COMPARISON OF *PERKINSUS MARINUS* INFECTION AND OYSTER CONDITION IN SOUTHEASTERN NORTH CAROLINA TIDAL CREEKS

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ABSTRACT

The Eastern oyster (*Crassostrea virginica*) is a commercially important species that also performs critical ecosystem functions, affecting water quality and providing habitat for fish and invertebrates. However, populations of *C. virginica* have drastically declined, especially over the last 50 years. In recent years, disease caused by the protozoan parasite, *Perkinsus marinus* (Dermo), has been a major factor contributing to the decline of oyster populations. Exposure to one or more environmental stressors, such as increased particulate loading and declining water quality, may adversely affect oysters, making them more susceptible to infection. This study compares *P. marinus* infection in intertidal oysters from three tidal creeks in southeastern North Carolina that vary in historic water quality conditions. Prevalence and intensity of *P. marinus* infection was compared over time and among creeks for both natural oyster populations and hatchery stock outplants using RFTM tissue assay. Oyster tissue condition, growth, and mortality were also compared. Infection was nearly 100% prevalent across sampling periods, among creeks, and in both hatchery and natural oysters. However, despite high prevalence, overall infection intensities were low. Infection intensity among the creeks did not follow historic water quality patterns, but did vary with specific factors. Temporal patterns of *P. marinus* infection and tissue condition were apparent in both hatchery and natural oysters. Infection intensities were highest and oyster condition was lowest during November 2005 while infection was at its lowest and condition was at its highest in February 2006. Infection levels and condition also differed between oyster types with hatchery oysters having higher infection intensity and lower condition than natural oysters. Infection intensity and oyster condition were also correlated with aspects

of water quality in the tidal creeks. The results of this study have implications not only for restoration, but also for understanding oyster and parasite biology in the intertidal environment. They suggest that conditions of the intertidal environment may impact seasonal cycles of *P. marinus* infection in the southeastern United States and may even affect the parasite itself. Overall, the results underscore the idea that a variety of factors likely interact to influence *P. marinus* infection levels in oysters and oyster health.

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DEDICATION

I would like to dedicate this thesis to my mother, Judy Colosimo, who although not here has been with me every step of the way. This would not have been possible without her. She is my strength and inspiration.

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INTRODUCTION

The Eastern oyster, *Crassostrea virginica,* has long been considered a commercially and ecologically important species. Oysters are of economic value and once supported a successful fishery, with peak harvests in Chesapeake Bay of 200 million bushels (Newell 1988, Mann 2000). Oysters serve vital ecosystem functions, forming reefs that serve as habitat for a variety of fish and invertebrates, often providing critical habitat for juveniles (Lenihan and Peterson 1998, Coen et al. 1999, Posey et al. 1999, Breightburg et al. 2000, Coen and Luckenbach 2000, Dame et al. 2000, Peterson et al. 2000). Oysters also affect water quality by filtering suspended materials from the water column (Gerritsen et al. 1994, Gottlieb and Schweigher 1996, Lenihan and Peterson 1998, Nelson et al. 2003, Cressman et al. 2004) and may impact estuarine food webs by exerting top-down control on phytoplankton (Ulanowitz and Tuttle 1992). However, over the past several decades populations of *C. virginica* have declined drastically. This decline can be attributed to a variety of factors, including overharvesting, disease, poor water quality, and habitat degradation (Andrews 1988, Ortega and Sutherland 1992, Ford and Tripp 1996, Gottlieb and Schweigher 1996, Breightburg et al. 2000, Mann 2000, Peterson et al. 2000). Since the 1950s, diseases caused by the protozoan pathogens *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) have been considered among the major factors contributing to the decline of oyster populations (Chu and Greene 1989, Paynter and Burreson 1991, Burreson and Ragone Calvo 1996, Ford and Tripp 1996, Mann 2000).

In North Carolina, the oyster pathogen most responsible for mortality of Eastern oysters in mid to high salinity areas is *Perkinsus marinus*, commonly called Dermo

