0 | 0.0 | 18.0 | 45.0 | 0.0 | 21.0 | 228.0 |
| 1993 | 2.7 | 67.0 | 62.0 | 2.0 | 189.0 | 47.0 | 0.0 | 91.0 | 147.0 |
| 1994 | 5.3 | 150.0 | 7.0 | 2.5 | 114.0 | 6.0 | 0.7 | 153.0 | 7.0 |
| 1995 | 5.3 | 31.0 | 51.0 | 2.5 | 48.0 | 3.0 | 1.0 | 31.0 | 113.0 |
| 1996 | 6.0 | 123.0 | 13.0 | 8.0 | 73.0 | 21.0 | 4.5 | 126.0 | 19.0 |
| 1997 | 18.0 | 96.0 | 47.0 | 22.0 | 71.0 | 107.0 | 10.0 | 115.0 | 312.0 |

Numbers in parenthesis correspond with station IDs in Figure one. NS means no samples were taken at that site during the corresponding year.

efficiency of 29.8% based on Levitan et al's (1991) estimator. These calculations strongly suggest that by aggregating the broodstock oysters into very dense populations, fertilization efficiency is greatly improved, and production of larvae on the reef is similar to that of the entire Great Wicomico system in predisease conditions.

### 1997 Field Studies

Surface temperature at the reef (station N1; Fig. 2) reached a maximum of 29.5°C on July 28th (Fig. 5). The difference between the surface and bottom temperature increased in a down river direction (station N1 to N3) away from the reef (Fig. 6). The maximum temperature difference occurred on July 28th for all three stations. As with the temperature, the difference in salinity between the surface and bottom water increased downstream (from N1 to N3; Fig. 6). Salinity at the three stations ranged from 12 to 18 ppt. The maximum difference encountered between surface and bottom samples was 3 ppt at station N2 and N3.

### *Oyster Reproductive Biology and Disease Status*

At the beginning of the study, both males and females were either in the late active or ripe stage of development (Fig. 7). Evidence of spawning (spent specimens) were first seen in the July 14th samples (Fig. 7). Most of the specimen sampled were completely spawned out by early September, with a large majority of them returning to the inactive stage by the end of September.

MSX was absent in all of the oysters examined. *Perkinsus* prevalence increased from 32% in June to 100% in July and continued at that level for the remainder of the study (Fig. 8). Intensity of *Perkinsus* infection increased from June to September, with the highest percentage of highly infected oysters occurring toward the end of the study.

### *Plankton Studies*

The number of oyster larvae observed in plankton samples ranged from a high of 37,362 ± 4,380 $m^{-3}$ on June 23rd at station

TABLE 4.

Patent tong survey data for 1995 and 1997 reported at number of market (>76 mm), small (<76 mm), and spat per square meter.

| Station | 1995 | | | 1997 | | |
|---|---|---|---|---|---|---|
| | Market | Small | Spat | Market | Small | Spat |
| Ingram (10) | 0.4 | 7.0 | 8.3 | 1.2 | 8.8 | 4.6 |
| Fleet Point (11) | 0.3 | 4.4 | 13.0 | 3.0 | 9.8 | 5.8 |
| Cranes Creek (7) | 0.8 | 12.0 | 6.5 | 1.9 | 14.0 | 15.0 |
| Sandy Point (5) | 1.6 | 22.0 | 10.0 | 0.9 | 37.0 | 62.0 |
| Shell Bar (4) | 0.1 | 4.1 | 11.0 | 1.6 | 27.0 | 103.0 |

Numbers in parenthesis correspond to the station IDs in Figure one.

