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Recruitment, gametogenesis and parasite (*Perkinsus marinus*) prevalence in the eastern oyster, *Crassostrea virginica*, within the Sapelo Island National Estuarine Research Reserve

Francis X. O'Beirn
Randal L. Walker
Michelle L. Jansen
Christopher R. Spruck

The University of Georgia

School of Marine Programs
Athens, Georgia

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Abstract

Year two of a monitoring program designed to chart oyster, *Crassostrea virginica* (Gmelin 1791), recruitment within the Sapelo Island National Estuarine Research Reserve was completed. Recruitment levels were established to serve a baseline data set for evaluating system health and augment management protocols. Recruitment levels of oysters in 1993 were considerably reduced compared to 1992, as were levels at sites in Wassaw Sound located 100 km to the north. The decrease in recruitment was attributed to a combination of elevated water temperatures in 1993 as well as natural recruitment fluctuations.

In an attempt to explain the various oyster spat recruitment patterns observed among sites in the Duplin River, the gametogenic cycles of oysters on three separate beds encompassing the length (8 km) of the river were determined. Gametogenic development and spawning at the Marsh Landing site (mouth of river) began two weeks earlier than at the Jack Hammock site (4 km upriver), which in turn occurred two weeks earlier than at the upper-river site (Flume Dock). Significant differences in hydrological data were detected between Marsh Landing and Flume Dock, with water temperature higher at the Flume Dock site and salinity and pH higher at Marsh Landing. Roughly twice as many females as males occurred at all sites. Different gametogenic patterns within the Duplin River might partially explain the reduction or absence of oyster recruitment in the Flume Dock site.

Estimation of the prevalence and intensity of the protozoan parasite, *Perkinsus marinus* (Dermo), in oysters was carried out monthly at the three sites on the Duplin River. Dermo prevalence levels in the oysters were high; however, the intensities of the parasite were such that the pathogen was not deemed to be a major inducer of mortality on the oyster beds. Oyster sizes did appear to be related to the presence of Dermo, with smaller oysters displaying lower prevalences of Dermo than the larger forms.

Keywords: bivalve, coast, disease, estuary, fishery, gametogenesis, intensity, mollusc, parasite, prevalence, recruitment, resource, saltmarsh, sex ratio, spawning, stock

List of Figures

List of Tables

iv

Table of Contents

Introduction

Because of their commercial importance and extended range (Burrel 1985), oysters have been studied more than perhaps any other marine organism. The accumulated body of knowledge available on this organism and its ubiquity within an area make it an appropriate bio-indicator of the health of a particular estuarine environment (Phelps and Mihursky 1986; Couch and Hessler 1989). For these reasons, the eastern oyster, *Crassostrea virginica* (Gmelin 1791), was chosen as an indicator organism to monitor the health of the Sapelo Island National Estuarine Research Reserve (SINERR). More specifically, oyster recruitment was used to monitor the health of the reserve. The results of the first year's study (O'Beirn *et al.* 1994a) indicated much spatial as well as temporal variation in oyster recruitment among sites. In 1992, oysters began settling in May-June, peaked in August, and ceased in October-November. Monthly samples indicated that recruitment on mean low-water and subtidal collectors had comparable numbers of setting oysters. In contrast, intertidal collectors, two hours above mean low-water mark, where most oysters naturally occur, exhibited lower numbers (Galstoff and Luce 1930; Galstoff 1964; O'Beirn 1995; O'Beirn *et al.* 1995). However, seasonal collectors deployed for the duration of the sampling period had significantly higher numbers of oysters intertidally (O'Beirn *et al.* 1994a). One striking difference in recruitment patterns occurred at the Flume Dock site, where overall recruitment was lower despite the presence of moderate numbers of adult oysters. In 1992, recruitment was lower by three orders of magnitude at Flume Dock when compared to the other two sites. One possible explanation for the different recruitment numbers among sites was that the gametogenic cycle was either delayed or retarded in the upper reaches of the Duplin River, and somehow this translated into a reduction in the levels of spatfall in this portion of the river.

Another possible explanation of recruitment failure could be differences among the sites in the intensity or prevalence of organisms which cause disease in oysters. 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

1

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, GA DNR, pers. comm.). In their study, Lewis *et al.* (1992) also reported a high prevalence of Dermo on the Duplin River (44%) and in creeks adjacent to Doboy Sound in both 1966 and 1987.

The present report describes the results of the monitoring study carried out on the SINERR during the 1993 spawning season (April-October, Heffernan *et al.* 1989a). It relates recruitment with continuous hydrographic data from two of the sites on the Duplin River. In addition, this study investigates whether differences in gametogenesis and prevalence of the protozoan parasite, Dermo, occurred at the three sites, and whether such differences may account for some of the observed variability in recruitment in the area.

Site Description

The work was carried out on the Duplin River within the Sapelo Island National Estuarine Research Reserve, Georgia (SINERR; Fig. 1). Located west of Sapelo Island, the SINERR incorporates portions of the southern and western parts of the island. The area is predominantly marsh with numerous tidal creeks. Salinities at the site historically average over 20 ppt. (Stevens 1983). Within the SINERR, the UGA Marine Institute has two monitoring stations, 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, measures water temperature, pH and salinity. Sampling sites were established in the area adjacent to these two stations. A third site, Jack Hammock, near Lumber Dock, is approximately halfway between the other two sites.

Materials and Methods

Recruitment Evaluation

Longitudinally grooved PVC tubing (1.9cm diameter) with embedded chips of calcium carbonate were used for the collection of spat. A 12-cm section of tubing was used for each

2

collector giving a sampling area of approximately 100 cm^2. Five replicate collectors were attached to a portable PVC frame which in turn was attached (using cable ties) to a fixed frame on site (see O'Beirn *et al.* 1994a for complete design specifications). At each of the three sites, recruitment was estimated at three tidal heights: subtidally, at mean low water and intertidally approximately two hours after mean-low water. The three tidal heights were chosen to reflect various environmental extremes to which newly set spat would be subjected, i.e., from a zone of lower risk of desiccation and higher risk of predation to a zone of higher risk of desiccation and lower risk of predation. Upon return to the laboratory, each collector was rinsed with saline water to remove extraneous matter. The collectors were then examined with a binocular microscope at 10X and the oysters enumerated. Monthly sampling consisted of replacing an array of collectors at each site from April 1993 until November 1993, after which sampling ceased. The seasonal collectors were deployed in April and left on site for the duration of the study.

Statistical Analysis

All of the raw data (numbers of recruits per 0.01m^2) were log transformed, a consequence of the high degree of variability of numbers over the sampling period (Sokal and Rohlf 1981). Analysis of Variance (ANOVA: α=0.05) and Tukey's Studentized Range Test (TSR) 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. The mean daily values for temperature, pH and salinity were pooled biweekly and subsequently were compared. All statistical analyses were carried out using PC SAS (SAS Inst. Inc. 1989).

Gametogenic Evaluation

Biweekly collection of oysters at the three aforementioned sites occurred from April 30 through December 14, 1993 during the major spawning season (Heffernan *et al.* 1989a) and monthly between December 1993 and May 1994. Oysters were collected from a standard

3

intertidal height at all sites and measured for shell height (hinge-to-lip) to the nearest 0.1 mm with Vernier calipers. The lower shell was removed and a gonadal tissue cross-section (ca. 0.5 cm in width) was dissected from each oyster at a standard area approximately 1 cm toward the hinge from the adductor muscle. The gonadal sections were held in Davidson's fixative for 48 hours 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 in thickness using a rotary microtome. Sections were stained with Harris Hematoxylin and counter-stained with Eosin (Howard and Smith 1983).

Qualitative Reproductive Analysis

Histological slides were examined with a Zeiss Axiovert 10 microscope (20X), sexed, and assigned to a development stage as described by Heffernan *et al.* (1989b), where Sexually Undifferentiated = 0; Male and Female Early Active = 3; Male and Female Late Active = 4; Male and Female Ripe = 5; Male and Female Partially Spent/Spawning = 2; Male and Female Spent = 1. Mean male and female monthly gonadal index (G.I.) values were computed for each of the three sites.

Quantitative Reproductive Analysis

Quantitative analysis of gonadal preparations was performed using Color Image Analyzed Densitometry Microscopy housed at Skidaway Institute of Oceanography, Savannah, Georgia (Kanti *et al.* 1993; O'Beirn 1995). Photomicroscope images (10X1.25 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

4

analyzed for percent gonadal area, percent of that gonadal area occupied by follicles, oocyte number per standard area, and mean oocyte diameter. Egg numbers per standard area were manually counted on the Trinitron screen from two areas within one field, and oocyte diameter of nucleated oocytes was measured directly on the screen. Males were analyzed for percent gonadal area and percent area occupied by follicles.