(Fisher et al. 1992, Chu and LaPeyre 1993, Ford and Tripp 1996). *P. marinus* was first detected in the Gulf of Mexico in the 1940's. Since then its range has extended to the Atlantic coast of the United States into the Chesapeake Bay and from there has spread further northward to Delaware Bay and southern New England (Andrews 1988, Burreson and Ragone Calvo 1996, Ford and Tripp 1996). *P. marinus* is a water-borne pathogen most likely acquired through oyster feeding since *P. marinus* is commonly found in tissues of the digestive system (Ford and Tripp 1996). The parasite may be transmitted from oyster to oyster as infective cells are released from dead or dying oysters (Mackin 1962, Chu 1996, Ford and Tripp 1996). *P. marinus* may cause mortality in oysters by producing proteases and other lytic substances, which results in the degradation of infected oyster tissues (Ford and Tripp 1996, Paynter 1996) and by blocking hemolymph vessels in infected oysters (Burreson and Ragone Calvo 1996, Ford and Tripp 1996). Prior to causing mortality, *P. marinus* infection has several sub-lethal effects, interfering with the physiological and metabolic processes of the oysters. For example, *P. marinus* is known to reduce shell growth (Burreson 1991, Paynter and Burreson 1991, Paynter 1996) and condition index of *C. virginica*, a measure of oyster health based on soft tissue to body cavity ratio (Burreson 1991, Paynter and Burreson 1991, Austin et al. 1993, Chu and LaPeyre 1993, Ford and Tripp 1996, Paynter 1996). Other sub-lethal effects of *P. marinus* include reduced reproductive capacity of oysters and altered biochemical composition, such as decreased free amino acid concentrations and depleted glycogen reserves (Chu and LaPeyre 1993, Ford and Tripp 1996, Paynter 1996).

The progression of *P. marinus* infection is influenced by a variety of environmental factors, especially temperature and salinity (Chu and Greene 1989,

Paynter and Burreson 1991, Fisher et al. 1992, Ford and Tripp 1996). *P. marinus* proliferates most rapidly at temperatures above 25^{o}C (Chu and Greene 1989, Fisher et al. 1992, Ford and Tripp 1996) and at salinities >15ppt (Chu and Greene 1989, Paynter and Burreson 1991, Ford and Tripp 1996). While temperature and salinity may interact to affect disease progression, temperature has been shown to have an overriding influence (Fisher et al. 1992, Chu 1996, Ford and Tripp 1996). In northern estuaries, highest infection prevalence and intensity typically occur in September immediately following maximum summer temperatures and decline with temperature during winter and spring as *P. marinus* becomes dormant. In the southeastern United States and Gulf region, infection periods are not as discrete because warmer temperatures are more persistent than in the northern regions. For oysters from all regions, disease related mortality tends to occur the second summer following infection (Chu and Greene 1989, Fisher et al. 1992, Ewart and Ford 1993, Ford and Tripp 1996).

In addition to disease, *C. virginica* is exposed to a wide range of natural and anthropogenic environmental stressors including extreme temperature and salinity, low dissolved oxygen (DO) concentrations, pollution, sediment loads, and nutrient inputs (Paynter 1996, Lenihan and Peterson 1998, Lenihan et al. 1999, Chu et al. 2002). Exposure to stressful environmental conditions has been shown to influence host-parasite interactions. Physiological stress induced by adverse environmental conditions may make a host more susceptible to parasitic infection and increase the likelihood of mortality from infection (Sousa and Gleason 1989, Lafferty and Kuris 1999). Oysters that are exposed to one or more environmental stressors are more susceptible to *P. marinus* infection and are more likely to succumb to infection (Chu and Hale 1994,

Anderson et al. 1996, Fisher et al. 1999, Lenihan et al. 1999, Chu et al. 2002). In an experiment exposing oysters to flow and hypoxia related stress, Lenihan et al. (1999) found that oysters subjected to low DO, high levels of sedimentation, and reduced flow speed had greater prevalence and intensity of *P. marinus* infection as well as increased mortality. Chemical contaminants in the environment (e.g. heavy metals, tributyltin) have been shown to enhance existing *P. marinus* infections and oyster mortality as well as increasing susceptibility of uninfected oysters to infection (Chu and Hale 1994, Anderson et al. 1996, Fisher et al. 1999). Exposure of oysters to pollutants suppresses their immune system function, reducing the number and activity of circulating phagocytic hemocytes and decreasing the production of reactive oxygen intermediates, key aspects of oyster immune response (Anderson et al.1992, Chu and LaPeyre 1993, Anderson et al. 1995, Anderson et al. 1996, Fisher et al. 1999, Chu et al. 2002).

Nutrient input and suspended particulates associated with storm water runoff may also adversely affect oysters and thus increase their vulnerability to *P. marinus* infection (Paerl et al. 1995, Lenihan and Peterson 1998, Lenihan et al. 1999, Mann 2000). Increases in nutrient loading have been shown to stimulate microalgal production, which in turn may cause a decrease in DO concentration due to decomposition of algae and can eventually lead to hypoxic/anoxic conditions especially in subtidal environments (Paerl et al. 1995, Lenihan and Peterson 1998, Mann 2000). Exposure to such conditions may have a variety of direct and indirect effects on oysters, causing a decline in overall physiological condition if it persists (Paynter 1996, Lenihan et al. 1999). High sediment loads reduce both the availability and quality of suspended food particles, reducing oyster

filtering efficiency with subsequent effects on the amount energy available for growth, reproduction, and physiological condition (Lenihan et al. 1999, Mann 2000).

