PREVALENCE OF Perkinsus marinus IN THE EASTERN OYSTER, Crassostrea virginica IN RELATION TO TIDAL PLACEMENT IN A GEORGIA TIDAL CREEK

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ABSTRACT: This experiment was designed to evaluate the effects tidal zonation and bottom placement of the eastern oyster, Crassostrea virginica, have on the prevalence and intensity of the oyster parasite, Perkinsus marinus, a suspected causative agent for subtidal oyster mortalities experienced in the coastal waters of Georgia. Twelve grow-out bags (1 m x 0.5 m; 12.7 mm mesh), each containing 200 oysters, were placed in Jointer Creek, Georgia in March 1992. Bags (3 replicates each) were placed intertidally and subtidally on the creek bottom and off-bottom. Ten oysters per bag were removed monthly for twelve months beginning March 1992, and were inspected for prevalence and intensity of Perkinsus marinus, using the thioglycollate method. Oyster mortality and shell length data were also evaluated.

Neither prevalence (p = 0.3505) nor intensity levels (p = 0.2993) of $Perkinsus\ marinus$ in oysters were significantly different among the treatments. Although there were no significant differences in prevalence or intensity of the pathogen among treatments, the intertidal off-bottom treatment had the lowest values most frequently. $Perkinsus\ marinus\ was$ present in all replicates every month. Prevalence and intensity of infection followed the typically observed pattern of maximum values in summer and fall and minimum levels in winter.

Subtidal bottom oysters experienced higher mortalities (p = 0.0022), but the prevalence and intensity of *Perkinsus marinus* in oysters were not significantly different between treatments. It appears therefore, that the oyster parasite, *Perkinsus marinus* is not the discerning factor in the higher mortalities witnessed in oysters placed subtidally on the bottom in the southeastern U.S. coastal waters.

The eastern oyster, Crassostrea virginica, occurs subtidally along much of the eastern seaboard, but primarily in the intertidal zone in the southeastern U.S. (Dean, 1892; McKenzie and Badger, 1969; Harris, 1980; Burrell et al., 1984). Adult oysters transplanted from intertidal beds of Wassaw Sound, Georgia and placed in subtidal areas in coastal Georgia have suffered high mortalities (Oemler, 1889). The subtidal oyster mortalities have, in the past, been attributed to predation, parasitism by the boring sponge, Cliona celata, and high siltation in the subtidal zone (Harris, 1980; Burrell et al., 1984; Ofiara and Stevens, 1987). Recent ¹Corresponding Author

research, however, indicates that these factors may not be the major causes of adult oyster mortalities in the subtidal zone in the southeastern U.S. Adams et al. (1994) placed oysters in predator exclusion bags on and above various substrates in both intertidal and subtidal areas of coastal Georgia. Subtidal onbottom oysters suffered significantly higher mortalities than the other treatments. It was speculated that the oyster pathogen, *Perkinsus marinus*, 'Dermo', may be a contributing factor in the lower survival rates for oysters cultured subtidally on the bottom.

Perkinsus marinus affects many

populations of Crassostrea virginica along the eastern and Gulf coasts of the United States (Andrews, 1988). The parasite was detected in oyster populations in Georgia in 1966, and occurred in epizootic proportions between 1985-1987, when massive oyster mortalities occurred along the entire coastline (Lewis et al., 1992).

This study was initiated to determine the extent of the pathogen Perkinsus marinus in a Georgia tidal creek. It also examined the effects that bottom placement and tidal zonation had on the prevalence and intensity of the pathogen within oysters. Another perceived benefit of this study is that it will add valuable insight into the establishment of a growout protocol for oysters in a coastal Georgia maricultural operation.