Mean individual values for each data category were examined by the image analyzer. Mean monthly values were then computed and used in quantitative assessment of reproduction. Sex ratios were tested against a 1:1 ratio with a Chi-square statistic (Elliott 1977).

Statistical analysis of data was performed by ANOVA (α=0.05) and Tukey's SRT using SAS for PC computer (SAS Inst. Inc. 1989). Prior to statistical analysis, all percentage data were arcsine transformed.

Disease Evaluation

Oysters (N=20 per site) were collected from the same tidal height at Marsh Landing, Jack Hammock and Flume Dock from July 1993 to June 1994. Oysters were measured for shell height to the nearest 0.1 mm, and a portion of each animal's rectum and mantle was dissected. 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 (α=0.05) and Tukey's SRT using SAS for PC computer (SAS Inst. Inc. 1989). Prior to statistical analysis, all prevalence data were arcsine transformed.

Results

Marsh Landing

Oyster recruitment for 1993 was first recorded at the end of May and beginning of June at this site ($\bar{x}$=5.2±1.7SE). Overall, recruitment was low and increased to a maximum of only 20.3±4.2SE spat/0.01m^2 in August (Fig. 2). Hereafter, all spat means are given per 0.01m^2. Recruitment ceased in October after very low numbers were obtained in September ($\bar{x}$=2.1±0.6SE). For the pooled monthly data from each of the tidal heights, the low water collectors captured significantly more oysters than did those at the other two tidal heights (p=0.0008). For the seasonal data, there were no differences in numbers of recruits among the tidal heights (p=0.1089).

Jack Hammock

Recruitment at this site was extremely high at the first sampling in May ($\bar{x}$=301.5±51SE), after which it decreased precipitously. Extremely low levels were recorded for the rest of the study (Fig. 2). There were no differences in recruitment among the tidal heights at this site for the pooled monthly data (p=0.1002). For the seasonal data, there were no differences among the tidal heights in terms of oyster recruitment (p=0.0637).

Flume Dock

No oyster recruitment was obtained on any of the monthly or seasonal collectors at this site throughout 1993.

Inter-site Comparisons

Monthly data for the Jack Hammock and the Marsh Landing sites showed similar recruitment levels (p=0.1044). For the seasonal data, again there were no distinctions between the Jack Hammock and Marsh Landing sites (p=0.4192).

6

Hydrographic Analysis

Marsh Landing had significantly higher pH values throughout the year than Flume Dock (Fig. 3a) and significantly higher salinity values in most biweekly periods (Fig. 3b). The results of the ANOVAs carried out on the temperature data from the Duplin River revealed that the Flume Dock site showed significantly higher values than the Marsh Landing site in April, May, July and August (Fig. 3c).

Gametogenesis

Significantly more females than males occurred at all sites (Table 1). Roughly twice as many females as males occurred at Marsh Landing (1.00F:0.46M), and Flume Dock (1.00F:0.54M) sites, while the ratio was 1.00F:0.61M at the Jack Hammock site. The overall sex ratio for oysters in the Duplin River was 1.00F:0.54M, which differed significantly from a 1:1 ratio (Chi-square=66.70, p<0.05).

Qualitative Results

Ripe oysters were recorded at all three sites from April-June 1993 (Fig. 4). A gradual decrease in G.I.'s was observed in July and August at the Jack Hammock site, followed by an increase in September to 4.75. Ripe animals continued to be sampled at the other sites (Marsh Landing and Flume Dock) through August (Fig. 4). In subsequent samples, there appeared to be a lag in spawning activity (indicated by sharp declines in G.I. values) moving north along the Duplin River, i.e., spawning was first observed at Marsh Landing (mid-August), followed by Jack Hammock (early September) and Flume Dock (mid-September).

Gametogenic activity had ceased at all sites by November 1993. In 1994, an increase in activity was first recorded at Marsh Landing in February. Ripe animals were apparent at all sites by May (Fig. 4). Again, gametogenic activity was first observed at the Marsh Landing site with a lag of two weeks at the other sites.

Quantitative Results

The proportion of area occupied by gonads in the males (Fig. 5a) remained relatively high (80-100%) at all three sites from April through mid-August. However, gonadal content decreased sharply thereafter, initially at the Jack Hammock site and two weeks later at Marsh Landing. After another two-week period (October), the oysters at the Flume Dock site displayed a similar decrease in gonad content. These declines indicated a decrease in the potential area for the manufacture of male reproductive cells. The area occupied by follicles in oysters increased to approx. 85% at Flume Dock and Marsh Landing, and 75% at Jack Hammock in mid-August (Fig. 5b). 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.

From April through mid-June, all of the female reproductive parameters remained, for the most part, constant at all three sites (Fig. 6a-d). In July there was a sharp decrease in the gonad area at the Jack Hammock site (Fig 6a) and a sharp decline in mid-July at Marsh Landing (Fig. 6a). A more gradual decline also was witnessed in the follicle area as well as the oocyte diameter parameters from the Marsh Landing site (Fig. 6b,d). However, at the Marsh Landing site, the mean number of eggs per field appeared to increase over the aforementioned time period (Fig. 6c).

The gonad area and follicle area (Fig. 6a,b) at the Flume Dock site had consistently higher values than did those at the other two sites for the latter part of the summer (1993). Spawning at all sites was probably best indicated by a sharp decline in the mean number of eggs per field parameter (Fig. 6c) in mid-August. Declines in egg numbers were followed by declines in gonad and follicle areas in subsequent sampling periods (Fig. 6a-c).

Egg sizes (Fig. 6d) 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 and early in 1994.

Disease

Initial estimates of Dermo prevalence were high for all of the sites in July 1993 (Fig. 7a). These estimates ranged from 75% at Flume Dock to 95% at Marsh 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%). In December the percentage of oysters with Dermo at the Marsh Landing site declined sharply from 80 to 40% (Fig. 7a). Prevalences also decreased at the other two sites 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 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 quite variable among the three sites. However as with the prevalences, values tended to decrease as the sampling progressed (Fig. 7b). 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. 7b). 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). Significant differences among the sites were attained in July 1993 (p=0.0415), October 1993 (p=0.0480), December 1993 (p<0.0001), March 1994 (p=0.0054), and May 1994 (p=0.0031). However, overall there were no differences in the intensity of Dermo among the sites (p=0.5642).

The sizes of the oysters used in the Dermo study are presented in Fig. 7. Animals from Marsh Landing were generally smaller than those from the other two sites, particularly between

9

the months of November 1993 and March 1994. This is borne out by the statistical analysis (ANOVA) in which Marsh Landing ($\bar{x}$=70.8 mm) oysters are significantly smaller than oysters from both Flume Dock ($\bar{x}$=82.4 mm) and Jack Hammock ($\bar{x}$=77.8 mm) (p<0.0001).

Discussion

The most striking feature of the 1993 data, when compared to that of 1992, was the overall reduction in oyster recruitment at all sites examined. The difference pertained not only to the number of oysters found on the collectors (p<0.0001; Fig. 2), but also to the patterns of recruitment within the years. In 1992, peak recruitment at all sites invariably occurred in August (Fig. 2: O'Beirn et al. 1994a). In 1993 however, 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 recruitment whatsoever in 1993.

The lack of recruitment at the Flume Dock site in 1993 might warrant some cause for concern. However, given the extremely low numbers of oysters that actually recruited in 1992 at this site (O'Beirn et al. 1994a), the results of 1993 are not surprising. Overall recruitment was extremely low in 1993 not only in the Sapelo Reserve but along the entire Georgia coast. This is evidenced by the low rates of recruitment experienced in Wassaw Sound in a corresponding study (O'Beirn 1995).

Examination of the hydrographic data for the Duplin River in 1993 between the Marsh Landing and Flume Dock sites revealed some significant differences (Fig. 3a-c). 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 sufficient to account for the disparity of oyster recruits between the sites. Peak values obtained at Marsh Landing and Flume Dock were around 30°C. An increase in temperature to approximately 30°C was shown to increase oyster settlement by Lutz et al. (1970) and Hidu and Haskin (1971). Salinity differences throughout the year were more pronounced. The Marsh Landing site had significantly higher salinities than Flume Dock (Fig. 3b). However, the ranges of salinities were optimal for larval survival and recruitment viability (Hidu and Haskin 1971; Feeny 1984). The pH values at Flume Dock, although significantly lower, were still above neutral value and were not deemed detrimental to larvae (Calabrese and Davis 1966, 1970; Fig. 3a).