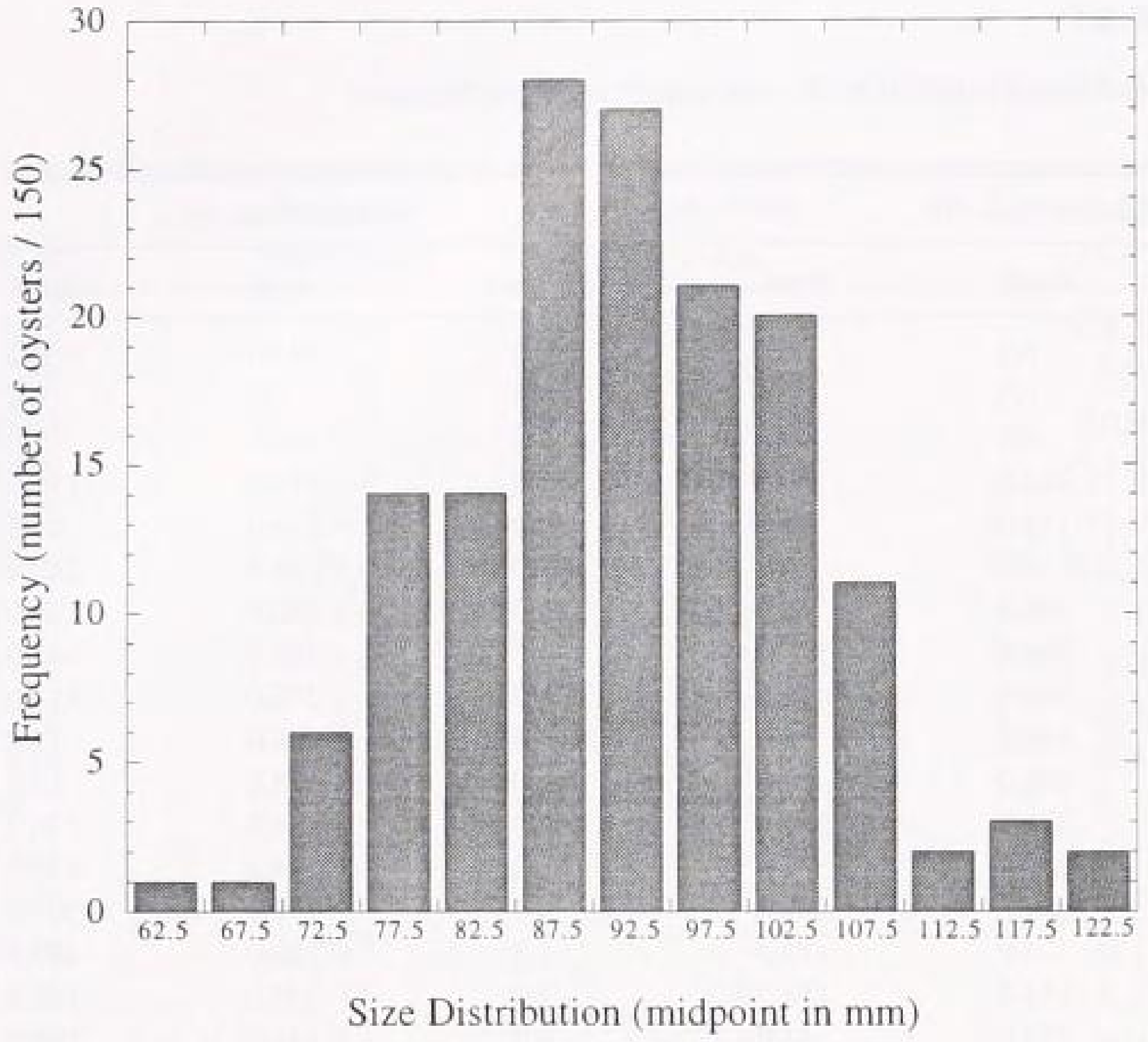

**Figure 4. Size frequency distribution of broodstock oysters on Shell Bar reef (n = 150).**

GW 2 to a low of 0 at all stations on several different sampling days. Larvae were most abundant at all stations on the 23rd and 30th of June, and on the 14th of July (Fig. 9). From the 14th of July onward, there was a continuous decrease in the number of larvae seen in the water column. Coefficient of variation for most samples was within the accepted limits of between 5 and 20% (Van Guelpin et al. 1982). Higher CVs were observed when larval abundance was below 10 $m^{-3}$.

The total number of larvae $m^{-3}$ was transformed to meet the assumptions of normality and homogeneity of variance. Differences in larval abundance between tidal stage and station were then compared with analysis of covariance (ANCOVA) using day of the year as the covariate. The power transformation ($X' = X^{0.20}$), recommended by Downing et al. (1987) for use in estimating zooplankton populations, was used. The use of this transformation met the assumptions of homogeneity of variance, but did not meet the assumptions of normality. Given that ANCOVAs are generally robust to non-normality (Underwood 1997), this transformation was considered valid and the resulting data were utilized in performing the ANCOVA.