METHODS

A total of 2400 eastern oysters, with shell heights 40-90 mm, were collected from the lease area of Satilla Sea Farms, Inc., Jointer Creek, near Brunswick, Georgia. Oysters were cleaned of any obvious epibionts, including oyster spat and were placed in 12 commercial oyster grow-out bags (1 m x 0.5 m; mesh size 12.7 mm). These bags were designed to contain the oysters as well as exclude potential predators, such as drills crabs and whelks. Four treatments, each with three replicate bags, were examined. Bags were placed subtidally (such that they were approximately 60 cm below the mean low water mark), on and off the bottom, and intertidally (such that they were approximately 1 meter above the mean low water mark), on and off the bottom. Intertidal oysters were placed so that they were exposed to air for approximately four hours during each tidal cycle. Offbottom treatment bags were attached with cable ties to iron trestles which held bags approximately 0.3 m above the creek bottom. The on-bottom bags were laid directly on the shell bottom. For the intertidal off-bottom treatment, the bags were positioned such that inundation by the incoming tide occurred simultaneously for both intertidal treatments.

Each month from March 1992 to February 1993, ten oysters were randomly removed from each bag. The oysters were measured for shell height, from beak to lip, to the nearest mm. The rectum and some mantle tissue of each oyster were dissected out and prepared for quantification of Perkinsus marinus infection using the thioglycollate method described by Ray (1963). Mortality counts were conducted in May, August, November, and February. Mortality was calculated using counts of the remaining live oysters in each replicate bag. Salinity and temperature measurements were recorded whenever possible, this resulted in approximately 20 readings per month. Analysis of Variance (ANOVA) and Tukey's Studentized Range Tests (SRT) were performed to determine differences between treatments in prevalence and intensity of Perkinsus marinus, shell height, and mortality. All statistical tests were performed for each monthly sample as well as the overall 12-month study period (pooled data). Prevalence was measured as the proportion of animals with Perkinsus marinus hypnospores present, and was arcsine transformed for statistical analysis (Sokal and Rohlf, 1981). Intensity of disease was measured on a scale of 0 to 6 (Quick and Mackin, 1971). The percent mortality within each treatment was calculated from counts of remaining live animals in the bags, and was arcsine transformed prior to statistical analysis. Statistical analysis was performed on a personal computer utilizing PC SAS (SAS Institute, 1989). A significance level of 5% (alpha = 0.05) was chosen for all tests.

RESULTS

The initial prevalence of 'Dermo' within the oysters and sizes of the oysters were not significantly different among the treatments (p = 0.7329 and 0.1727. respectively). Prevalence and intensity of Perkinsus marinus for the pooled data increased steadily from March to June 1992 (Fig. 1). Prevalence peaked from July through December 1992, with a slight decrease in August followed by an increase in September and October, after which it decreased steadily. Intensity was highest from June to October 1992, with a small decline in August (Fig. 1). The results of ANOVA showed no significant difference in prevalence (p = 0.3505): Table 1a) or intensity (p = 0.2993; Table 1b) of Perkinsus marinus in ovsters among treatments for the pooled data. Pooled mean prevalence ranged from 86.2% in ovsters from the intertidal offbottom to 90.4% in oysters from the intertidal bottom (Table 1a). Pooled mean intensity ranged from means of 2.5 for oysters from intertidal off-bottom to 2.8 from the intertidal bottom (Table 1b). No significant differences were evident among the treatments from the ANOVA for disease prevalence in each of the twelve monthly comparisons (Fig. 2).

The results of the ANOVA showed significant differences in intensity (Fig. 3),

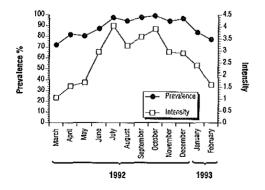


Figure 1. Mean prevalence and intensity of the pooled data for the four treatments over the entire study period.

among treatments in the May sample (p = 0.0231) and in the July sample (p = 0.0330). The Tukey test did not differentiate among treatments for the May sample and the separation in the July sample was between the two extremes (4.6 for intertidal bottom and 3.3 for subtidal bottom).

