The Journal of the Elisha Mitchell Scientific Society, 113(1), 1997, pp. 22–36

REPRODUCTIVE BIOLOGY AND PARASITE (*Perkinsus marinus*) PREVALENCE IN THE EASTERN OYSTER, *Crassostrea virginica,* WITHIN A GEORGIA TIDAL RIVER

FRANCIS X. O'BEIRN,[1] RANDAL L. WALKER,[2]* and MICHELLE L. JANSEN[2]

[1]*Cooperative Fish and Wildlife Research Unit*
Department of Fisheries and Wildlife Science
Virginia Tech, Blacksburg, VA 24061-0321
[2]*Shellfish Aquaculture Laboratory*
University of Georgia Marine Extension Service
20 Ocean Science Circle
Savannah, GA 31411-1011

Abstract: Recruitment, in 1992, of the eastern oyster, *Crassostrea virginica,* at one site (Flume Dock) within the Sapelo Island National Estuarine Research Reserve (SINERR) was lower than other sampling sites. Recruitment allied with gametogenesis and parasite (*Perkinsus marinus*) prevalence within the oysters was examined in 1993, in an attempt to explain the differences observed the previous year. Sampling occurred at three sites (Marsh Landing, Jack Hammock, and Flume Dock) along the Duplin River within the SINERR. Oyster recruitment in 1993 was reduced in all sites within the SINERR. No oyster recruitment was recorded at the Flume Dock site during any monthly collections or on collectors deployed for the duration of the sampling study. Oysters developed gametogenically and spawned at the Marsh Landing site two weeks prior to their occurrence at the Jack Hammock site. Gametogenic development in oysters at the Flume Dock site was retarded a further two weeks where the males appeared to spawn six weeks after the females, which could account for reduced recruitment levels at this site. Approximately, twice as many females as males occurred in all sites. Monthly sampling of prevalence and intensity of *Perkinsus marinus* (Dermo) revealed high levels of both parameters. Smaller oysters did display lower prevalences of Dermo than larger forms.

Key Words: oyster, *Crassostrea virginica,* recruitment, disease, reproduction, environment, Georgia, *Perkinsus marinus.*

INTRODUCTION

Eastern oyster, *Crassostrea virginica,* recruitment was evaluated in 1992 as part of a monitoring study of the Sapelo Island National Estuarine Research Reserve (SINERR). The results of that study (O'Beirn et al., 1994a) indicated much spatial and temporal variation in oyster recruitment among three sites chosen along the Duplin River within the reserve. A striking difference in recruitment patterns among the sites was the overall lower recruitment occurring in the upper-most

* Corresponding author.

site (Flume Dock) on the river, despite the presence of moderate numbers of adult oysters. Three orders of magnitude lower recruitment occurred at Flume Dock in 1992 when compared to the other two sites. We advance some possible hypotheses for the variations in oyster recruitment among the sites:

1. Oyster gametogenic cycle was either delayed or retarded in the upper reaches of the Duplin River, hence, this could possibly translate into a reduction in the levels of spatfall in this portion of the river.

2. Stresses attributable to prevalence and intensity of a disease-causing organism in oysters at the sites, e.g., the protozoan parasite *Perkinsus marinus* (Dermo).

3. Differences attributable to hydrographic parameter (temperature, salinity, and pH) variations might also account for recruitment variation among the sites.

This report describes the results of the monitoring study carried out on the SINERR during the 1993 spawning season (April–October, Heffernan et al., 1989). It investigated differences in oyster gametogenesis and prevalence of the protozoan parasite, Dermo, at the three study sites and related oyster recruitment observed with continuous hydrographic data from two of three study sites on the Duplin River Georgia.

MATERIALS AND METHODS

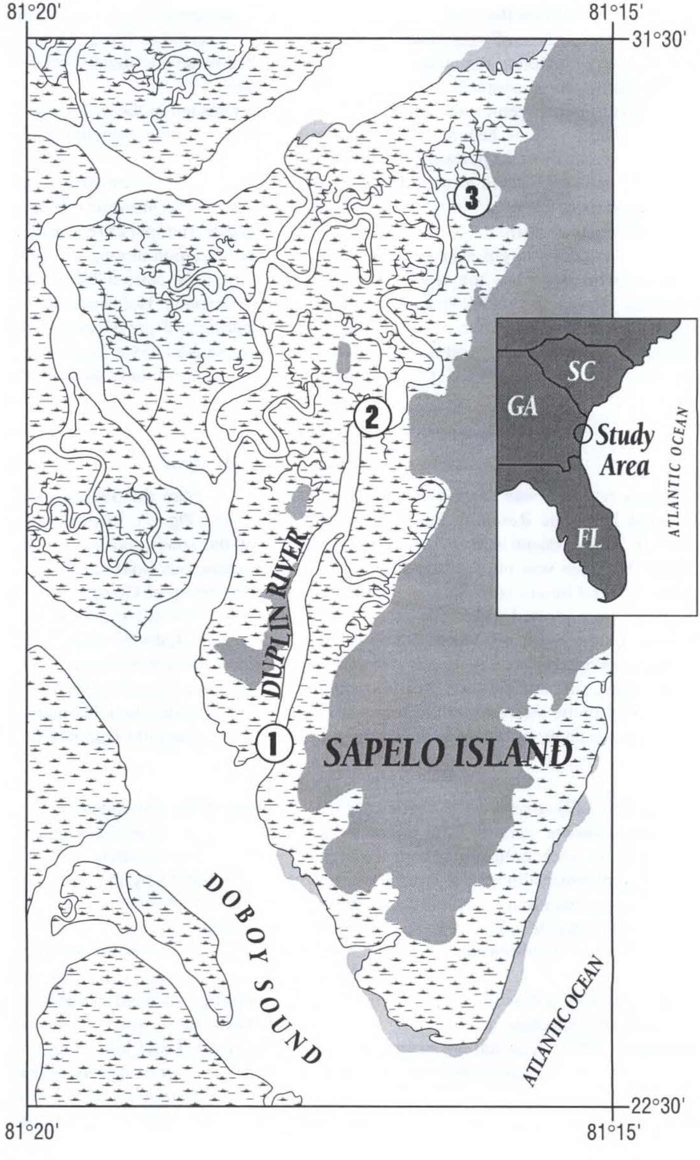
Site Description and Hydrographic Analysis