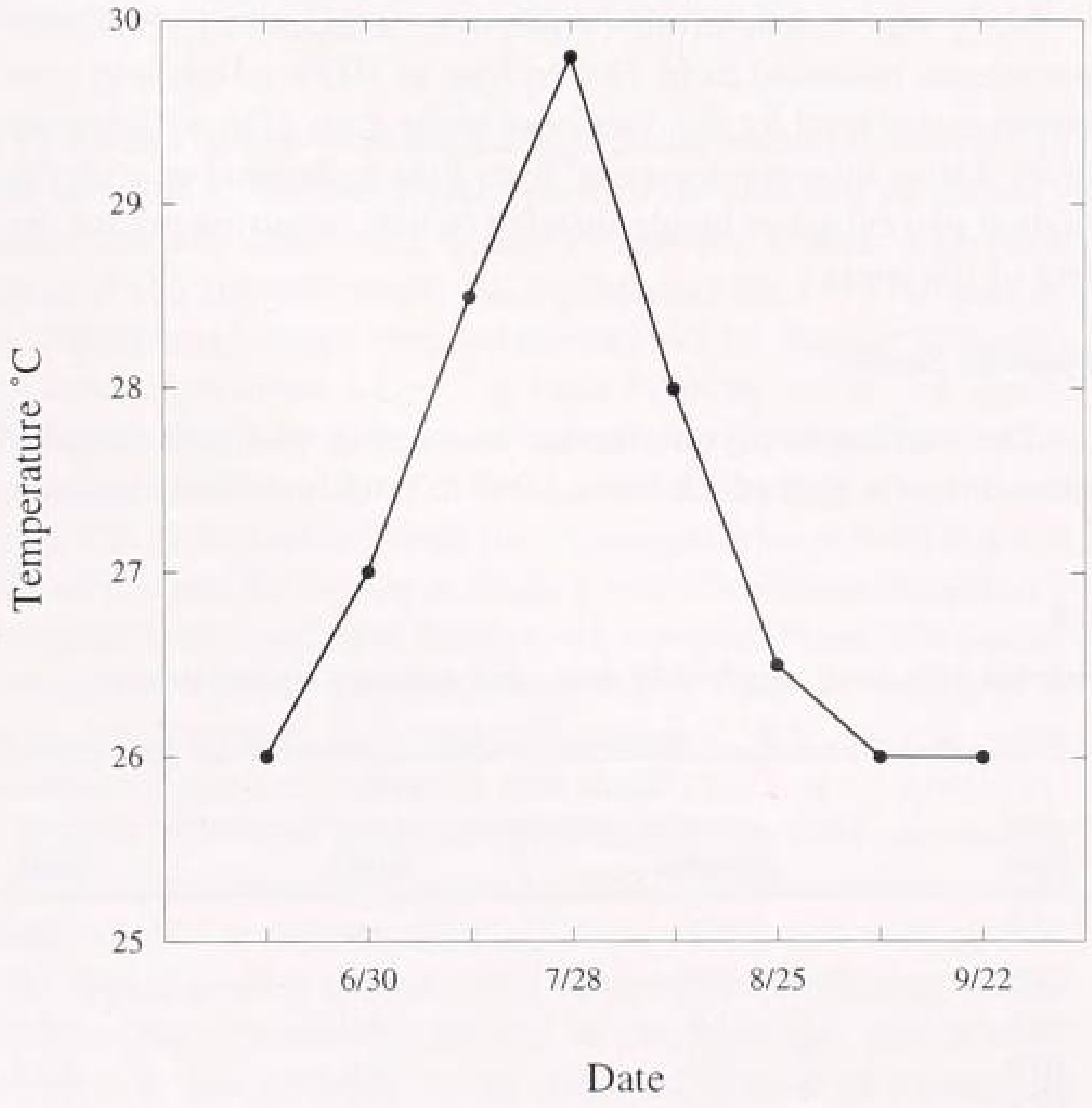

**Figure 5. Surface temperature measured at station N1 from June 23 to September 22, 1997.**

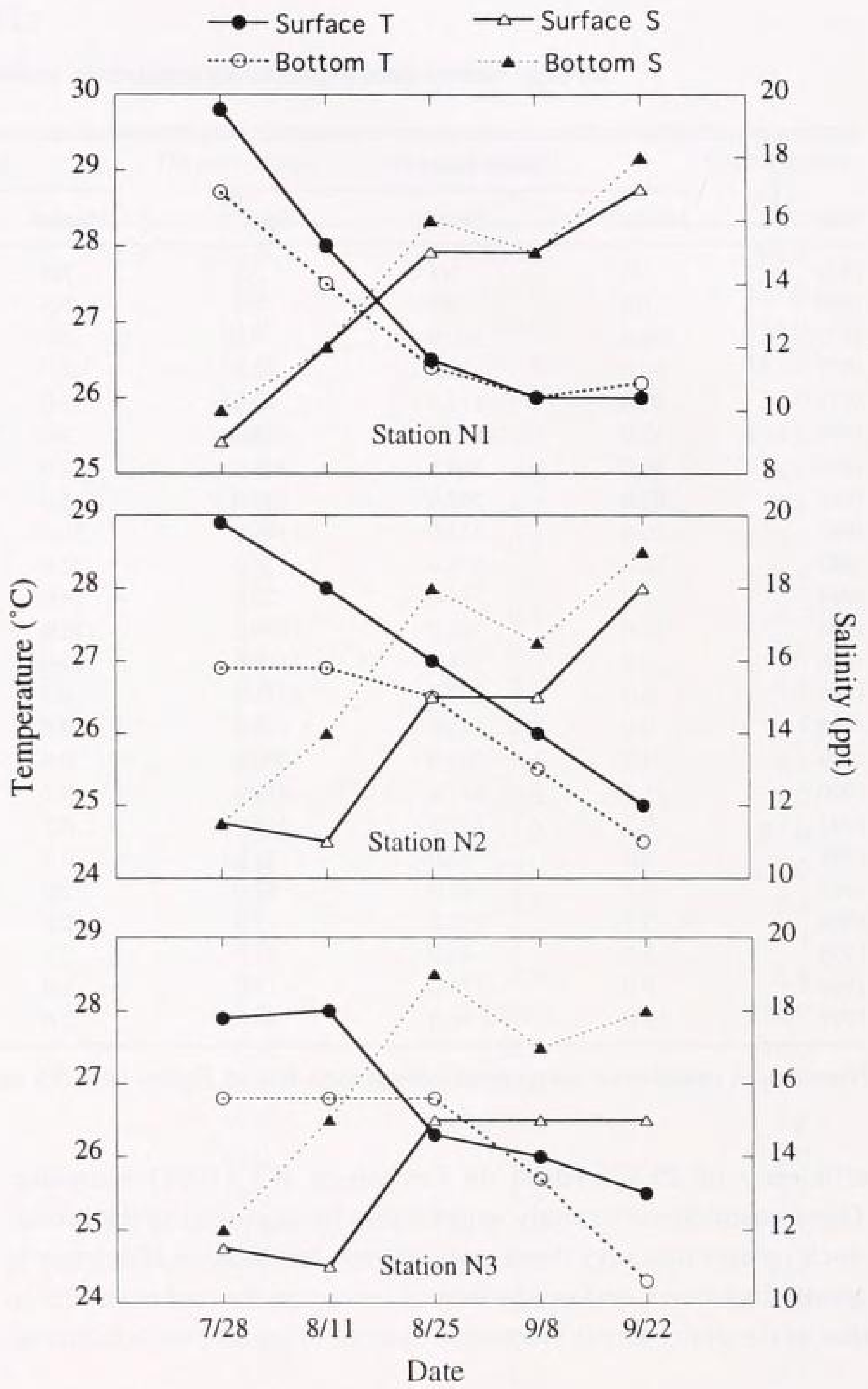

**Figure 6. Surface and bottom temperature and salinity for all three stations (N1–N3) measured from July 28 to September 22, 1997.**

There was a significant difference in larval concentration between tidal stages ($p < .01$) and stations ($p < .05$), with no interaction between the two factors ($p = .55$). Student Newman Keuls (SNK) multiple comparison test for station effect showed that there were significantly more larvae at GW-1 than at the other two stations (Table 5A). There was no difference in larval abundance between GW 2 and GW 3. The SNK for tidal stage showed there were significantly more larvae during the flood tidal stage than during the ebb or slack onto flood stages. (Table 5B). No differences were found between any of the other tidal stages.

### *Circulation Studies*

The direction, mean velocity, and distance traveled by the drifters on each sampling day, for each fix during the day, was re-