Shell height of the pooled data revealed significant differences among the treatments (Table 2). The intertidal onbottom were significantly smaller (x =70.1 mm) than subtidal on-bottom oysters (x = 72.6 mm). Both were significantly

(a) Prevelance

P-value = 0.3505

IOFF	SOFF	SON	ION
86.2%	88.7%	89.1%	90.4%

(b) Intensity

P - value = 0.2993

IOFF	SOFF	SON	ion
2.5 ± 0.10	2.6 ± 0.10	2.7 ± 0.10	2.8 ± 0.10

Table 1. Results of the ANOVA and Tukey Studentized Range Test on the pooled data for; (a) mean prevalence of Prerkinsus marinus infection in oysters and (b) mean intensity of Perkinsus marinus infection in the oysters. Treatments connected by the same line are not significantly different. SOFF-Subtidal Off-Bottom, SON-Subtidal On-Bottom. IOFF-intertidal Off-Bottom and ION-intertidal On-Bottom.

smaller than oysters grown off-bottom, which were not significantly different from each other. ANOVA and Tukey's SRT for the monthly readings of shell height revealed no significant differences in size between treatments from March 1992 to June 1992 (Table 3). From July 1992 to February 1993, oysters from offbottom treatments were consistently larger than bottom treatments (Table 3).

ANOVA and Tukey's tests of arcsine transformed mortality data revealed

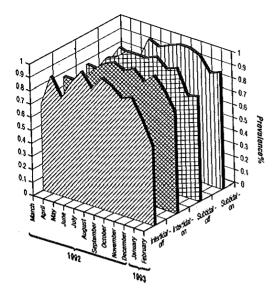


Figure 2. Mean prevalence levels for the individual treatments over the entire study period.

significant differences (Table 4). The apparent decrease in the cumulative mortality totals for November to February, may be attributed to more live oysters counted during the latter time period than were counted during the former, for the treatments concerned. This may have been a result of including newly settled or unaccounted spat on the experimental ovsters that had grown to approximately 50 mm, i.e., the sampling size. Oyster

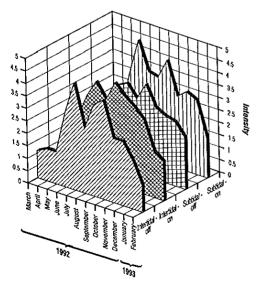


Figure 3. Mean intensity levels for the individual treatments over the entire study period.

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10N	SON	IOFF	SOFF
70.1 mm ± 0.58	$72.6 \mathrm{mm} \pm 0.59$	74.5 mm ± 0.59	75.6 mm ± 0.62

Table 2. Results of the ANOVA and Tukey Studentized Range Rest on the pooled data for shell height ± standard error. Treatments connected by the same line are not significantly different. SOFF-Subtidal Off-Bottom, SON-Subtidal On-Bottom, IOFF-Intertidal Off-Bottom and ION-Intertidal On Bottom.

mortality per treatment, on a cumulative basis, demonstrated that the on-bottom treatments had significantly higher mortality than the off-bottom treatments (Table 4). The highest incidence of mortality for all of the treatments occurred in the August-November time period (Table 4), with the opening period of the study (March-May) having the next highest increase in mortality.

Ambient river salinity ranged from 21 to 31 ppt, while water temperature varied from 10 to 31° C (Fig. 4).

DISCUSSION

The most frequently documented pattern of Perkinsus marinus prevalence is similar to that found in this experiment. To summarize, increasing infection prevalence or intensity in spring are followed by peak levels in latesummer/fall and subsequently the levels decrease during winter (Andrews and Hewatt, 1957; Mackin, 1962; Crosby and Roberts, 1990). In Virginia and Delaware, infections may disappear during late winter (Andrews and Hewatt, 1957; Andrews, 1988), further south however, they may persist (South Carolina, Georgia, Florida, Louisiana, and Texas: Andrews and Hewatt, 1957; Quick and Mackin, 1971; Burrell et al., 1984; Crosby and Roberts, 1990; and this study). This difference in seasonal patterns of infection was largely thought to be a result of temperature fluctuations (Andrews and Ray, 1988). However, there have been many infection patterns documented that