Oyster research was carried out on the Duplin River within the Sapelo Island National Estuarine Research Reserve, Georgia (SINERR; Fig. 1), located to the west of Sapelo Island and incorporating a portion of the south and west of the island. The area was predominantly salt marsh with numerous tidal creeks. Two environmental monitoring stations, operated by the University of Georgia Marine Institute, were located within the SINERR; one located at Marsh Landing and the other at Flume Dock off Moses Hammock. At each site, a Hydrolab Data Sonde I unit, suspended from a float one meter below the water surface, measured water temperature, pH, and salinity. Recruitment and gametogenic sampling sites were established in the area adjacent to these two stations. A third site, Jack Hammock, was approximately half-way between the other two sites, along the Duplin River.

Recruitment Evaluation

Longitudinally-grooved PVC tubes (1.9 cm diameter) with embedded chips of calcium carbonate were used for the collection of spat. A 12-cm section of tubing was used for each collector giving a sampling area of approximately 100 cm^2. Recruitment was estimated, at three sites, at each of three tidal heights: subtidally, at mean low-water and intertidally approximately two hours after mean-low water, using five replicate collectors (see O'Beirn et al., 1994a for complete design specifications). Upon return to the laboratory, each collector was rinsed with saline water to remove extraneous matter. Oyster enumeration on the collectors was carried out with a binocular microscope at 10×. Sampling consisted of replacing an array of collectors at each site on a monthly basis from April 1993 until November 1993. Seasonal collectors were deployed in April and left on site for the duration of the study (one complete spawning season; Heffernan et al., 1989).

All raw data (numbers of recruits per 0.01 m^2) were log transformed, a consequence of the high degree of variability of recruit numbers within each sampling period (Sokal and Rohlf, 1981). Analysis of Variance (ANOVA: α = 0.05) and


81°20'
81°15'
31°30'
SC
GA
FL
Study Area
ATLANTIC OCEAN
3
2
1
DUPLIN RIVER
SAPELO ISLAND
DOBOY SOUND
ATLANTIC OCEAN
22°30'
81°20'
81°15'

Tukey's Studentized Range Test (when appropiate) were performed on these data to determine inter- and intra-site differences in recruitment. ANOVAs and multiple range tests were also carried out on the hydrological data from the Marsh Landing and Flume Dock sites on the Duplin River. Mean daily values for temperature, pH and salinity were pooled biweekly and subsequently compared. All statistical analysis were carried out using PC SAS (SAS Inst. Inc., 1989).

Quantitative Reproductive Analysis

Biweekly collection of oysters (n = 30) at the three sites occurred from 30 April through 14 December, 1993, during the major spawning season (Heffernan et al., 1989) and monthly between December 1993 and May 1994. Oysters, collected from a standard intertidal height (just above the mean low water mark) at all sites, were measured for shell height (hinge-to-lip) to the nearest 0.1 mm with Vernier calipers. A gonadal tissue cross-section (ca. 0.5 cm in width) was dissected from each oyster at a region approximately 1 cm toward the hinge from the adductor muscle. The gonadal sections were held in Davidson's fixative for 48 hr under refrigeration, rinsed with 50% ethanol, and stored in 70% ethanol. Tissue samples were dehydrated in an alcohol series, cleared in toluene, and embedded in paraplast. Sections were cut 7–10 μm thick using a rotary microtome. Sections were stained with Harris Hematoxylin and counter-stained with Eosin (Howard and Smith, 1983).

Quantitative analysis of gonadal preparations was performed using Color Image Analyzed Densitometry Microscopy housed at Skidaway Institute of Oceanography, Savannah, Georgia (O'Beirn et al., 1996a). Photomicroscope images (10 × 1.25 optivar) were viewed and captured by a Hitachi Model DK-7000 SU-3 Chip CCD camera. The images were then viewed on a Trinitron video monitor. The image analyzer is capable of performing detailed area measurements within the blue thresholds (operator controlled). One field of view per specimen was analyzed.

An operator-controlled marker was used to edit non-gonadal tissue (e.g., intestines and digestive diverticula) in evaluation of percent gonadal area per field of view. Females were analyzed for percent gonadal area, percent of that gonadal area occupied by follicles, oocyte number per standard area, and mean oocyte diameter. Egg number per standard area was manually counted from the screen and oocyte diameter of nucleated oocytes was measured directly on the screen using the associated software. Males were analyzed for percent gonadal area and percent area occupied by follicles. Statistical analysis of data was performed by one-way ANOVA ($\alpha = 0.05$) using SAS for PC computer (SAS Inst. Inc., 1989). Prior to statistical analysis, all percentage data were arcsine square-root transformed. Sex ratios were tested against a 1:1 ratio with a Chi-square statistic (Elliott, 1977).