11

Because the Duplin River and tidal creeks lose approximately 40% of their water volume on an outgoing tide (Ragotzkie and Bryson 1955), it is possible that larvae were flushed out on an ebb tide.

The tidal marsh systems, indicative of the Georgia coastline and in particular the oyster reefs located therein, are highly specialized environments (Bahr 1976). Oysters form the basis of a rich and diverse community which has adapted to extremes in both temperature and salinity. Fluctuations in these hydrological parameters usually do not adversely affect the reefs unless such changes are protracted. However, a similar change (albeit relatively small) could have a major effect on the more sensitive larval oyster. The prolonged increase in temperature witnessed in 1993 compared to 1992 could have resulted in reduced viability of the larvae, resulting in a reduction in the overall recruitment.

A corresponding study of recruitment in Wassaw Sound, Georgia (O'Beirn 1995), 100 km north of the Duplin River, evidenced similarly reduced levels, which suggests that naturally occurring variations in recruitment could account for 1993's numbers. Such natural fluctuations in annual recruitment 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. In general, the year 1993 could have been a year of reduced recruitment of oysters within the Duplin River and coastal Georgia. Recruitment success can be due, in no small way, to successful gametogenesis and subsequent spawning of gametes in the established population.

The general pattern of gametogenesis of oysters in this study is comparable to results found in oyster beds studied in Wassaw Sound (Heffernan et al. 1989a; O'Beirn 1995), St. Catherines Sound and Sapelo Island, Georgia (Durant 1968). A continuous buildup of gametogenic material followed by a single major spawning event were evidenced in all studies. However, in this study the major spawning event was retarded by up to a month compared with the other studies in the region. Similar reproductive patterns have been indicated for North Carolina (Chestnut and Fahy 1952) and South Carolina (McNulty 1953). This pattern is in

12

contrast to the bimodal spawning periods (spring and fall) which are typical of oysters in the Gulf of Mexico from Florida (Hayes and Menzel 1981); Louisiana (Menzel 1951; Hopkins *et al.* 1953); Texas (Hopkins 1931), and Mexico (Rogers and Garcia-Cubas 1981). In northern Atlantic coast oyster populations, gametogenesis started later and peaked earlier than in southern oyster populations (Coe 1932; Loosanoff 1942; Kennedy and Battle 1964; Kennedy and Krantz 1982; Heffernan *et al.* 1989a; this study).

According to the qualitative results, oyster spawning was successive and separated by a two-week delay between each of the three oyster beds along the Duplin River (Fig. 4). However, the cause(s) of this differential spawning is unknown. Significant differences in water temperature, salinity, and pH were observed between the two extreme sites (Flume Dock and Marsh Landing). Differences in spawning of oyster populations have been observed within beds of the Chesapeake Bay. Oysters in beds from the lower Chesapeake Bay region began spawning a month earlier in 1977 and 1978 than did those at five sites from the western Chesapeake Bay and eight beds from the upper Chesapeake Bay area (Kennedy and Krantz 1982). Water temperatures were slightly higher at the lower Chesapeake Bay sites, but Kennedy and Krantz (1982) hypothesized that spawning was not regulated by temperature alone (Geise 1959). They cited food-related chemicals and temperature-food stimulus as other possible regulatory factors.

The pH levels obtained during the course of the study, while different at the two sites, were within the levels of tolerance by oyster larvae as determined by Calabrese and Davis (1966, 1970). Davis (1958) determined that the optimum salinity range for larval development of oysters was closely related to the salinities at which the adults were conditioned. Despite the disparate salinities observed at the two sites over the course of the study, salinity is not deemed a major factor in the differences of gametogenesis observed between the sites. Durant (1968) found similar gametogenic development patterns and rates in oysters from medium and high saline areas in coastal Georgia.

Major spawning occurred later in the fall at all sites, yet spawning success did not reflect recruitment success. Oysters clearly spawned late in the season as expected; however, with the exception of a peak in recruitment in August at Marsh Landing site, oyster recruitment levels remained low after the initial peak levels achieved in May at Jack Hammock Site. The peak level in recruitment at Marsh Landing site was only marginally larger than the initial peak in May and was significantly lower, by an order of magnitude, than the 1992 peak (Fig. 2; O'Beirn *et al.* 1994a). In 1991 and 1992, peak recruitment levels (O'Beirn *et al.* 1994a; O'Beirn *et al.* 1995) generally occurred later in the season after the period of peak spawning (Durant 1968; Heffernan *et al.* 1989a; this study). Yet in 1993, peak spawning occurred in late summer with no corresponding peak recruitment except at the Marsh Landing site. In 1993, salinity and water temperatures in the Duplin River were significantly higher than in 1992 (J. Mensler, UGA Marine Institute, pers. comm.). Yet the mean temperature difference was only 1 to 2°C higher overall - well below 35°C where temperatures begin to negatively affect embryonic development (Taylor 1980). Major spawning at Marsh Landing started in mid-August (Fig. 4), and peak oyster recruitment levels occurred in August at this site; however, major spawning commenced two weeks later at Jack Hammock. Flume Dock spawning was experienced two weeks later still. Consequently, poor recruitment at the Flume Dock site may be due to rapidly declining water temperatures, which have been shown to affect the survival of oyster larvae (Davis and Calabrese 1964).

It is clear that the primary spawning events occurred later than usual (August-October), which perhaps accounts for the overall reduced recruitment levels for 1993. However, this theory is based on the assumption that much of the larvae setting in the Duplin River originated there. Allied to this is the observed lag in spawning at the Flume Dock site. Spawning occurred as late as October, and given a minimum of two weeks for larval development, temperature conditions in late October might be less than ideal for larval development (Fig. 3).

Another aspect highlighted by the quantitative reproductive data (Figs. 5 and 6) is the fact that the mass spawning events at all three sites occurred earlier in females (Fig. 6c) than males

(Fig. 5a). In fact, the primary male spawn occurred two weeks and six weeks after the females spawned at Marsh Landing/Jack Hammock and Flume Dock, respectively (Fig. 5b), which could account for the large spawn witnessed in the qualitative data (Fig. 4). It would seem unlikely that male gametes were not in the water when the eggs were present. However, this hypothesis cannot be totally discounted since the delay in spawning was so profound, particularly at the Flume Dock site, where no recruitment was observed. These spawning patterns exhibited by the males and females are surprising given the findings of other investigators. Galstoff (1964) described how the male oysters will spawn continuously throughout the reproductive season. This is followed by a large continuous spawn in males at the end of the same season which can last for several hours (Galstoff 1964). This characteristic discharge of male gametes is also exhibited by the populations in this study (Fig. 5). Loosanoff (1937) and Galstoff (1964) report that males respond to spawning stimuli prior to females, presumably to initiate a concomitant response in the females. For this reason the delayed detection of major spawns in males in this study is highly surprising and noteworthy.

The patterns of prevalence and intensities of *Perkinsus marinus* in oysters appeared to mimic those of oysters from other regions. High levels of Dermo occurred in the summer and fall of 1993 followed by a decrease in winter. Levels appeared to rise again in late spring and early summer.

The sudden decrease in prevalences of Dermo in oysters at the Marsh Landing site in December 1993 could be attributable to any one of or a combination of three factors: 1) the high prevalences and intensities of Dermo in the preceding months resulted in high oyster mortality (Fig. 7) and therefore, only oysters with low infection levels were sampled in December, 2) the decrease was a result of a drop in temperature and salinity typically observed in coastal Georgia during winter (Fig. 3), both of which have been shown to retard Dermo infection (Andrews and Ray 1988), and 3) a decrease in Dermo prevalence was a consequence of sampling smaller oysters at the Marsh Landing site in December.

15

The first two factors probably are not the primary causative agents for the observed decline in prevalences at the Marsh Landing site. Similar factors existed at the other two sites, neither of which evidenced a precipitous decline in the levels of Dermo during December. Both Flume Dock and Jack Hammock did experience large decreases in prevalences in January, which is probably attributable to hydrographic factors (Fig. 3). The most probable cause of the sudden decline in Dermo levels at the Marsh Landing site in December is the significantly 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 are attributable to exogenous environmental factors) in that inconsistency in the sizes of the oysters retrieved from any of the three sites would make it difficult to attribute any differences in the prevalence or intensity of the parasite among the sites to some environmental factor. Smaller oysters are less susceptible to Dermo infestation than older, larger forms (Ray 1953). The sizes of the oysters at the Marsh Landing site would easily fall within the range of juvenile oysters (60-70 mm), which had only set a few months previous to the sampling (O'Beirn 1995). However, the results do seem to support the claim that younger oysters are less susceptible to Dermo infection than older ones. Yet another possible cause for the disparity in Dermo prevalence among the sites could be due to 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 than normal salinities observed at the Marsh Landing site during the latter portion of 1993 (S. Smith, Marine Institute, Sapelo Island, pers. comm.). The salinities in 1993 were significantly higher than 1992 for the months September through December (p<0.01). Also the salinities were significantly higher at the Marsh Landing site than at the Flume Dock site for much of the same period (Fig. 3b). While the range of salinities experienced falls within those deemed tolerable for oysters 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 must be addressed.