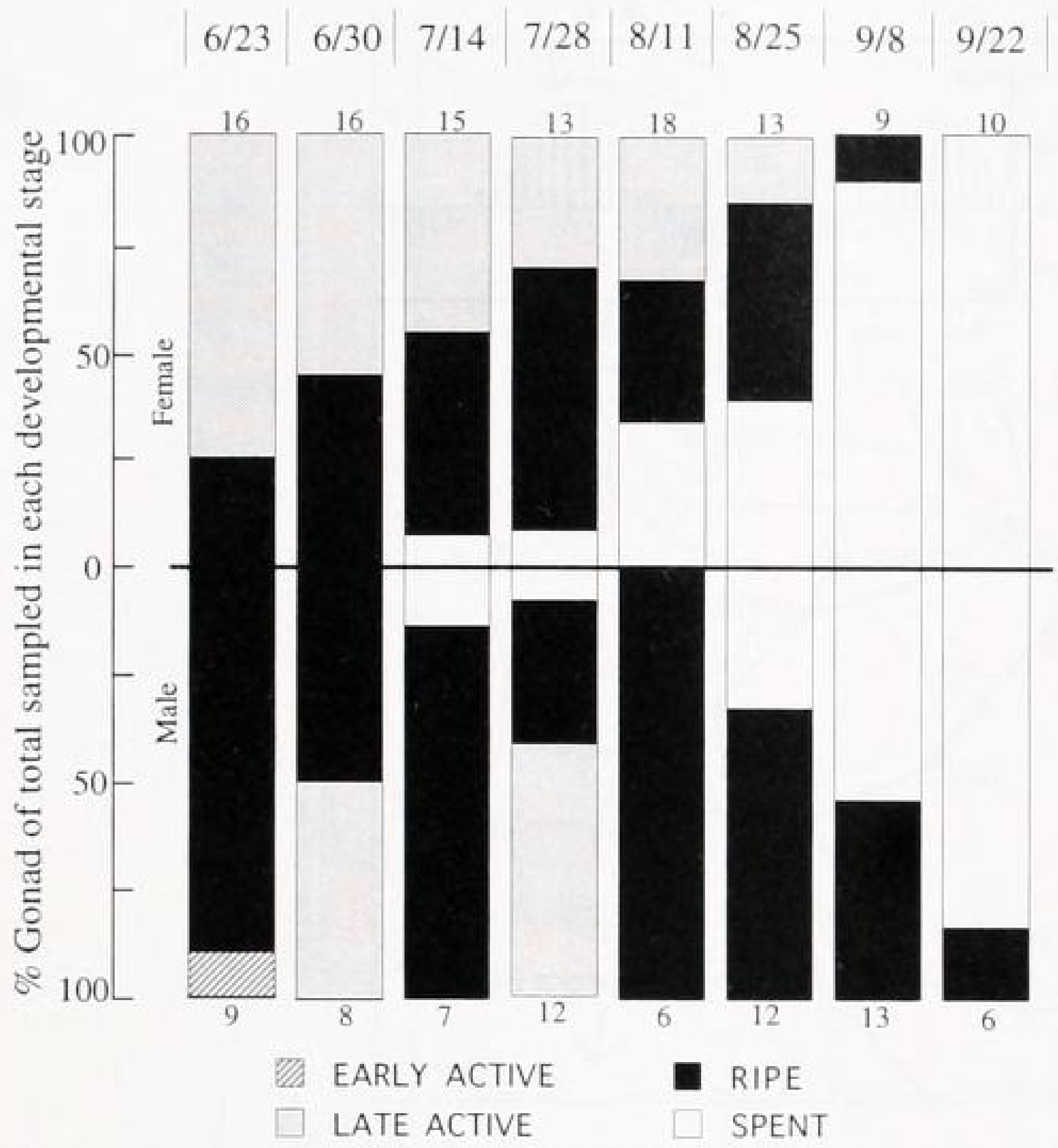

**Figure 7. Seasonal changes in gonadal development by sex in *Crassostrea virginica* oysters collected on the reef from June 23 to September 22, 1997. Number of male and female oysters sampled on each day are represented by the numbers above and below each bar.**

corded and/or estimated. Tidal cycle was recorded as the stage or stages of the tide occurring between a particular observation and the previous observation. For example, if the tide was ebbing for the first half of a drifter deployment, then changing to slack water for the second half, it was recorded as E-S. If the tide was flooding for the entire deployment, then tidal stage was recorded as F. Mean velocities recorded by the drifters ranged between 0 and 15.9 cm/sec. Maximum predicted tidal current was between 10 and 20 cm/sec on all sampling days.