←

FIG. 1. Map of the Duplin River within the Sapelo Island National Estuarine Research Reserve, indicating the three sampling sites: 1. Marsh Landing, 2. Jack Hammock, and 3. Flume Dock.

Disease Evaluation

Oysters (n = 20 per site) were collected from the same tidal height at Marsh Landing, Jack Hammock, and Flume Dock on a monthly basis, from July through December, 1993. Oysters were measured for shell height to the nearest 0.1 mm and a portion of the rectum and mantle were dissected from each animal. These tissues were placed in fluid thioglycollate media (Ray, 1963) and incubated for two weeks before analysis. Prevalence was determined by the presence of hypnospores in the tissue sample after staining. Intensity was measured on a scale of 0–6, as described by Quick and Mackin (1971), whereby, 0 represented no infection and 6 corresponded to very high infection levels.

Statistical analysis of data was performed by ANOVA ($\alpha = 0.05$) using SAS for PC computer (SAS Inst. Inc., 1989). Prior to statistical analysis, all prevalence data were arcsine square-root transformed.

RESULTS

Recruitment Evaluation

Marsh Landing.—Oyster recruitment for 1993 was first recorded at the end-of-May/beginning-of-June at this site ($\bar{x} = 5.2 \pm 1.7$ SD spat/0.01 m^2). Hereafter, all values given are mean number of spat (±SD) per 0.01 m^2. Overall, recruitment was low and increased to a maximum of only 20.3 ± 4.2 in August (Fig. 2). Recruitment ceased in October after very low numbers were obtained in September (2.1 ± 0.6). Low water collectors collected significantly more oysters than the other two tidal height collectors ($p = 0.0008$). There were no differences in numbers of recruits among the tidal heights, for the seasonal data ($p = 0.1089$).

Jack Hammock.—Recruitment was high at the first sampling in May (301.5 ± 51), after which it decreased precipitously. Low levels of recruitment were recorded for the rest of the study (Fig. 2). No differences existed in recruitment among the tidal heights at this site for the monthly data ($p = 0.1002$). There were no differences among the tidal heights in terms of oyster recruitment, for the seasonal data ($p = 0.0637$).

Flume Dock.—No oyster recruitment was obtained on any of the monthly or seasonal collectors throughout 1993.

Hydrographic Analysis

Marsh Landing had significantly higher pH values throughout the year than Flume Dock (Fig. 3a). Marsh Landing had significantly higher salinity values in most biweekly periods than the Flume Dock site (Fig. 3b). The peak temperatures at both sites were around 30°C. The results of the ANOVAs carried out on the temperature data from the Duplin River revealed significantly higher values from the Flume Dock site over the Marsh Landing site in April, May, July and August (Fig. 3c).

Quantitative Reproductive Analysis

Significantly more females than males occurred at all sites (Table 1). Roughly, twice as many females as males occurred at Marsh Landing (2.16F:1M) and Flume Dock (1.85F:1M) sites, while the ratio was 1.64F:1M at the Jack Hammock

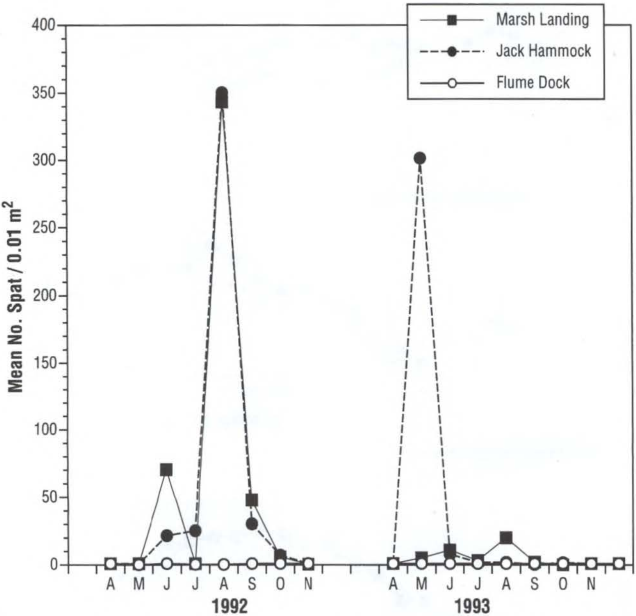


FIG. 2. Mean monthly oyster spat recruitment (pooled over tidal heights) for the three sites in 1992 and 1993.

site. The overall sex ratio for oysters in the Duplin River was 1.84F:1M, differing significantly from a 1:1 ratio (Chi-square = 66.70, $p < 0.01$).

The proportion of area occupied by gonads in the males (Fig. 4a) remained high (80–100%) at all three sites from April through mid-August. A sharp decline in gonad content was initially observed at the Jack Hammock site followed by the Marsh Landing site two weeks later. After a further two-week period (October), the oysters at the Flume Dock site also displayed a similar decrease in gonad content. These declines indicate a decreased in the potential area for the manufacture of male reproductive cells. The area occupied by follicles in male oysters increased to approx. 85% at the Flume Dock and Marsh Landing sites, and 75% at the Jack Hammock site in mid-August (Fig. 4b). A sharp decline was then witnessed at Jack Hammock and Marsh Landing at the beginning of September followed by a more gradual decline at the Flume Dock site. Regeneration of spermatozoa appears to have occurred at Marsh Landing and Jack Hammock in October. Again a lag was apparent at the Flume Dock site, with possible regeneration of spermatozoa in mid-November.