When comparing the results of this study to those obtained in other records of Dermo in the Duplin River and vicinity (Hoese 1968; Lewis *et al.* 1992), it is appreciated that the handling of the oysters and the subjectivity of the analysis will make comparisons somewhat nebulous. In this study, prevalence levels of Dermo in January 1994 (50-64%) are higher than those observed in January 1967 (15%) by Hoese (1968) and in January 1966 (44%) by Lewis *et al.* (1992). Two of the sites sampled by Lewis *et al.* (1992) in November 1987 are adjacent to the Marsh Landing site, i.e., Back River and South River. Both appeared to have high incidences of Dermo (100% prevalence) and had values comparable to those obtained at the Marsh Landing site in November 1993 (84%). It is unclear exactly where Hoese (1968) sampled his oysters, therefore comparisons are impractical.

Regarding the intensities of Dermo in oysters, comparisons between the two studies (this study and Lewis *et al.* 1992) are difficult to make because researchers employed different scales of enumeration. Lewis *et al.* (1992) used a scale of 1-9 (very light - very heavy), whereas this study used the scale determined by Quick and Mackin (1971), 0-6 (no infection - very heavy infection). Both studies had moderate-to-low levels of Dermo, i.e., approximately a value of 4 for Lewis *et al.* (1992) and a value of 2 for this study.

Overall, levels of Dermo maintain a high prevalence in oysters of the Duplin River and vicinity on a seasonal basis. These findings are consistent with the findings of other studies in the region (Hoese 1968; Lewis *et al.* 1992; O'Beirn *et al.* 1994b). While the prevalence of Dermo is high, intensities remain moderate which suggests that the parasite is not directly responsible for oyster mortalities. Consequently, the oyster beds on the Duplin River do not appear to be unduly stressed.

When compared to previous year's data, the results of the 1993 recruitment study highlight the importance of such monitoring studies. The fluctuations in recruitment levels and patterns could either be attributed to natural endogenous influences or to anthropogenic exogenous factors. Determining the effect of such factors is made easier by having the "control" sites located at SINERR. The Duplin River, part of a National Estuarine Reserve, is a relatively

17

pristine natural example of the Carolinian Biogeographic Province. It represents an invaluable comparative tool for assessing the impact of anthropogenic activities on other areas within this province. The levels of recruitment experienced 100 km further north on the Georgia coast closely mimicked those obtained in the Duplin River. Therefore, the drastic reduction in recruitment can be attributable to some natural (cyclical recruitment variations) or hydrological factor (increased temperature) and not some anthropogenically induced factor.

References

Andrews, J.D. and S.M. Ray. 1988. Management strategies to control the disease caused by *Perkinsus marinus*. Amer. Fish. Soc. Special Publication18: 257-264.

Bahr, L.M. Jr. 1976. Energetic aspects of the intertidal oyster reef community at Sapelo Island, Georgia, (U.S.A.). Ecology 57: 121-131.

Burrell, V.G. 1985. Oyster culture: In: Huner, J.V. and E.E. Brown (eds.). Crustacean and molluscan aquaculture in the United States. pp. 235-273.

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.

Calabrese, A. and H.C. Davis. 1970. Tolerances and requirements of embryos and larvae of bivalve molluscs. Helgolander Wiss. Meeresunders. 20: 553-564.

Chestnut, A.F. and W.E. Fahy. 1952. Studies on the setting intensities of oysters in Bogue Sound, North Carolina. Proc. Natl. Shellfish. Assoc. 43: 79-89.

Coe, W.R. 1932. Sexual phases in the American oyster (*Ostrea virginica*). Biol. Bull. 63: 419-441.

Couch, D. and T.J. Hessler. 1989. Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest). Olympia oyster. Biol. Rep. U.S. Fish and Wildl. Serv. 17 pp.

Davis, H.C. 1958. Survival and growth of clam and oyster larvae at different salinities. Biol. Bull. 114: 296-307.

Davis, H.C. and A. Calabrese. 1964. Combined effects of temperature and salinity on development of eggs and growth of larvae of *M. mercenaria* and *C. virginica*. Fish. Bull. 63: 643-655.

Durant, J. 1968. The effects of temperature and salinity upon the gonadal cycle of *Crassostrea virginica* (Gmelin) in Georgia waters. In: Linton, T.L. (ed.), Feasibility Study of Methods for Improving Oyster Production in Georgia. Mar. Fish. Div., Georgia Game and Fish Comm. and University of Georgia. Completion Report 2-10-R, Brunswick, Georgia 176 p

Elliott, J.M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association Scientific Publication 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.

Galstoff, P.S. 1964. The American oyster, *Crassostrea virginica* Gmelin. Fishery Bulletin 64: 1-480.

Galstoff, P.S. and R. Luce, 1930. Oyster investigations in Georgia. Document 1077. Report of the Commissioner of Fisheries, Appendix 5. U.S. Bureau of Fisheries, U.S. Dept. of Commerce, Washington, DC.

Gauthier, J.D., T.M. Soniat and J.S. Rodgers. 1990. A parasitoligical survey of oysters along salinity gradients in coastal Lousiana. J. World Aquacult. Soc. 21(2): 105-115.

Geise, A.C. 1959. Comparative physiology and annual reproductive cycles of marine invertebrates. Ann. Rev. Physiol. 21: 547-576.

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.

Hayes, P.F. and R.W. Menzel. 1981. The reproductive cycle of the early setting*Crassostrea virginica* (Gmelin) in the northern Gulf of Mexico and its implications for population recruitment. Biol. Bull. 160: 80-88.

Heffernan, P.B., R.L. Walker and J.L. Carr. 1989a. Gametogenic cycles of three marine bivalves in Wassaw Sound, Georgia: II. *Crassostrea virginica* (Gmelin, 1791). J. Shellfish Res. 8: 61-70.

Heffernan, P.B., R.L. Walker and J.L. Carr. 1989b. Gametogenic cycles of three bivalves in Wassaw Sound, Georgia: I. *Mercenaria mercenaria* (Linnaeus, 1758). J. Shellfish Res. 8: 51-60.

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.

Hoese, H.D. 1968. Studies on the parasitic oyster fungus, *Labyrinthomyxa* sp. in Georgia salt waters. In: Linton, T.L. (ed.). Feasibility study of methods for improving oyster production in Georgia. Marine Fisheries Division, GA Game and Fish Comm. and University of Georgia, Completion Report 2-10-R.102-120. pp.

Hopkins, A.E. 1931. Factors influencing the spawning and setting of oysters in Galveston Bay, Texas. Bull. Bur. Fish. 47: 55-58.

Hopkins, S.H., J.G. Mackin and R.W. Menzel. 1953. The annual cycle of reproduction, growth, and fattening in Louisiana oysters. Proc. Natl. Shellfish. Assoc. 44: 39-50.

Howard, D.W. and C.S. Smith. 1983. Histological techniques for marine bivalve mollusks. NOAA Technical Memorandum NMFS-F/NEC-25. 97 pp.

Kanti, A., P.B. Heffeman and R.L. Walker. 1993. Gametogenic cycle of the southern surf clam, *Spisula solidissima similis* (Say), from St. Catherines Sound, Georgia. J. Shellfish Res. 12: 255-261.

Kennedy, V.S. and H.I. Battle. 1964. Cyclic changes in the gonad of the American oyster, *Crassostrea virginica* (Gmelin). Can. J. Zool. 42: 305-321.

Kennedy, V.S. and L.B. Krantz. 1982. Comparative gametogenic and spawning patterns of the oyster, *Crassostrea virginica* (Gmelin), in central Chesapeake Bay. J. Shellfish Res. 2: 133-140.

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.

Loosanoff, V.L. 1942. Seasonal gonadal changes in the adult oysters, *Ostrea virginica*, of

   Long Island Sound. Biol. Bull. 129: 546-561.

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.

McNulty, J.K. 1953. Seasonal and vertical patterns of oyster setting off Wadmalaw Island,

   S.C. Contributions from Bears Bluff Laboratory, No. 15, 17 pp.

Menzel, R.W. 1951. Early sexual development and growth of the American oyster in

   Louisiana waters. Science 113: 719-721.

O'Beirn, F.X., 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. University of Georgia, School of Marine Programs, Marine Technical Report No.