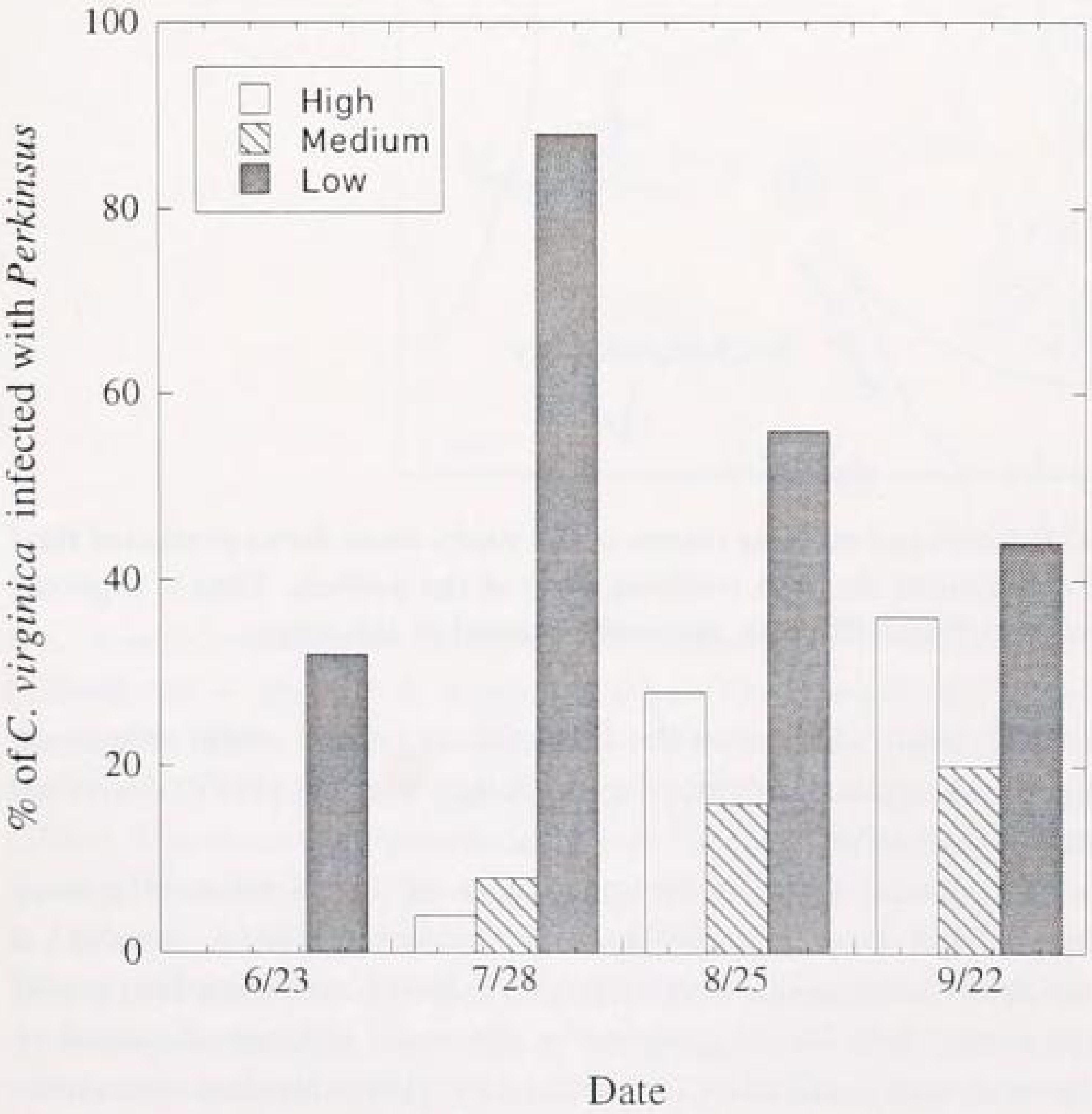

**Figure 8. Progression of *Perkinsus* infections in broodstock oysters over the 1997 reproductive season (n = 25).**

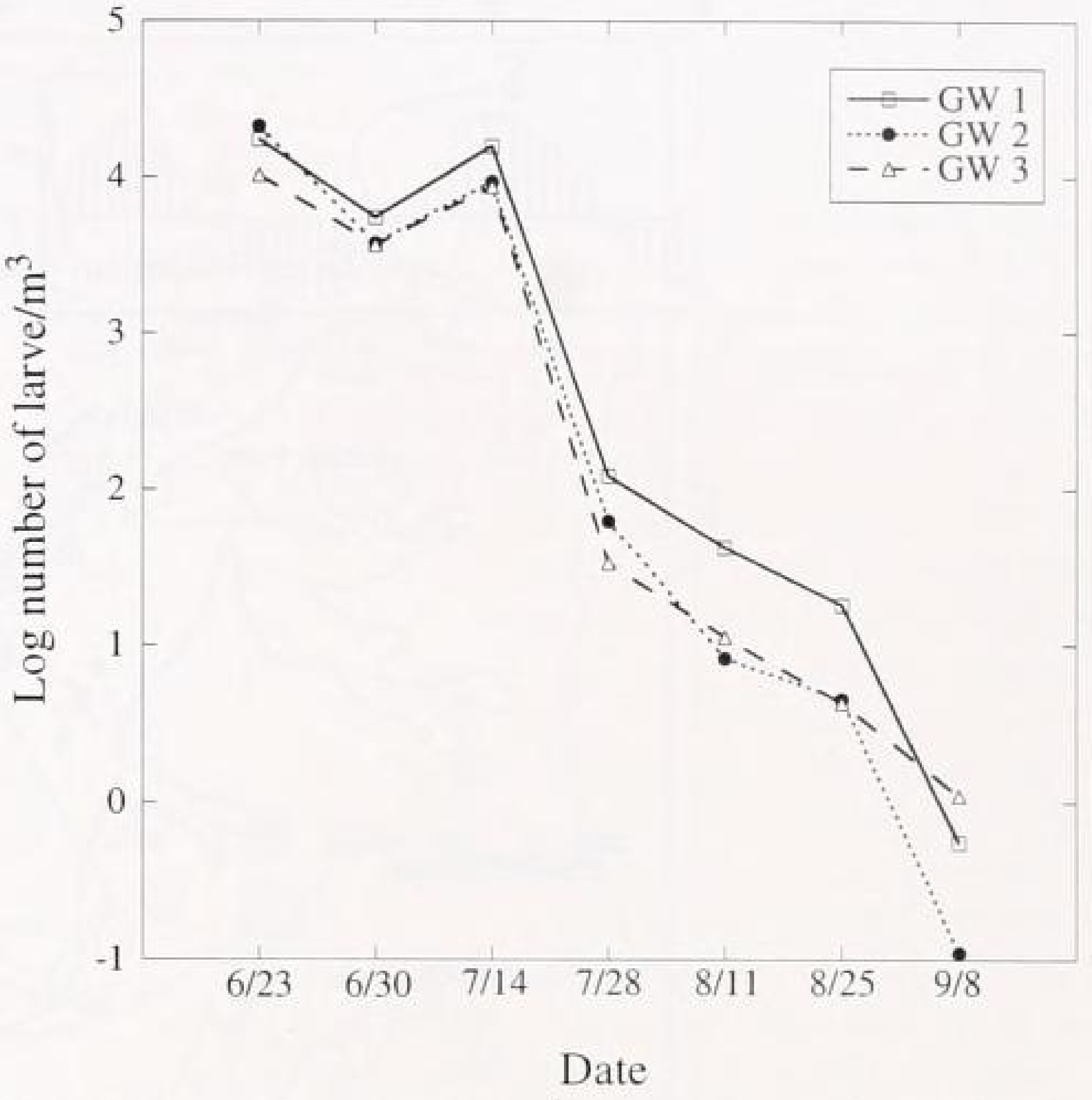

**Figure 9. Log number of larvae m$^{-3}$ averaged over each sampling day (averaged over all four stages of the tide).**

From the 14 drifter tracks analyzed, four patterns of movement were observed (Fig. 10A–D). Four of the 14 drifters followed the predicted tidal current, traveling downstream during the ebb tide, remaining approximately in the same place during slack water, then traveling upstream with the flooding tide. One drifter out of those four remained in the channel and drifted toward marker 9 before turning with the tide (Fig. 10A). The other 3 drifters remained in the channel, further upstream from Sandy Point (Fig. 10B provides an example).

Ten of the 14 drifters analyzed did not follow the predicted tide. Of these 10, 7 traveled downstream with the predicted tide, but turned westward, away from the channel, several hours before the predicted tide turned (see Fig. 10C for example). The other three drifters traveled downstream until they arrived in the shallow region west of Sandy Point. Despite being repositioned, they essentially remained in the same area even during a flood tide (e.g., see Fig. 10D). These patterns of movement illustrated in Figures 10C and 10D indicate that some local retention of larvae is occurring upstream of Sandy Point.