All of the female reproductive parameters, from April through mid-June, remained relatively constant at the three sites (Fig. 5a–d). At the beginning of July there was a sharp decrease in the gonad area at the Jack Hammock site (Fig 5a).

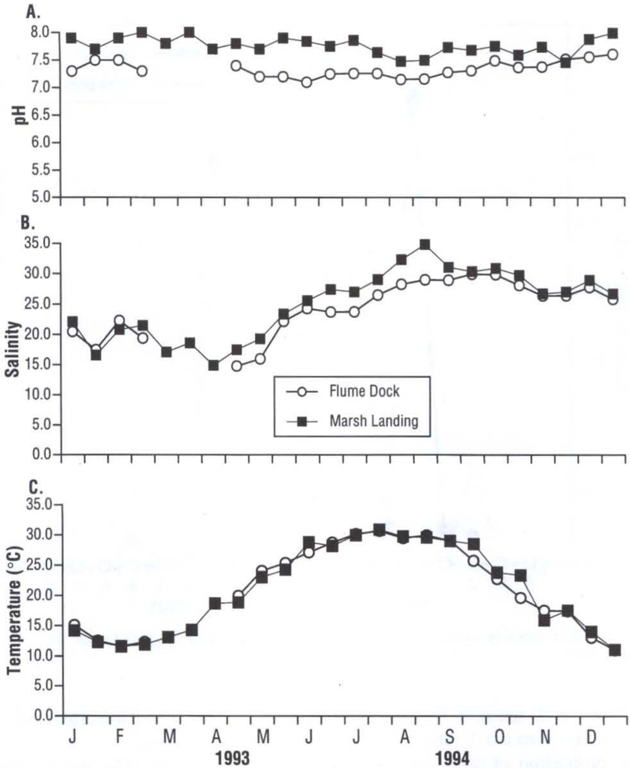


FIG. 3. Mean biweekly hydrographic data collected from the Marsh Landing and Flume Dock monitoring stations over the course of the study.

Table 1. Total numbers of oysters collected and sex ratios at each site within the Duplin River, Sapelo Island, Georgia.

Site	Total (n)	Males (n)	Females (n)	F:M	Chi-square
Marsh Landing	221	70	151	2.16:1	29.69*
Jack Hammock	288	109	179	1.64:1	17.01*
Flume Dock	313	110	203	1.85:1	27.63*
Overall	822	289	533	1.84:1	66.70*

* $P < 0.01$.

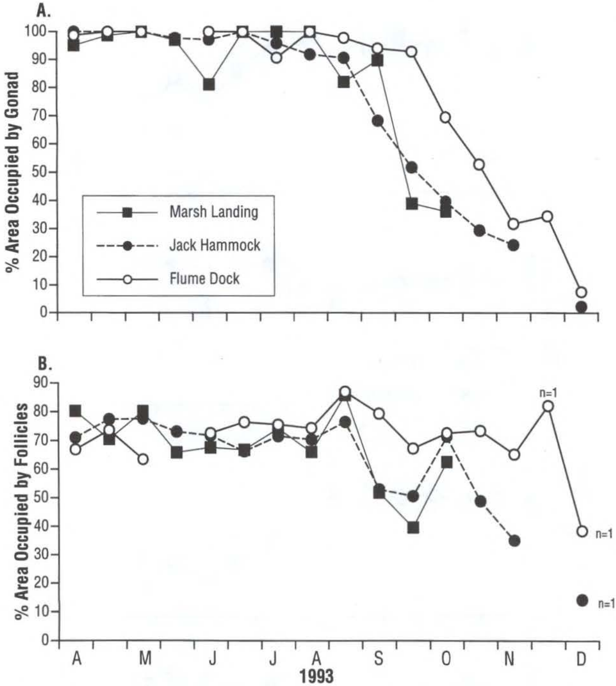


FIG. 4. Male reproductive parameters from the three sites: (A) Gonad Area and (B) Follicle area.

There was a sharp decline in mid-July at Marsh Landing (Fig. 5a). A gradual decline was also witnessed in the follicle area as well as the oocyte diameter parameters through July at all sites (Fig. 5b, d). However, at the Flume Dock site, the mean number of eggs per field appeared to increase over the aforementioned time period (Fig. 5c).

The gonad area and follicle area (Fig. 5a,b) at the Flume Dock site had consistently higher values than the other two sites for the latter part of the summer (1993). Spawning at all sites was best indicated by a sharp decline in the mean number of eggs (Fig. 5c) in mid-August. Declines in egg numbers were followed by subsequent sampling period declines in gonad and follicle area (Fig. 5a–c).

Egg sizes (Fig. 5d) remained between 30–35 μm at all three sites during the summer months in 1993. Greater variability was apparent among the sites toward the end of 1993.