   94-2, 42 pp.

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.

O'Beirn, F.X. 1995. Reproduction and recruitment of the eastern oyster, *Crassostrea*

   virginica, in coastal Georgia. Ph.D. Dissertation, University of Georgia, Athens GA.

   163 pp.

O'Beirn, F.X., P.B. Heffernan and R.L. Walker. 1995. Preliminary recruitment studies of

   Crassostrea virginica in coastal Georgia and their potential applications. Aquaculture 136:

   231-242.

Phelps, H.L. and J.A. Mihursky. 1986. Oyster (*Crassostrea virginica*, Gmelin) spat

   settlement and copper in auwfuchs. Estuaries 9: 127-132.

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 and 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.

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

Rogers, P. and A. Garcia-Cubas. 1981. Evolution gonadica a nivel histologico del ostion *Crassostrea virginica* (Gmelin, 1791) del sistema fluvio lagunar Apasta-Pom, Laguna de Terminos, Campeche, Mexico. An. Inst. Cien. Del Mar y Limnol. Univ. Nacl. Auton. Mexico 8: 21-42.

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

Sokal, R.R. and F.J. Rohlf. 1981. Biometry. Second Edition. W.H. Freeman and Company, New York. 859 pp.

Stevens, S.A. 1983. Ecology of intertidal oyster reefs: Food distribution and carbon nutrient flow. Ph.D. Dissertation, Institute of Ecology, University of Georgia, Athens, GA.

Taylor, B. 1970. Responses of early development stages of *Crassostrea virginica* to heat shock. Master Thesis, Georgia State University, Atlanta.
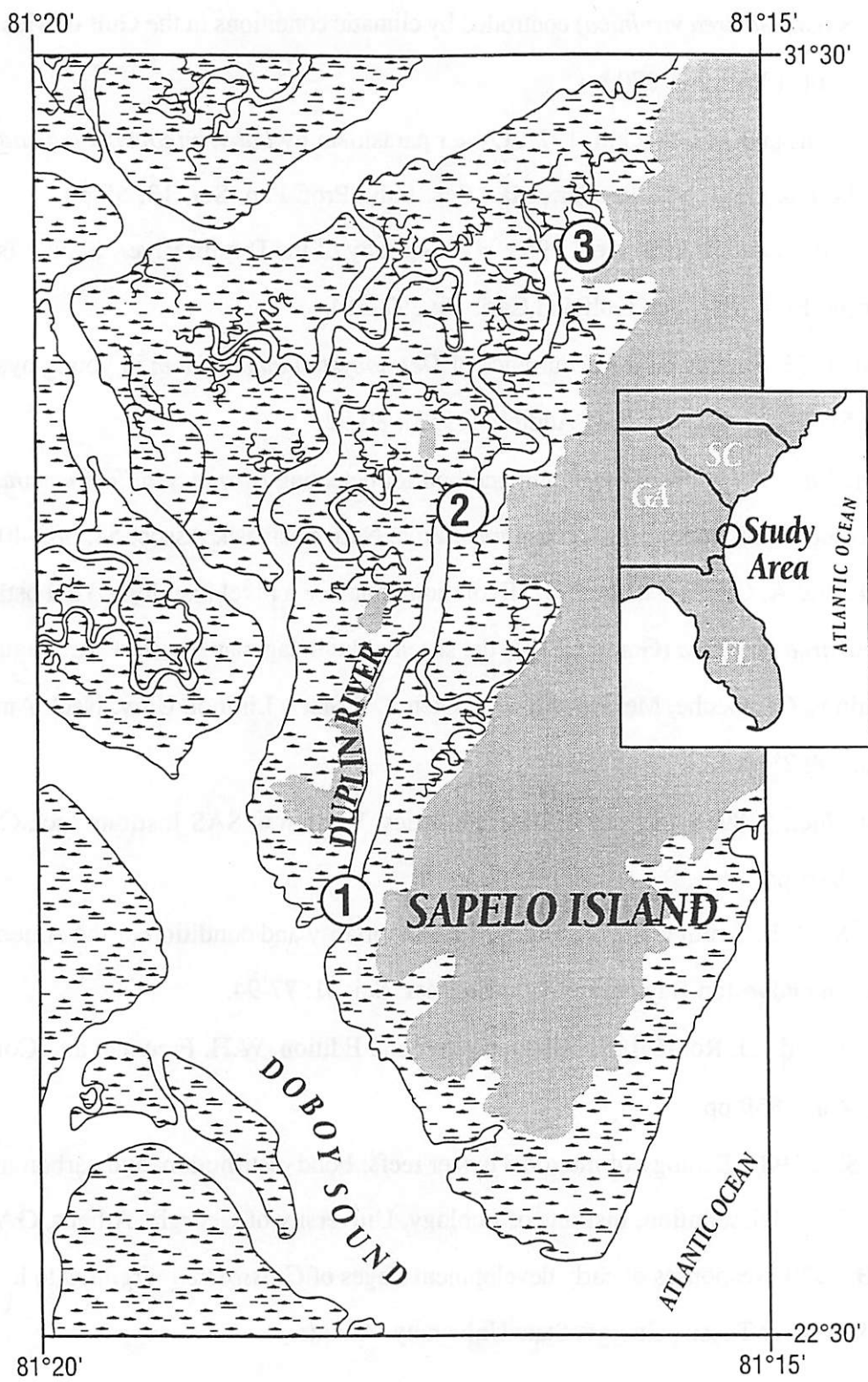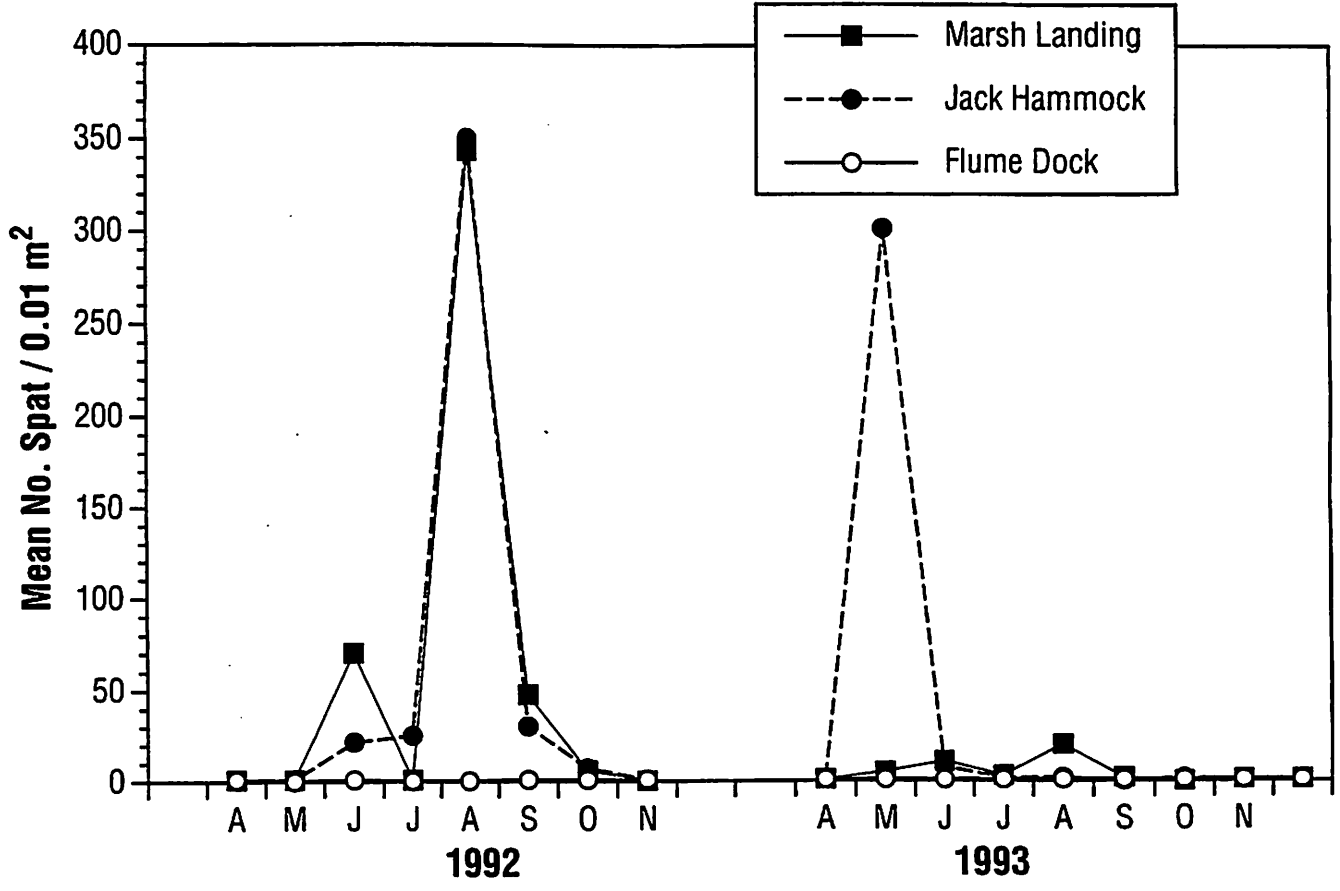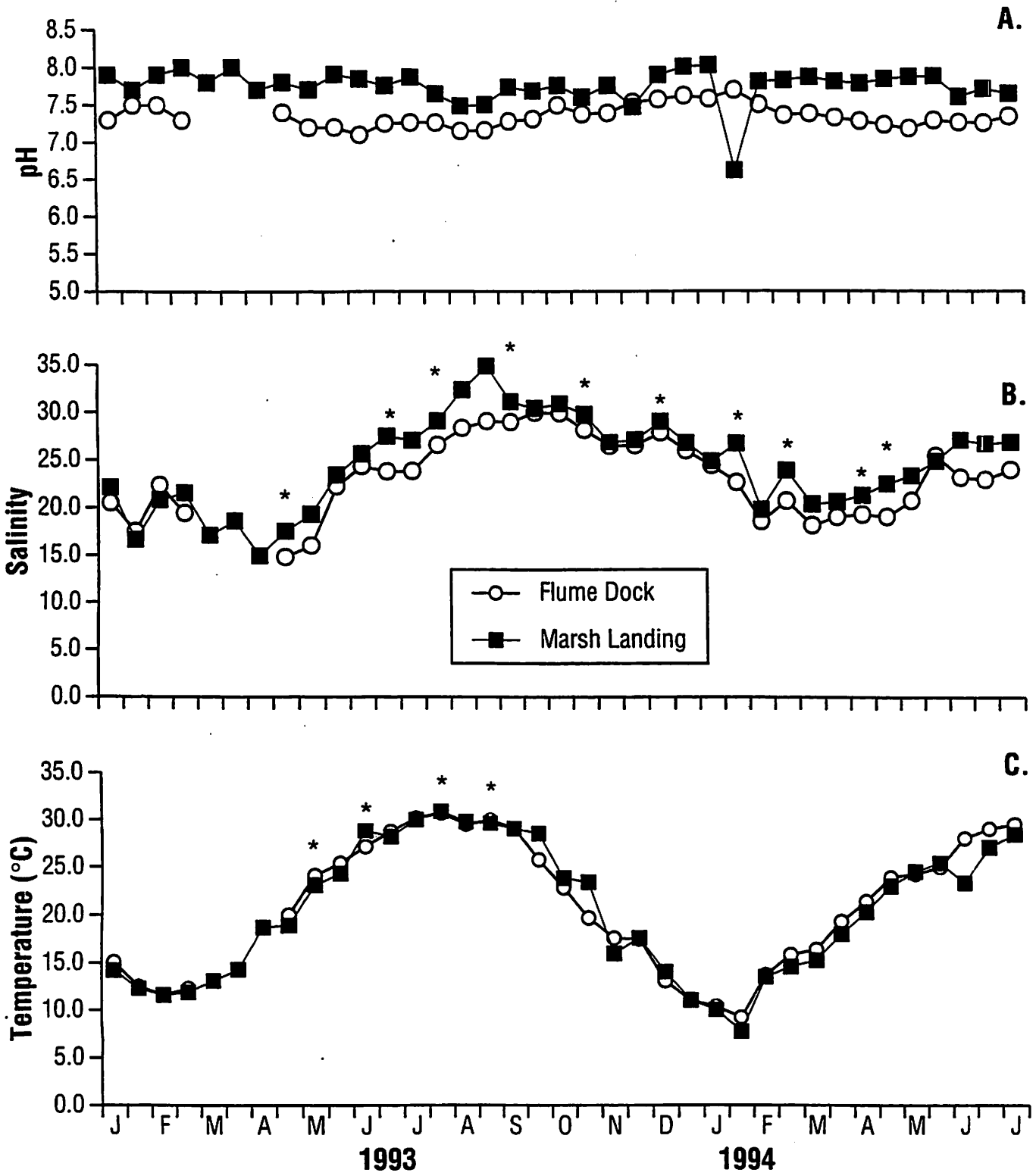
81°20'   81°15'