**TABLE 5.**

**SNK multiple comparisons test on larval abundance (p = .05).**

| | | | | |
|---|---|---|---|---|
| **A. Ranking of Larval Abundance by Station** | | | | |
| | High | | Low | |
| Station | GW-1 | GW-2 | GW-3 | |
| **B. Ranking of Larval Abundance by Tidal Stage** | | | | |
| | High | | | Low |
| Tidal stage | Flood | Slack-ebb | Ebb | Slack-Flood |

Stations/tidal stages underlined by the same line are statistically the same.

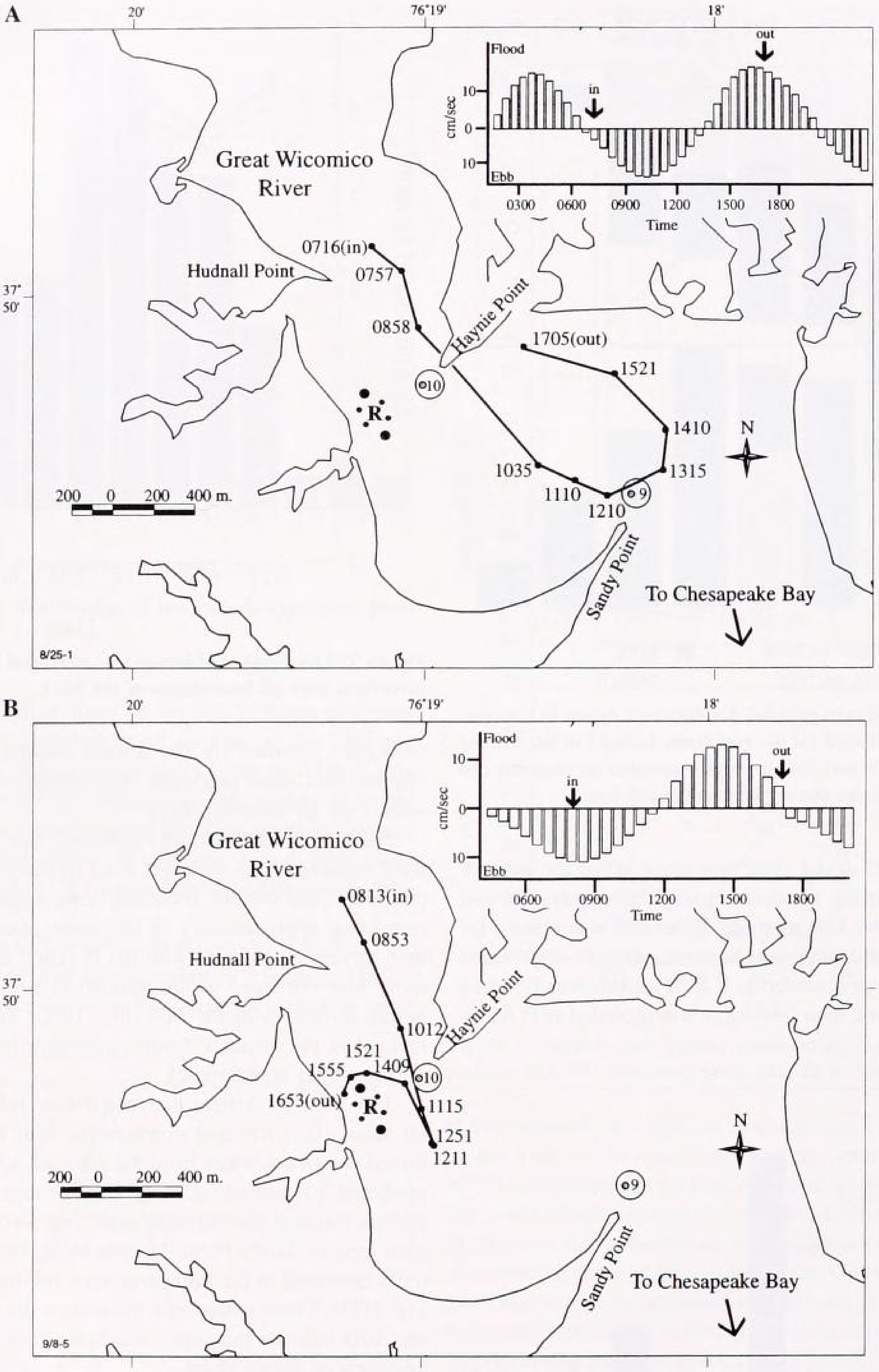

**Figure 10. Representative examples of the four patterns of drifter tracks (A–D) observed over the course of the study. Inset shows predicted tidal currents for Sandy Point, with arrows representing approximate times of deployment (in) and retrieval (out) of the drifters. Time is reported in Eastern Standard (E.S.) Military time. R denotes the location of the reef and 9 and 10 mark the main channel in the river.**

## DISCUSSION

### *Egg Production*

Egg production estimates for broodstock oysters planted on the reef were found to be similar to estimates of production seen throughout the entire system in historic times (when oysters were abundant in the river). Though the estimates are similar they should be viewed with caution because of our inability to offer better values for disease- and salinity-related modifiers of fecundity. These are both discussed in Mann and Evans (1998) and are widely acknowledged in the literature as having major effects on the bioenergetics of oysters, and yet they are still poorly described in a quantitative sense.