Disease Evaluation

Initial estimates (July 1993) of Dermo prevalence were high for all of the sites (Fig. 6a). These estimates ranged from 75% at Flume Dock to 95% at Marsh

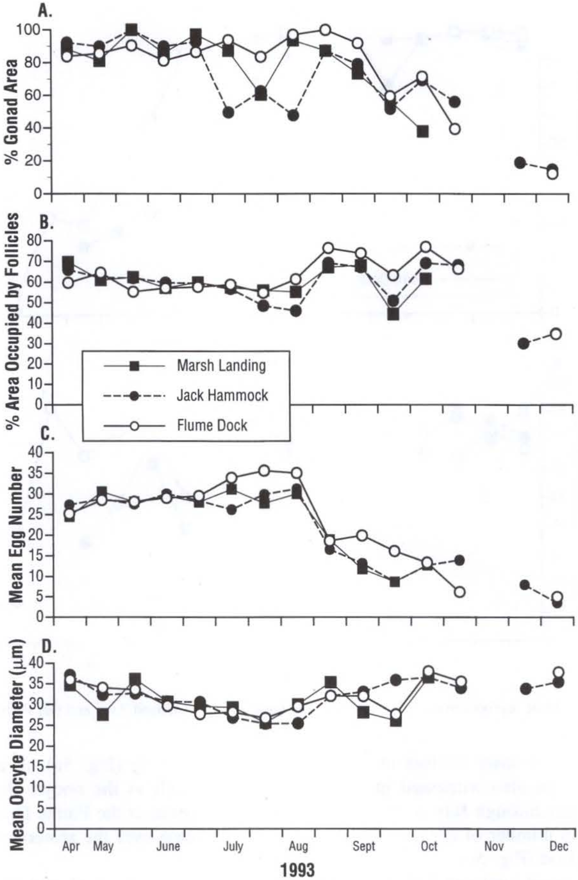


FIG. 5. Female reproductive parameters from the three sites: (A) Gonad Area, (B) Follicle Area, (C) Mean Number of Eggs, and (D) Mean Egg Diameter.

Landing. Peak prevalences were attained in September and December at the Jack Hammock site (100%), in September at Flume Dock (95%), and in October at Marsh Landing (100%). There was a sharp decline in December in the proportion of oysters with Dermo at the Marsh Landing site. The proportion decreased from 80% in November to 40% in December (Fig. 6a). The other two sites also had decreases in prevalences, however, they were delayed by one month. Minimum levels of prevalence were observed in March and May for the Marsh Landing site (0%), in January and June at the Flume Dock site (50%), and in June 1994

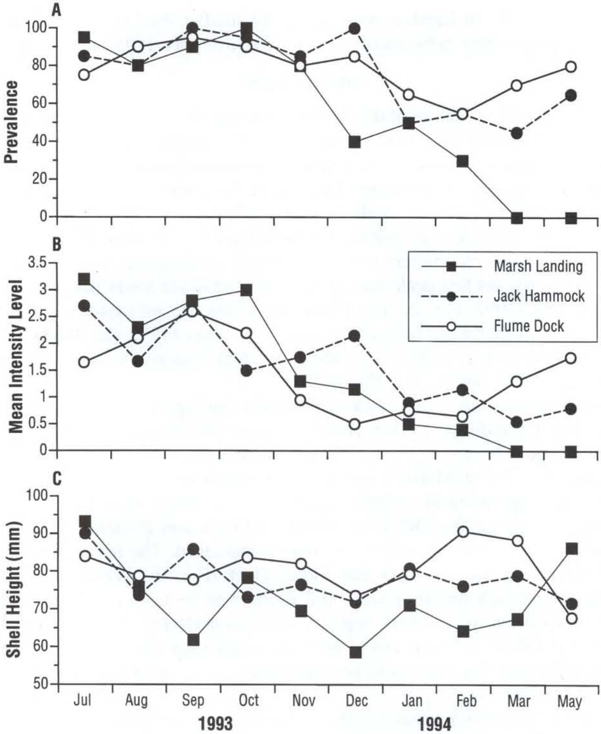


FIG. 6. Prevalence (A) and Intensity (B) of the protozoan parasite, *Perkinsus marinus,* and Mean Sizes of oysters (C) used in their evaluation.

at the Jack Hammock site (20%). No significant differences were attained among the sites when the prevalences were pooled by ANOVA (p = 0.144).

Intensity levels throughout the study were variable among the three sites. However as with the prevalences, there did appear to be a tendency for the values to decrease as the sampling progressed (Fig. 6b). Maximum intensity at the Marsh Landing site was achieved in the first month of sampling (July 1993) with a value of 3.2. Peak intensity values were realized in September at both of the other two sites, i.e., Flume Dock (2.6) and Jack Hammock (2.8) (Fig. 6b). The decrease in the intensity levels through the Duplin River appeared to continue from January through March when minimum values were attained at Flume Dock in December 1993 (0.5), and in March and May at Marsh Landing (0), and in June at the Jack Hammock site (0.2).

The sizes of the oysters used in the Dermo study are presented in Fig. 6c.

Animals from Marsh Landing were generally smaller than those from the other two sites, particularly between the months of November 1993 and March 1994.

DISCUSSION

The most striking feature of the 1993 recruitment data was the overall reduction in oyster recruitment at all sites examined, when compared to the 1992 data (Fig. 2). The difference between the two years, was not only confined to the numbers of oysters found on the collectors, but also to the patterns of recruitment within the years. Peak recruitment at all sites in 1992, invariably occurred in August, while in 1993, maximum recruitment occurred in May at the Jack Hammock site, with low levels of recruitment thereafter. Marsh Landing did attain peak recruitment in August, yet this peak was an order of magnitude lower than the peak the previous year (1992; Fig. 2). The Flume Dock site had no oyster recruitment in 1993. One fact that was consistent between the years (1992 and 1993) was that the recruitment levels at Marsh Landing and Jack Hammock were considerably greater than those observed at Flume Dock.