31°30'

DUPLIN RIVER

③

②

① SAPELO ISLAND

DOBOY SOUND

ATLANTIC OCEAN

SC

GA

○ Study Area

FL

ATLANTIC OCEAN

22°30'

81°20'   81°15'
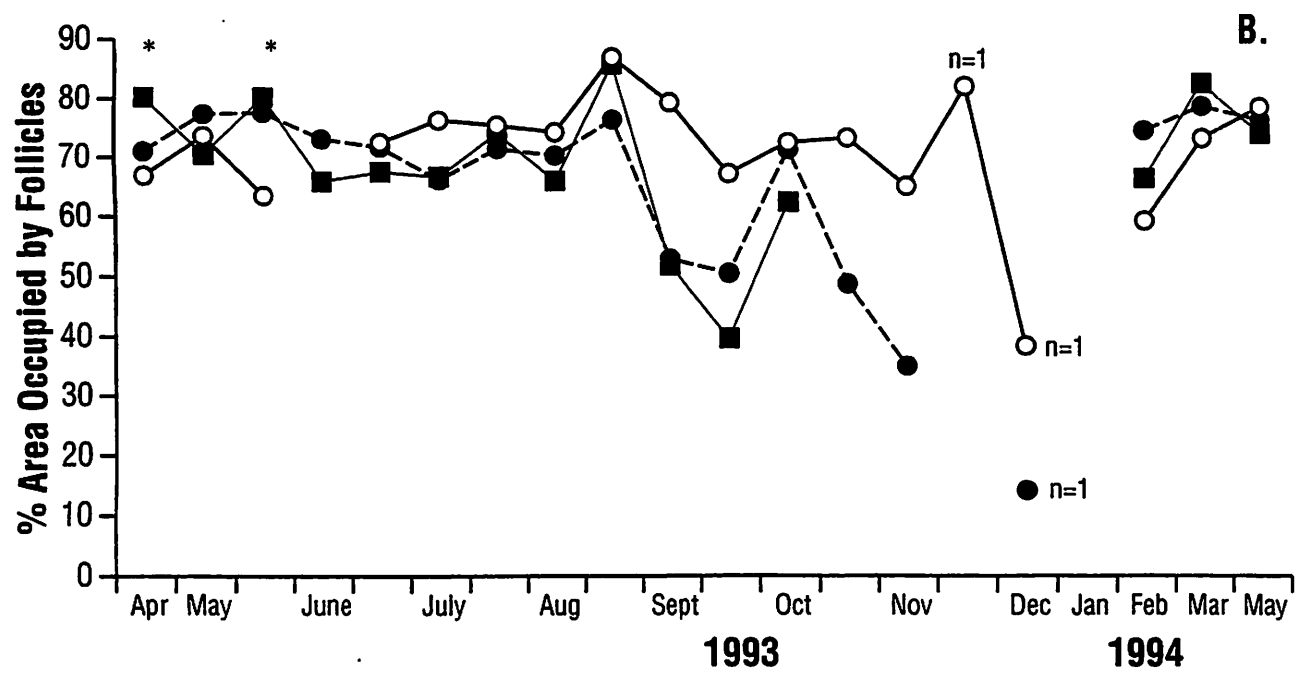
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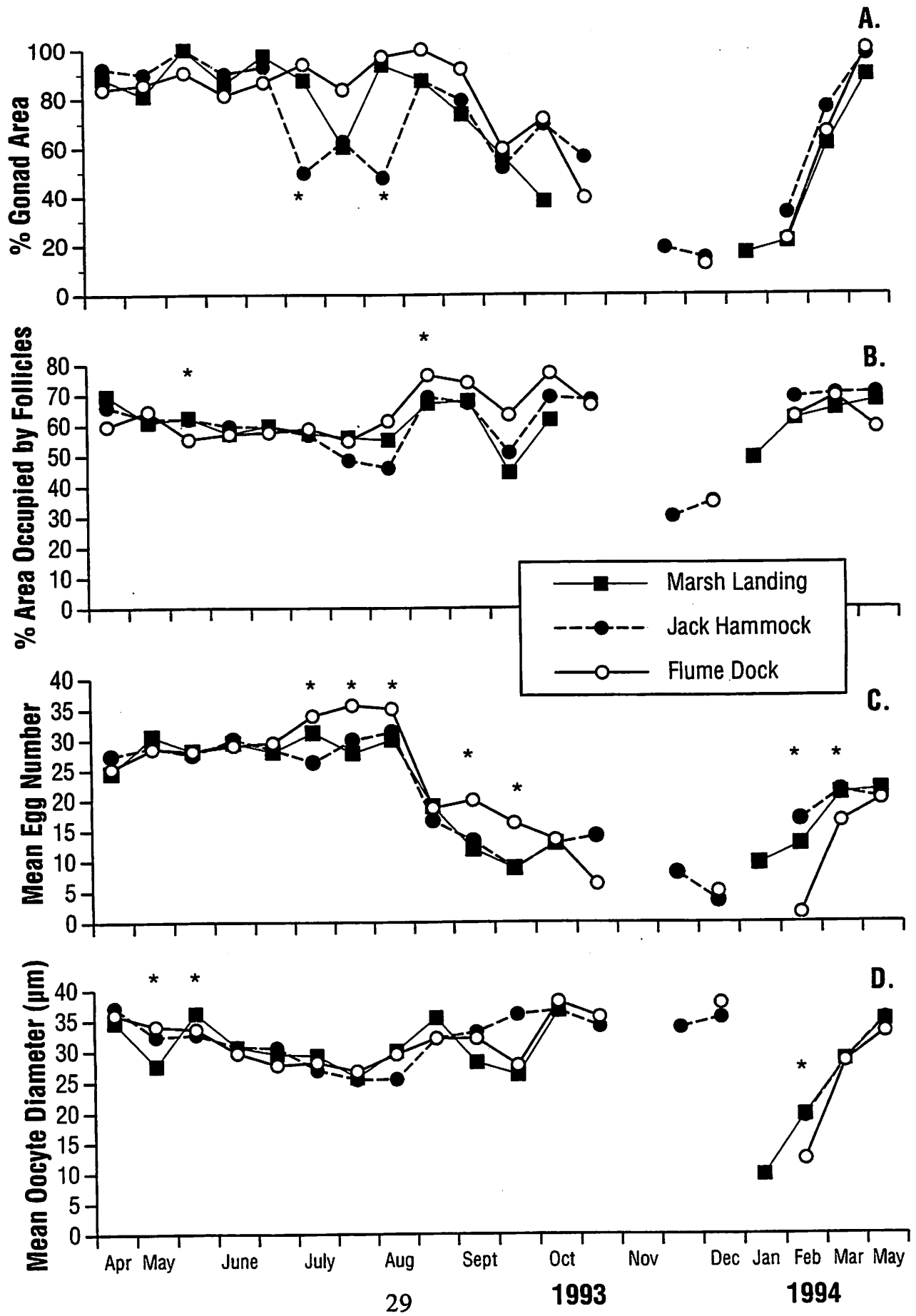Figure 2.   25
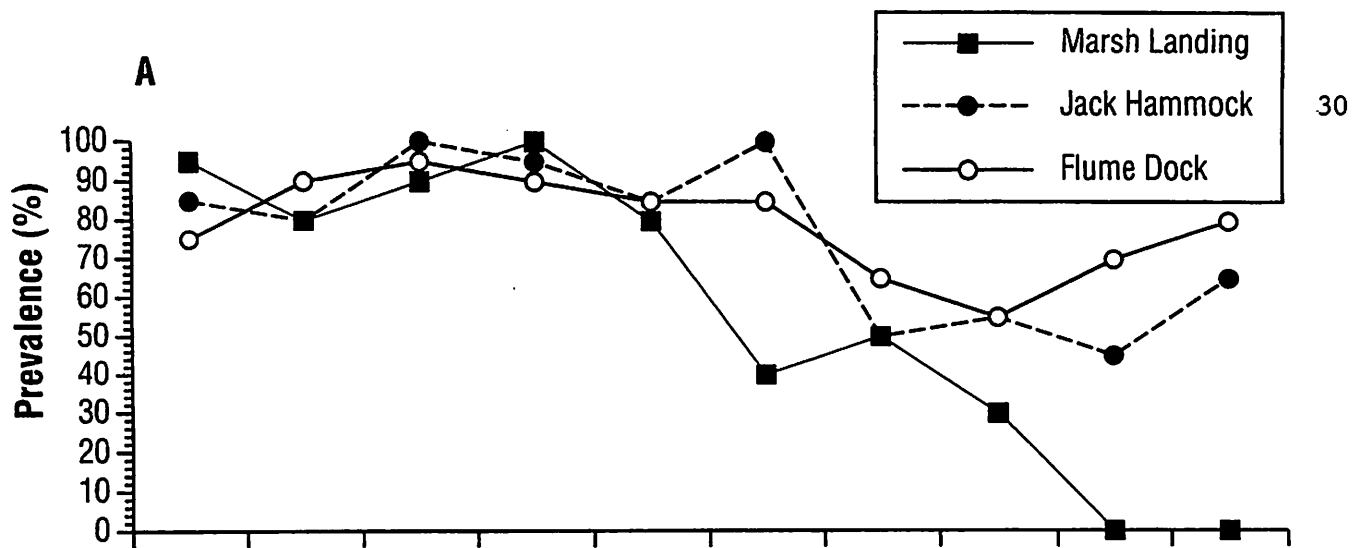
Figure 3.