The model used in the calculation of fertilization efficiency taken from Levitan's (1991) work on echinoderms, involves a series of assumptions concerning synchrony and completeness of spawning, half-life of gametes in the water column, dispersal or dilution, and probability of fertilization given absolute concentrations of sperm and eggs. There is a notable absence of models in the literature describing fertilization efficiency for sessile bivalves. The employment of the Levitan model in the current application

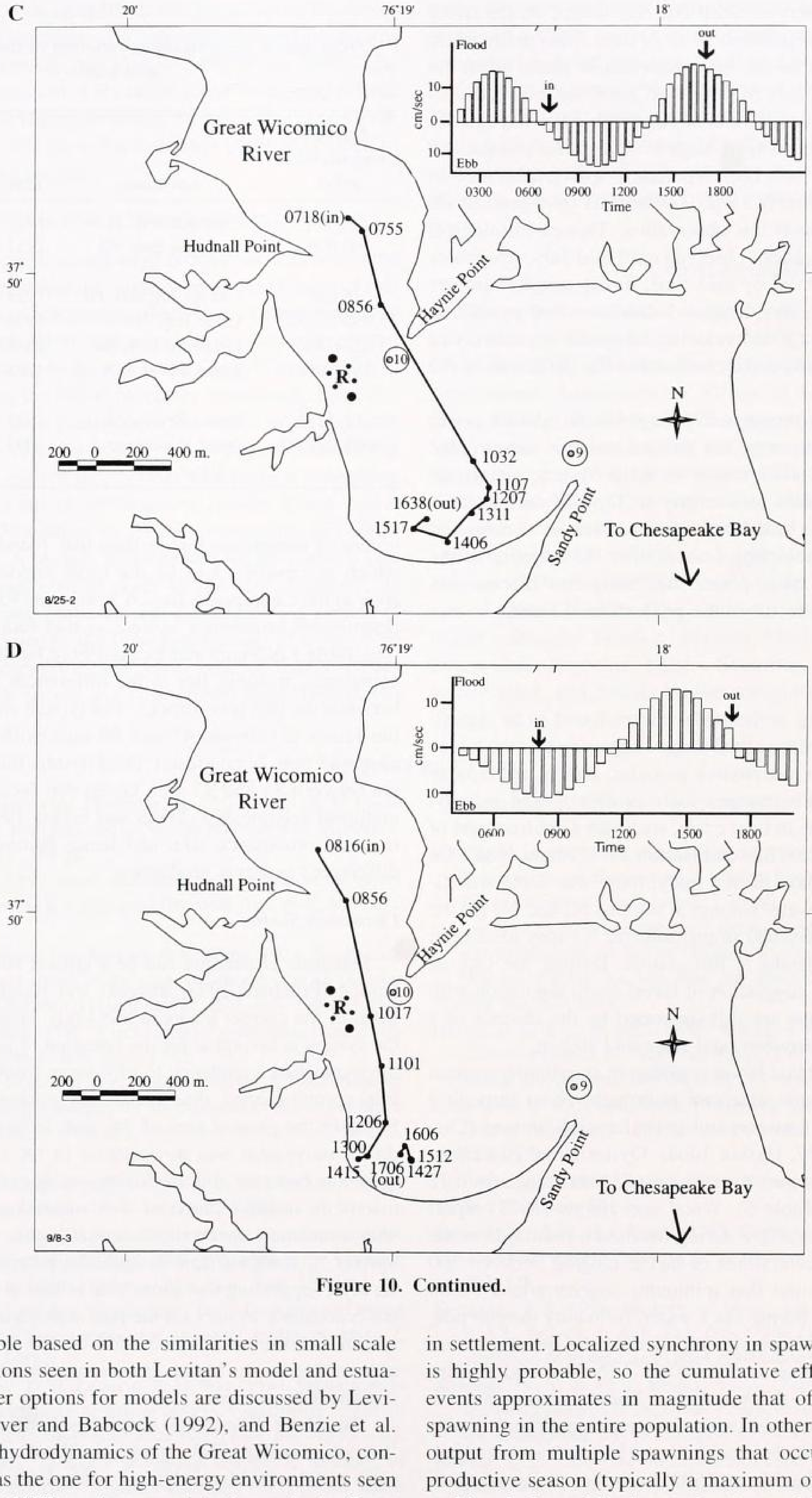

**Figure 10. Continued.**

was deemed reasonable based on the similarities in small scale hydrodynamic conditions seen in both Levitan's model and estuarine oyster reefs. Other options for models are discussed by Levitan et al. (1991), Oliver and Babcock (1992), and Benzie et al. (1994). Based on the hydrodynamics of the Great Wicomico, contrasting models such as the one for high-energy environments seen in Denny and Shibata (1989) are inappropriate for use in the Great Wicomico system. The current model assumes synchrony in spawning throughout the entire oyster population; however, local synchrony is more appropriate when discussing these populations. The lack of synchrony throughout the population is demonstrated by the variation in developmental stage seen in this and other studies (e.g., Haven and Fritz 1985) that observe spatial variation in settlement. Localized synchrony in spawning on the other hand is highly probable, so the cumulative effect of these localized events approximates in magnitude that of a single synchronous spawning in the entire population. In other words, the cumulative output from multiple spawnings that occurs throughout the reproductive season (typically a maximum of 2–3 per season based on historical spatfall observations) are within an order of magnitude of the single synchronous spawning event estimate of production.

### *Oyster Reproductive Biology and Disease Status*

Both histological evidence and the presence of significant number of larvae in plankton samples indicate spawning of the transplanted broodstock oysters on Shell Bar reef. Based on the larval abundance data, and an estimated 14 to 21 days spent in the water column by *C. virginica* larvae, inferences can be made about the timing of spawning. Oysters from the reef probably spawned continuously from mid-June to the end of July, with a few late spawnings occurring in the beginning of August. *C. virginica* populations in the Chesapeake Bay have been reported to spawn from June to August (Kennedy and Krantz 1982). Gonad data from broodstock oysters on the reef support this observation. Though histological evidence of spawning was not observed until mid-July, ripe males and females were observed by mid-June. Small sample numbers (25 oysters per sampling day compared with a resident population of approximately $1.1 \times 10^6$ individuals) and spatial asynchrony of spawning on the reef may have confounded the definition of the first spawning event.

*Perkinsus* infections progressed through the broodstock population throughout the summer, but did not result in catastrophic mortalities. The effect of *Perkinsus* on adult oysters, mainly reduced fecundity, increases as intensity of the disease increases (Choi et al. 1994). In the broodstock oysters, intensity was highest toward the end of the sampling season, after the majority of the spawning had already taken place, suggesting that disease was probably not a limiting factor in the production of larvae.

### *Plankton Studies*

Larval concentrations at the surface were found to be significantly higher during the flood tidal stage, suggesting that the larvae are acting as more than just passive particles. Larvae are capable of depth regulating with changes such as density and salinity, associated with a change in tidal cycle, such that a net transport of larvae upriver is possible (Hidu and Haskin 1978, Mann 1988). On most of the sampling days in this study, there was some stratification occurring in the water column at stations N2 and N3 (in the channel). The lack of physical stratification at N1 may have been due to the shallower depths at this station. Despite the lack of stratification at N1, the suggestion of larval depth regulation with the changes in tidal stage are still supported by the absence of a significant interaction between tidal stage and station.