Temperatures at the Flume Dock site tended to be higher in the summer months than at Marsh Landing (Fig. 3c). However, these differences were not sufficiently large to account for the disparity of oyster recruits between the sites. Peak temperatures recorded at Marsh Landing and Flume Dock were around 30°C. An increase in temperature to approximately 30°C was shown to increase oyster settlement in a hatchery by Lutz et al. (1970) and Hidu and Haskin (1971). Salinity differences throughout the year were more pronounced. The Marsh Landing site tended to have higher salinities than Flume Dock (Fig. 3b). Again, the ranges of salinities were such that they were still deemed to be within optimal limits for larval survival and recruitment viability (Hidu and Haskin, 1971; Feeny, 1984). Yet, the pH values at Flume Dock, although lower than those at Marsh Landing, were not deemed low enough to be detrimental to larvae (Calabrese and Davis, 1966, 1970; Fig. 3a).

Given that recruitment was similarly reduced in a corresponding study in Wassaw Sound, Georgia, 100 km north of the Duplin River (O'Beirn et al., 1996b), naturally occurring variations in recruitment must be considered. Such natural fluctuations in recruitment on a yearly basis have been observed in the Chesapeake Bay (Haven and Fritz, 1985) and at North Inlet, South Carolina (Kenny et al., 1990). These studies have documented years of high recruitment followed by years of greatly reduced levels of spatfall.

Recruitment success is greatly dependant upon successful gametogenesis and subsequent spawning of gametes in the source/established population. Major oyster spawning occurred later in the fall at all sites, yet spawning activity did not reflect subsequent recruitment. Oyster recruitment levels remained low after the initial peak levels achieved in May at Jack Hammock Site. The peak recruitment level in August at Marsh Landing site was only marginally larger than the earlier recruitment value observed in May and was lower by an order of magnitude than the value observed at this site in the previous year, 1992 (Fig. 2; O'Beirn et al., 1994a).

It is clear from the gametogenic data (Figs. 4 and 5), that the primary spawning events observed in this study occurred later in the year than usual (Heffernan et al. 1989), thus accounting for perhaps the overall reduced recruitment levels for

1993. Yet, spawning events, as witnessed by the histological analysis, did not appear to result in subsequent recruitment events. Also, the early season recruitment cannot be accounted for by a reduction in gametogenic parameters values, indicative of spawning.

Another aspect highlighted by the quantitative reproductive data (Figs. 4 and 5) is the fact that the mass spawning events at all three sites occurred earlier in females (Fig. 5c) than males (Fig. 4a). The primary male spawn occurred two weeks and six weeks after the females at Marsh Landing/Jack Hammock and Flume Dock, respectively (Fig. 4b). It would seem unlikely that male gametes were not in the water when the eggs were present. Galtsoff (1964) described how the male oysters will spawn continuously throughout the reproductive season. This was followed by a large continuous spawn in males at the end of the same season, which can last for several hours (Galtsoff, 1964). This characteristic end of season discharge of male gametes appeared to be exhibited by the populations in this study (Fig. 4). Loosanoff (1937) with *Mercenaria mercenaria* and Galtsoff (1964) with *Crassostrea virginica,* both report that males responded to spawning stimuli prior to the females presumably to initiate a concomitant response in the females. For this reason the delayed detection of major spawning behavior in males in this study was highly surprising and noteworthy.

The protozoan parasite, *Perkinsus marinus* (Dermo), was first recorded in coastal Georgia in 1966 (Lewis et al., 1992). Between 1985 and 1987, Dermo occurred in epizootic proportions resulting in high oyster mortalities along the Georgia coast (Lewis et al., 1992). These mortalities resulted in a near total collapse of the oyster industry within the state (Brad Williams, Georgia Department of Natural Resources, Brunswick, GA, pers. comm.). In their study, Lewis et al. (1992) also reported a high prevalence of Dermo (44%) in oysters on the Duplin River in both 1966 and 1987. In this study, the overall patterns of prevalence and intensities of Dermo in oysters exhibited over time did appear to mimic those of other regions (Andrews and Hewatt, 1957; Mackin, 1962; Quick and Mackin, 1971; Crosby and Roberts, 1990). High levels of both parameters occurring in the summer and fall of 1993 were followed by a decrease in winter, with the levels appearing to rise again in late spring and early summer.

The sudden decrease in prevalence of Dermo in oysters at the Marsh Landing site in December 1993 could be attributable to the smaller size of oysters sampled at this site during this period. These data would seem to confound one of the hypotheses of this study, (i.e., differences in Dermo levels among sites were attributable to exogenous environmental factors). Inconsistency in the sizes of the oysters retrieved from any of the three sites could make it difficult to attribute any differences, in the prevalence or intensity of the parasite among the sites, to some environmental factor. Smaller oysters have been deemed to be less susceptible to Dermo infestation than older, larger forms (Ray, 1953). The sizes of the oysters attained at the Marsh Landing site could fall within the size range of juvenile oysters (60–70 mm), which had only set a few months prior to the sampling (O'Beirn et al., 1996a,b). One other possible cause for the disparity in Dermo prevalence among the sites could be caused by some inhibitor of Dermo prevalence present at the Marsh Landing site. Such an inhibitor as proposed by Hoese (1963) could be related to the higher salinities observed at the Marsh Landing site during the latter portion of 1993 when compared to other years (S.