Time	P - value	TREATMENTS			
March	0.1727	ION 68.1 ± 2.34	SON 72.5 ± 1.62	SOFF 72.9 ± 1.37	IOFF 73.4 ± 1.93
April	0.1371	ION 74.0 ± 2.28	SON 76.3 ± 2.13	IOFF 77.1 ± 1.83	SOFF 81.2 ± 2.18
Мау	0.9872	SON 76.2 ± 1.93	SOFF 76.7 ± 1.71	ION 76.7 ± 1.74	IOFF 77.0 ± 2.12
June	0.5655	SON 78.1 ± 1.75	ION 80.0 ± 1.55	IOFF 80.6 ± 2.19	SOFF 82.0 ± 2.02
July	0.0108	ION 70.9 ± 1.63	IOFF 74.0 ± 1.63	SOFF 76.8 ± 2.07	SON 79.8 ± 2.03
August	0.0102	SON 65.8 ± 2.23	ION 67.0 ± 1.88	IOFF 70.5 ± 1.57	SOFF 74.5 ± 1.96
September	0.0332	ION 66.6 ± 2.15	SON 72.0 ± 1.47	IOFF 72.5 ± 1.83	SOFF 74.9 ± 2.28
October	0.0615	ION 67.9 <u>±</u> 1.84	IOFF 68.8 ± 1.53	SOFF 70.7 ± 2.10	
November	0.0378	SON 68.2 ± 1.86	ION 68.9 ± 1.86	IOFF 70.0 ± 2.20	SOFF 76.3 ± 2.27
December	0.0001	SON 67.5 ± 1.46	ION 67.6 ± 1.52	IOFF 74.3 ± 2.15	SOFF 79.0 ± 2.67
January	0.0374	ION 67.2 ± 1.58	SON 67.7 ± 2.22	SOFF 69.8 ± 1.89	
February	0.0001	ION 65.3 ± 1.44	SON 68.3 ± 3.17	IOFF 76.0 ± 1.97	SOFF 78.0 ·± 1.99

Table 3. Results of the ANOVA and Tukey SRT groupings on the monthly data for shell height ± standard error. Treatments connected by the same line are not significantly different.

do not follow this cycle (Soniat, 1985; Craig et al., 1989; Powell et al., 1992). Other research indicated that *Perkinsus* marinus infection is highly correlated with large scale, long-term climatic conditions (Gauthier et al., 1990), and points to salinity as the most important factor for both prevalence and intensity (Powell et al., 1992). The distinct drop in prevalence in August appears to coincide with a salinity drop in the same month (Fig 1 and Fig., 4). Unfortunately, there are

no hydrographic data for the month of July. The intensity levels for this experiment also appear to follow a four-phase cycle of infection intensity as described Crosby and Roberts (1990).

Mortalities of oysters in the subtidal zone in coastal Georgia have in the past been accredited to predation, mismanagement of the oyster beds, and siltation (Harris, 1980; Ofiara and Stevens, 1987). Adams et al. (1991) speculated that Perkinsus marinus may account for the

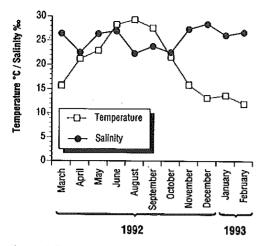


Figure 4. Temperature and salinity means recorded at the study site for the duration of the study.

observed low survival rates of the subtidal bottom oysters. As a consequence of being inundated for extended periods, subtidal oysters are probably exposed to water borne pathogens for longer periods of time than intertidal oysters.

The results of this study revealed no significant differences in *Perkinsus marinus* prevalence or intensity occurring in oysters planted subtidally or intertidally in bottom or off-bottom treatments. This is in agreement with Gibbons and Chu (1989) in Virginia, and Burrell et al. (1984) in South Carolina, who found no significant differences in Dermo levels between subtidal or intertidal oysters. This study also shows that proximity to the bottom

May	SOFF	ЮH	SON	ЮFF
P-value = 0.001	10.1%	19.3%	27.1%	29.5%
	SOFF	IOFF	ION	SON
August				
P-value < 0.0001	10.1%	25.3%	36.1%	39.8%
November				
P-yalue < 0.0001	SOFF	IOFF	ION	SON
	25.5%	30.0%	52.0%	56.6%
February	·			
P-yable < 0.0001	SOFF	IOFF	ION	SON
	25.9%	28.9%	42.4%	50,5%