It has been proposed that larval retention in an estuarine system and subsequent movement upstream is brought about through a combination of passive transport and active larval swimming (Carriker 1951, Kunkle 1957, Haskin 1964). Oyster larval concentrations reported in the literature over the past 75 years range from 12 $m^{-3}$ to 660,000 $m^{-3}$ (Table 6). Wood and Hargis (1971) report larval abundance at the surface during maximum flood tide in the James River, with concentrations of larvae ranging between 300 and 800 $m^{-3}$. They found that minimum concentrations (<100 $m^{-3}$) were encountered during slack water, following the ebb tide. The highest larval concentrations reported in the literature were recorded in Delaware Bay as 660,000 $m^{-3}$ (Nelson and Perkins 1931) and 125,500 $m^{-3}$ (Nelson 1927). These numbers are extremely high when compared with concentrations found in this study, but the date of the observations must be taken into account. In a more recent study by Mann (1988) in the James River, much lower concentrations of between 12 and 113 $m^{-3}$ were reported. The concentration of larvae found, reported in this study, is extremely high when compared with other reports made for years after the onset of disease and decimation of broodstock oyster populations in the Chesapeake Bay subestuaries. Though not of the same order of magnitude seen in historical times, the present report of concentration of larvae in the Great Wicomico is still several orders of magnitude higher than that found in the James River, which is considered to be the most important oyster-producing river in the Chesapeake Bay. A few James River reefs have similar densities of broodstock oysters as that found on Shell Bar Reef (see Table 1 of Mann and Evans 1998), but the difference in larval abundance probably lies in the differences in size and fecundity between the two broodstocks. The typical size of oysters found in the James is between 45 and 60 mm, with only a few reaching above 85 mm. In contrast, typical oysters found on Shell Bar Reef are between 85 and 95 mm. Given that fecundity and size have a nonlinear relationship (Mann and Evans 1998), small differences in mean broodstock size, and hence fecundity, can lead to vast differences in larval production.

**TABLE 6.**

**Oyster larval concentrations reported in the literature over the past century.**

| Larval Concentration m^-3 | Location | Year | Source |
|---|---|---|---|
| 125,000 | Barnegat Bay, NJ | 1927 | Nelson, 1927 |
| 660,000 | Barnegat Bay, NJ | 1931 | Nelson and Perkins, 1931 |
| 16,680 | Lanoka Lagoon, NJ | 1938 | Carriker, 1951 |
| 13,360–37,300 | Great Bay, NJ | 1939 | Carriker, 1951 |
| 625–2400 | Delaware Bay, NJ | 1656 | Haskin, 1964 |
| 300–800 | James River, VA | 1965 | Wood and Hargis, 1971 |
| 12–113 | James River, VA | 1985 | Mann, 1988 |
| 17,000–37,500 | Great Wicomico River, VA | 1997 | This study |

### *Circulation Studies*

Estuarine circulation can be a critical component of larval retention (Pritchard 1953, Ruzecki and Hargis 1989). The general trend of the current tracks in this study suggest that circulation in the system is favorable for the retention of larvae. The majority of the tracks had a tendency to turn away from the channel, prior to tidal current change, thus the drifters remained upstream of Sandy Point, in the general area of the reef. In several instances, initial drifter movement was downstream in the channel. On only one occasion, however, did the drifter continue traveling in the channel toward the mouth of the river. Settlement data were in concordance with circulation observations in that higher settlement was found upriver of the sand spit in both the patent tong and shellstring surveys, suggesting that some local retention of larvae produced by the broodstock oysters on the reef was occurring. The number of spat $m^{-2}$ on the bottom recorded from the patent tong survey were at least three times higher at stations upstream compared with the stations downstream.

The Great Wicomico River has historically been termed a ''trap-type estuary,'' along with the Piankatank River, also in Virginia and the St. Mary's River in Maryland (Manning and Whaley 1954). Andrews (1979), defines a trap-type estuary as one that has a low-flushing rate, small tidal amplitudes, and restricted entrances. Though these characteristics are important, local circulation, dictated by both topography and tidal currents, has been shown to be an important component of larval retention (Carter 1967). Larval retention is not, however, limited to trap-type estuaries. The James River, for example, has proved to be a good seed-producing river, with larvae that are produced in the lower reaches being moved upstream and subsequently settling on upstream oyster beds (Ruzecki and Hargis 1989, Mann 1988). The important feature of retention in the James River is the tidal-related gyre-like circulation in Hampton Roads. Thus, retention of larvae can occur in estuaries that have characteristics in direct contrast to the "typical" trap-type estuary.

### *Impact of Broodstock Seeding from a Management Perspective*

The demonstrated positive impact of broodstock addition to the Great Wicomico reef prompts the question as to the efficacy of this approach for widespread restoration efforts in the Chesapeake Bay and elsewhere. In comparison with prior reef construction efforts, the addition of broodstock to the reef in the Great Wicomico River approximately doubled the initial monetary investment, however, the ecological impact on replenishment was rapid in comparison. In just 1 y observed settlement of spat was comparable to historic conditions. Increased spatfall in 1997 has stimulated a resurgence of interest in deployment of substrate on private leased oyster grounds in the Great Wicomico in 1998 in anticipation of continued high recruitment. A blanket endorsement for the construction and broodstock enhancement of reefs should still, however, be avoided. Location of any proposed reef construction is critical to its possible success. Though the typical definition of a trap-type estuary is useful in general location, knowledge of historic oyster reef topography can be critical at a finer spatial scale. Indeed, our study supports the use of both historical data sets and circulation studies of a target system as a common precursor to management decisions involving reef construction. Location definition should be followed by numerical estimation of optimal stocking density. The current study stands as an example of how a modest (compared with historical numbers) number of large oysters planted at a high density of 300 $m^{-2}$ can have an immediate impact in an effort to restore decimated oyster populations to historic conditions.

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