Smith, Marine Institute, Sapelo Island, pers. comm.). Also, the salinities were substantially higher at the Marsh Landing site than at the Flume Dock site for much of the same period in 1993 (Fig. 3b). While the range of salinities experienced at all sites fall within those deemed tolerable for Dermo by other authors (Soniat et al., 1989; Gauthier et al., 1990; Powell et al., 1992), the question of whether the significant increase in salinity from one year to another might prove debilitating to the parasite might be addressed. Races of Dermo may respond differently to varying environmental conditions (Bushek and Allen, 1996).

Overall, it appears, based on the findings herein, the levels of Dermo exhibit a high prevalence in oysters on the Duplin River throughout the year. These findings are consistent with the findings of other studies in the region (Hoese, 1968; Lewis et al., 1992; O'Beirn et al., 1994b). However, while the prevalences were high, the intensities remained moderate suggesting that risk of oyster mortalities attributable to the presence of the parasite, are not high. Consequently, the oyster beds on the Duplin River did not appear to be unduly stressed.

The question of larval flushing out of (or into) a large tidal creek (i.e., the Duplin River) on an ebb tide cannot be ruled out, given that the Duplin River and tidal creeks exchange approximately 40% of their water volume in a single tidal cycle (Ragotzkie and Bryson, 1955). It is conceivable on an outgoing tide, that more water (and larvae) could be removed from the upper portions of the river than lower down, given the shallow and narrow nature of the uppermost reaches. Also, the lack of recruitment after spawning events, suggested that gametes and/or larvae were removed from that portion of the Duplin River. The hydraulics of the Duplin River undoubtably influences the fate of oyster larvae, given that they may be at the vagaries of such forces for two to three weeks. As we are unaware of the circulation patterns within the Duplin River, we are unwilling to speculate further regarding the fate of oyster larvae into and out of the system. A comprehensive investigation into physical attributes and circulation patterns within the Duplin River might address some of these questions and prove enlightening.

The primary goal of this study was an attempt to relate the low oyster recruitment levels at the uppermost site on the Duplin River to gametogenic and/or parasite (Dermo) prevalence in the adult oysters as well as hydrographic characteristics adjacent to the sampling site. Much interesting information was gathered during this study, such as; reduced recruitment in the Duplin River when compared with 1992 data; elevated temperatures at the Flume Dock site throughout the summer of 1993; and delayed gametogenesis and spawning witnessed at Flume Dock site when compared with the other two sites. However, based on our findings, it is clear that no one variable can be deemed causal in reducing the number of oyster recruiting at the Flume Dock site.

Acknowledgments: The authors wish to thank Dr. James Alberts, Dr. Alice Chalmers, Julia Mensler, Sean Smith and the staff of the University of Georgia Marine Institute on Sapelo Island for their cooperation throughout the course of this study. The assistance of the Georgia Department of Natural Resources is also acknowledged. Mary Sweeney and Christopher Spruck carried out some of the histological analysis. Anna Boyette is thanked for work on the figures. Dorset Hurley and Deborah Moroney and two anonymous reviewers are thanked for reviewing the manuscript. This work was primarily supported by USDC NOAA Grant NO. 2731RE337-049 (Sapelo Island National Estuarine Research Reserve) with additional support from the University of Georgia Marine Extension Service and the Georgia Sea Grant College Program under Grant No. NA84AA-D-00072.

REFERENCES CITED

ANDREWS, J. D., AND W. G. HEWATT. 1957. Oyster mortality studies in Virginia II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. Ecol. Monog. **27**:1–27.

BUSHEK, D., AND S. K. ALLEN JR. 1996. Races of *Perkinsus marinus*. J. Shellfish Res. **15**:103–107.

CALABRESE, A., AND H. C. DAVIS. 1966. The pH tolerance of embryos and larvae of *Mercenaria mercenaria* and *Crassostrea virginica*. Biol. Bull. **131**:427–436.

———, AND ———. 1970. Tolerances and requirements of embryos and larvae of bivalve molluscs. Helgolander Wiss. Meeresunders. **20**:553–564.

CROSBY, M. P., AND C. F. ROBERTS. 1990. Seasonal infection intensity cycle of the parasite *Perkinsus marinus* (and absence of *Haplosporidium* spp.) in oysters from a South Carolina Salt Marsh. Diseases Aquat. Org. **9**:149–155.

ELLIOTT, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biol. Assoc. Sci. Publ. No. 25. 160 pp.

FEENY, C. F. 1984. Effects of salinity on the vertical distribution of the larvae of *Crassostrea virginica* (Gmelin) and *Ostrea equestris* (Say). J. Shellfish Res. **4**:88–89.

GALTSOFF, P. S. 1964. The American oyster, *Crassostrea virginica* Gmelin. Fish. Bull. **64**:1–480.

GAUTHIER, J. D., T. M. SONIAT, AND J. S. RODGERS. 1990. A parasitological survey of oysters along salinity gradients in coastal Louisiana. J. World Aquacult. Soc. **21**(2):105–115.