Table 4. Cumulative oyster mortality estimates for March 1992 to February 1993, with results of ANOVA and Tukey SRT groupings. Treatments connected by the same line are not significantly different.

does not affect prevalence or intensity of the pathogen in oysters. During the first four months of sampling, there were no significant differences in shell height; however, from July 1992 until February 1993, there is a pattern of significantly larger animals coming from the offbottom treatments, and smaller oysters from the bottom treatments. This suggests faster growth rates in off-bottom treatments, attributable perhaps, to greater access to food in a less stressed environment (Beaven, 1953; Matthiessen and Toner, 1966; Shaw, 1971; Manzi et al., 1977; Adams et al., 1991). Mean shell height dropped from 80.2 mm in June to 69.5 mm in August 1992 (Table 3). The decrease in size may have resulted from a bias of selecting the larger oysters earlier in the study, selecting spat that had grown to approximately 50 mm later in the study, a higher mortality rate in larger oysters, or a combination of the above. However, it is important to note that, no relationship was revealed between shell height and infection level, thus concurring with Crosby and Roberts (1990), who determined that Perkinsus marinus infection is not related to size in adult oysters.

Analysis of oyster mortality data (Table 4) indicates that survival was better for off-bottom treatments than on-bottom treatments. Despite the fact that the subtidal on-bottom treatment among others, experienced an apparent increase in numbers for the November-February time period as evidenced by the decrease in the cumulative mortality totals, this treatment still had the highest mortality for pooled data. The studies of Adams et al. (1991, 1994) and MacKenzie (1981) also revealed significantly higher mortalities for subtidal bottom oysters. During this experiment, oysters from subtidal onbottom treatment were partially covered with mud, and mortalities may have resulted from suffocation (MacKenzie,

1981). However, it must be pointed out that the mortalities experienced for the subtidal on-bottom treatment were not significantly higher than those recorded intertidally on the bottom (Table 4). Slitation was not witnessed in these intertidal bags as the bags were laid upon a predominantly shell substrate. Also, during the monthly sampling the bags were cleared of any obvious mud and sllt. In the studies of Adams et al. (1994), oysters planted subtidally on-bottom on a variety of substrate types experienced heavy mortalities even in the absence of siltation.

As with other studies, the highest mortalities were experienced in the August-November period. Prevalences and intensities were also highest in this period and the preceding period. Oysters in coastal Georgia are subject to high physiological stress during the summer months due to the high ambient water temperatures (>30° C), and the prolonged spawning season (Hefferman et al., 1989). Thus, it is highly likely that Perkinsus marinus infection combined with physiological stress contributed to the observed mortalities.

Although there were no significant differences in prevalence or intensity of Perkinsus marinus in oysters grown in any of the four treatments, trends were noted. The intertidal on-bottom treatment had the highest prevalence and intensity of Perkinsus marinus in more of the monthly samples than did the other treatments. The intertidal off-bottom had the lowest disease prevalence and intensity in the majority of monthly samples. It would appear from these trends that relocation to an intertidal off-bottom site would minimize Perkinsus marinus infection during the summer months.

Adams et al. (1991) and O'Beirn et al. (1994a) note that the set of oyster spat is extremely high in intertidal off-bottom areas, which in the case of Adams et al. (1991), resulted in heavy fouling of oysters located there. This fouling greatly reduces the marketability of the oysters as singles. However, lower spat settlement in the study area is typical of the upper regions of coastal Georgia tidal creeks (O'Beirn et al., 1994b).

The results of this study do reiterate the recommendations of Adams et al. (1991) whereby, a combination of intertidal and subtidal placement may maximize growth and survival of oysters. Intertidal on-bottom placement during the June to September period will reduce fouling pressures by newly settled oyster spat, while not having an appreciable increase in the prevalence of Perkinsus marinus. Subsequent subtidal off-bottom placement will maximize growth and survival of the oysters. In addition, this study suggests an alternative protocol whereby, the oysters may be transplanted from the larger bodies of water (sounds, rivers and creeks) and placed intertidally off-bottom in the headwaters of the creeks to promote high survivability as well as take advantage of the lower spat fouling indicative of such areas.

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