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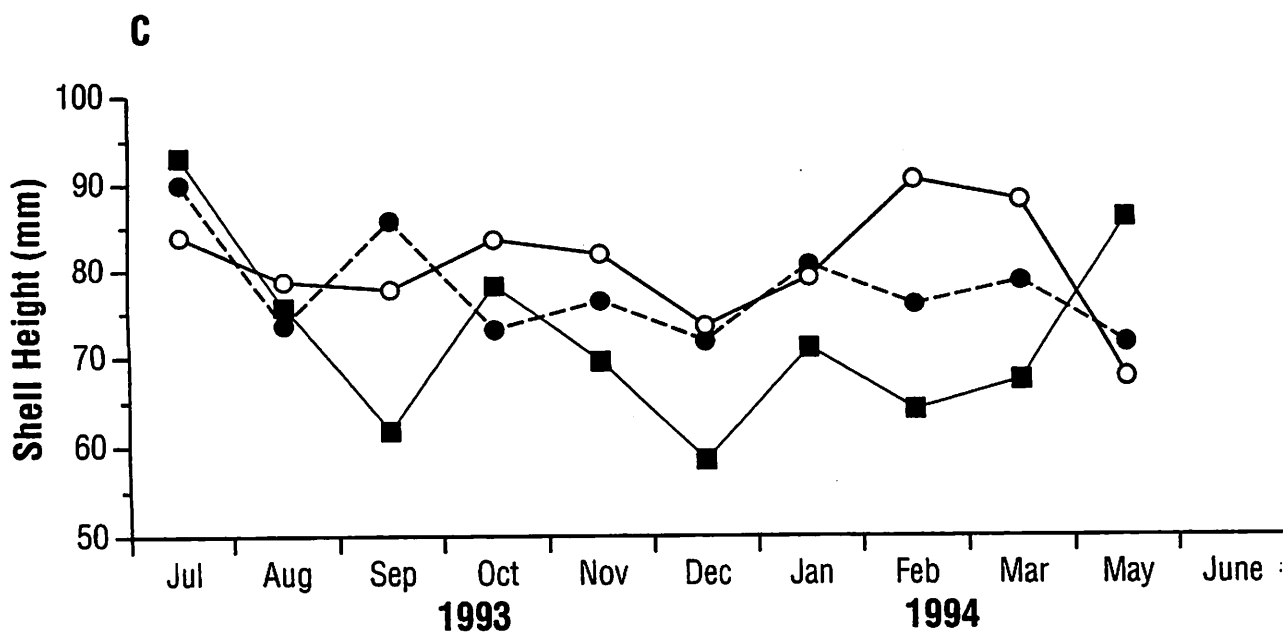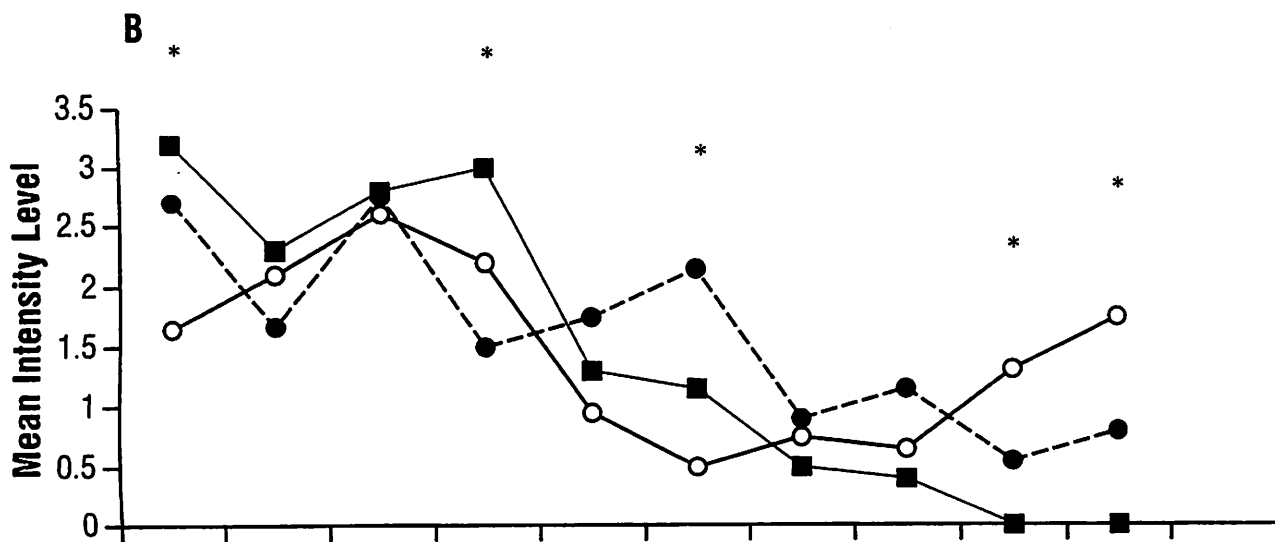
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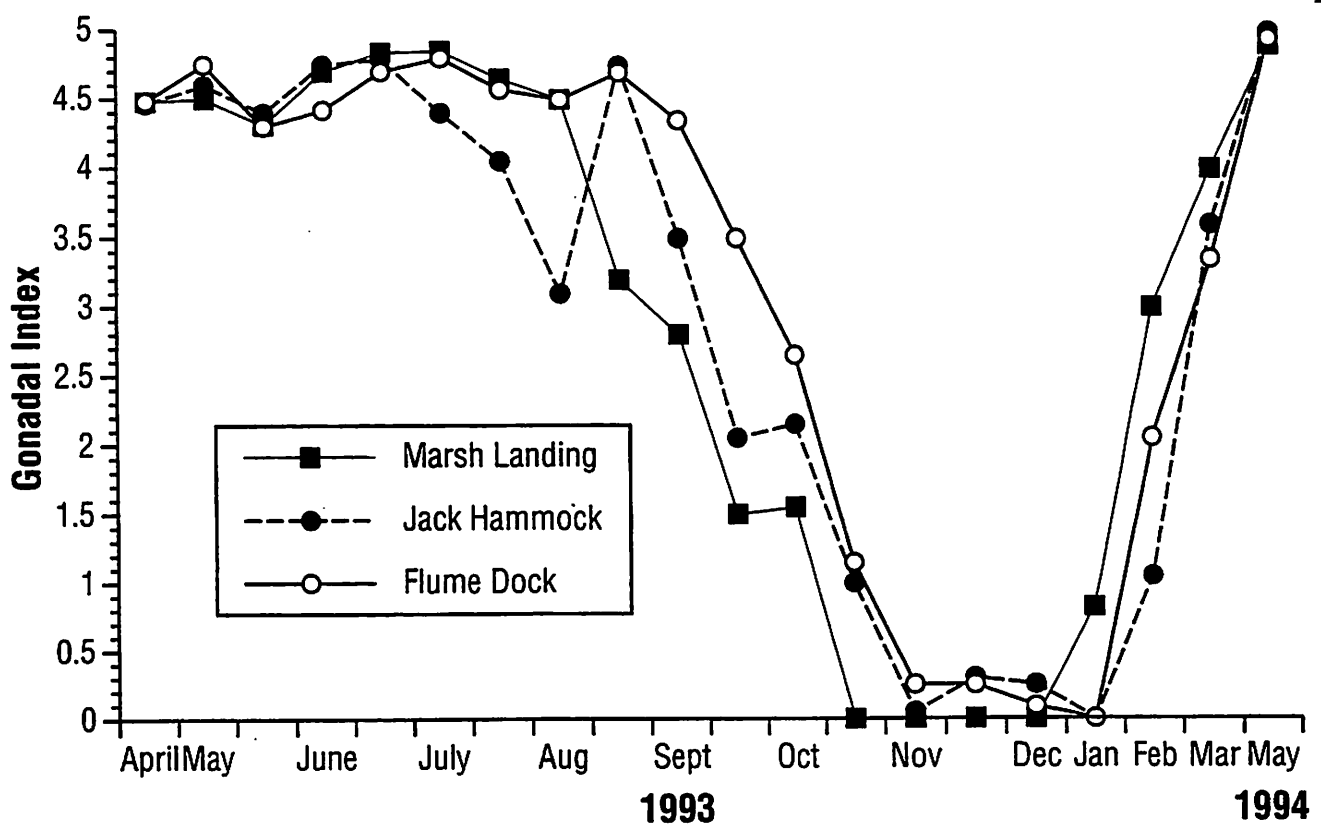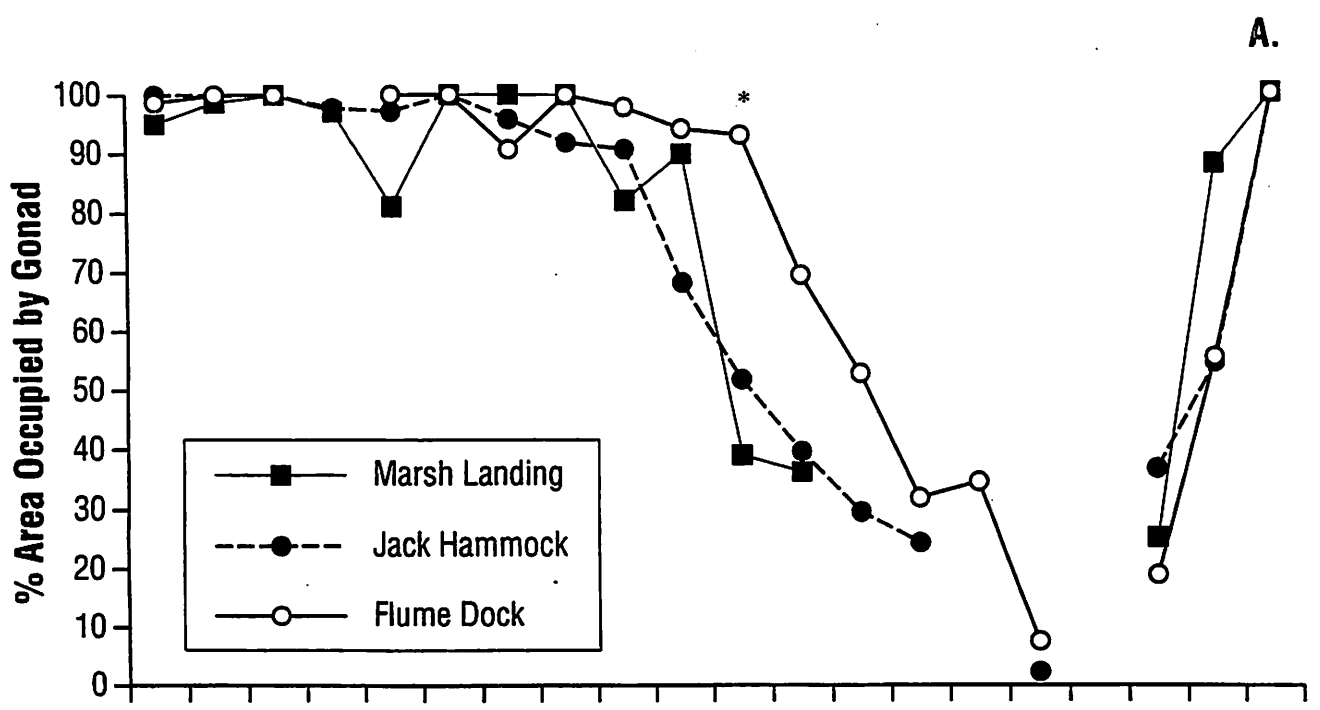
Figure 5.

Figure 6.

Table 1: Total numbers of oysters collected and sex ratios at each site within the Duplin
River, Sapelo Island, Georgia. * p<0.01

Site	Total	Males (n)	Females (n)	M:F	Chi-square
Marsh Landing	221	70	151	0.46:1	29.69*
Jack Hammock	288	109	179	0.61:1	17.01*
Flume Dock	313	110	203	0.54:1	27.63*