HAVEN, D. S., AND L. W. FRITZ. 1985. Settling of the American oyster, *Crassostrea virginica*, in the James River, Virginia, USA: temporal and spatial distribution. Mar. Biol. **86**:271–282.

HEFFERNAN, P. B., R. L. WALKER, AND J. L. CARR. 1989. Gametogenic cycles of three bivalves in Wassaw Sound, Georgia: I. *Crassostrea virginica* (Linnaeus, 1758). J. Shellfish Res. **8**:61–70.

HIDU, H., AND H. H. HASKIN. 1971. Setting of the American oyster related to environmental factors and larval behavior. Proc. Natl. Shellfish. Assoc. **61**:35–50.

HOESE, H. D. 1963. Absence of *Dermocystidium marinum* at Port Aransas, Texas, with notes on an apparent inhibitor. Tex. J. Sci. **15**(1):98–103.

———. 1968. Studies on the parasitic oyster fungus, *Labyrinthomyxa sp.* in Georgia salt waters. Pp. 102–120 in T. L. Linton (ed.), Feasibility study of methods for improving oyster production in Georgia. Mar. Fish. Div., GA Game Fish. Comm., Univ. Georgia, Compl. Rep. 2-10-R.

HOWARD, D. W., AND C. S. SMITH. 1983. Histological techniques for marine bivalve molluscs. NOAA Technical Memorandum NMFS-FINEC-25. 97 pp.

KENNY, P. D., W. K. MICHENER, AND D. M. ALLEN. 1990. Spatial and temporal patterns of oyster settlement in a high salinity estuary. J. Shellfish Res. **9**:329–339.

LEWIS, E. J., F. G. KERN, A. ROSENFIELD, S. A. STEVENS, R. L. WALKER, AND P. B. HEFFERNAN. 1992. Lethal parasites in oysters from coastal Georgia with discussion of disease and management implications. Mar. Fish. Rev. **54**:1–6.

LOOSANOFF, V. L. 1937. Spawning of *Venus mercenaria* (L.). Ecology **18**:506–515.

LUTZ, R. A., H. HIDU AND K. G. DROBECK. 1970. Acute temperature increase as a stimulus to settling in the American oyster, *Crassostrea virginica* (Gmelin). Proc. Natl. Shellfish. Assoc. **60**:68–71.

MACKIN, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publ. Inst. Mar. Sci., Univ. Texas **7**:132–229.

O'BEIRN, F. X., C. C. DEAN, AND R. L. WALKER. 1994b. Prevalence of *Perkinsus marinus* in the eastern oyster, *Crassostrea virginica*, in relation to tidal placement in a Georgia tidal creek. Northeast Gulf Sci. **13**(2):79–87.

———, P. B. HEFFERNAN, AND R. L. WALKER. 1994a. Settlement of *Crassostrea virginica:* A tool for Monitoring the Aquatic Health of the Sapelo Island National Estuarine Research Reserve. Univ. Georgia, School Mar. Prog., Mar. Tech. Rept. No. 94-2. 42 pp.

———, ———, AND ———. 1996b. Recruitment of the eastern oyster in coastal Georgia: patterns and recommendations. N. Am. J. Fish. Mag. **16**:413–426.

———, ———, ———, AND M. L. JANSEN. 1996a. Young-of-the-year oyster, *Crassostrea virginica* reproduction in coastal Georgia. Estuaries **19**:651–658.

POWELL, E. N., J. D. GAUTHIER, E. A. WILSON, A. NELSON, R. R. FAY, AND J. M. BROOKS. 1992. Oyster disease and climate change. Are yearly changes in *Perkinsus marinus* parasitism in oysters (*Crassostrea virginica*) controlled by climatic conditions in the Gulf of Mexico? Mar. Ecol. **13**(3):243–270.

QUICK, J. A., JR., AND J. G. MACKIN. 1971. Oyster parasitism by *Labyrinthomyxa marina* in Florida. Fla. Dept. Nat. Resour., Mar. Res. Lab., Prof. Pap. Ser. 13. 55 pp.

RAGOTZKIE, R. A., AND R. A. BRYSON. 1955. Hydrography of the Duplin River, Sapelo Island, Georgia. Bull. Mar. Sci. Gulf Carib. **5**:297–314.

RAY, S. M. 1953. Studies on the occurrence of *Dermocystidium marinum* in young oysters. Natl. Shellfish. Assoc. Conv. Address (1953):80–92.

———. 1963. A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. Proc. Natl. Shellfish. Assoc. **54**:389–404.

SAS, INST., INC. 1989. SAS User's Guide. Statistics. Version 6. SAS Institute Inc., Cary, N.C. 846 pp.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. Second Edition. W. H. Freeman and Company, N.Y. 859 pp.

SONIAT, T. M., L. E. SMITH, AND M. S. BRODY. 1989. Mortality and condition of the American oyster in Galveston Bay, Texas. Contrib. Mar. Sci. **31**:77–94.

Received 31 March 1997

EDITOR'S COMMENTS

The Journal of the Elisha Mitchell Scientific Society, the official publication of the North Carolina Academy of Science, is now up to date. Local, national, and international manuscripts are welcome and will be given prompt peer review. While the journal is printed in black and white, color photos are also welcome; however, cost for their production rests with the author. Regular figures should be submitted as high resolution *black* and *white* glossy prints. Present page charges are $30/page. Authors lacking publication support funds may request a waiver for all or part of the page charges. Such requests should be made to the editor at the time of submission.