The tidal creek estuaries of southeastern North Carolina have historically varied in their levels of nutrient input and turbidity, a parameter often related to differences in total suspended solids (TSS). While some of the creeks have experienced high nutrient loading and particulate inputs (e.g. Hewletts Creek), others have historically low inputs (e.g. Pages Creek) (Mallin et al. 1998, Mallin et al. 2005). The elevated nutrient concentrations, high chlorophyll *a* levels, and turbidity characteristic of some of the tidal creeks may adversely affect oyster physiological health and possibly render oysters in those estuaries more susceptible to *P. marinus* infection.

Oyster populations in this study are intertidal. Disease dynamics and their interactions with environmental conditions have been poorly studied in intertidal systems. Most of the studies on *P. marinus* infection in oysters have been conducted in subtidal areas such as Chesapeake Bay (e.g. Chu and Greene 1989, Burreson 1991, Paynter and Burreson 1991). Oysters residing in intertidal habitats are subject to different conditions and different potential environmental stressors than their subtidal counterparts. At low tide, intertidal oysters are exposed to stressors such as extreme air temperatures, decreased DO due to valve closure, and desiccation. While intertidal conditions can affect oysters, such conditions may also impact disease (Milardo 2006). Much remains to be learned about the effects of *P. marinus* infection on oysters in intertidal systems and the effects of the intertidal habitat on the parasite itself.

The main objective of this study is to determine if a relationship exists between the variation in historic water quality conditions evidenced across the tidal creek system

and levels of *P. marinus* infection in oysters. Additional objectives include 1.) determination of the effects of background water quality on oyster physiological condition (i.e. tissue condition, growth, mortality, and reef metrics) in intertidal habitats 2.) comparison of infection prevalence and intensity among resident and hatchery- raised oysters 3.) determination of temporal patterns of infection in intertidal southeastern estuaries and 4.) correlation between infection levels and oyster condition. *A priori* predictions were that oysters in creeks with historically higher levels of nutrient inputs and particulates would have higher prevalence and intensity of infection (i.e. Hewletts >Howe >Pages; see methods) and lower overall condition (i.e. Hewletts< Howe< Pages). Given the adverse effect of *P. marinus* on the Eastern oyster, controlling susceptibility to this pathogen is considered essential to restoring oyster populations. Understanding the influence of anthropogenic stressors on disease susceptibility will aid in the development of more efficient restoration strategies.

METHODS

Overview

Temporal and spatial patterns of disease incidence and oyster growth, survivorship, and condition were examined among resident oysters and hatchery-reared oysters in an intertidal system. Examining oysters from natural populations provided data on the present levels of *P. marinus* infection in each of three tidal creeks. Yet these infection levels may be the result of past events or conditions, including mortality of susceptible individuals. Including hatchery oysters allowed for a comparison of disease incidence and current infection rates among the creeks using oysters with similar history.

However, there is the possibility that hatchery oysters may not respond in the same manner when exposed to *P. marinus* as would oysters from natural populations, requiring inclusion of both resident and hatchery-reared individuals in this study.

Study Sites

This study was conducted in three tidal creeks located in New Hanover County, North Carolina- Pages Creek, Howe Creek, and Hewletts Creek (Figure 1). There were six sampling sites within each of the three creeks- three natural oyster reefs (R1, R2, and R3) and three hatchery oyster deployment sites (C1, C2, and C3). The selected sites were mid-intertidal and were located in the mid-section of each tidal creek. Salinity (28ppt-36ppt) and tidal regime (-0.6 ft. to +1.2 ft.) were consistent for all of the sampling sites within and among the creeks. However, background landscape (e.g. human population, % development, impervious surface coverage) and water quality characteristics (e.g. nutrient levels, chlorophyll *a*, turbidity) differed among the three creeks (Mallin et al. 1998, Mallin et al. 2000, Cressman et al. 2003). Hewletts Creek watershed had the most development and the greatest amount of impervious surface coverage (Mallin et al. 1998, Mallin et al. 2000) (Table 1), usually associated with greater storm water runoff. Hewletts Creek had the highest overall orthophosphate and nitrogen concentrations of the three creeks, with the lowest levels occurring in Pages Creek (Mallin et al. 1998, Mallin et al. 2003, Mallin et al. 2005) (Table 1). While certain areas in each of the tidal creeks are subject to algal blooms, and low DO and hypoxia occasionally occurs in all three of the creeks, algal blooms (evidenced by chlorophyll *a* levels) tend to be more common in

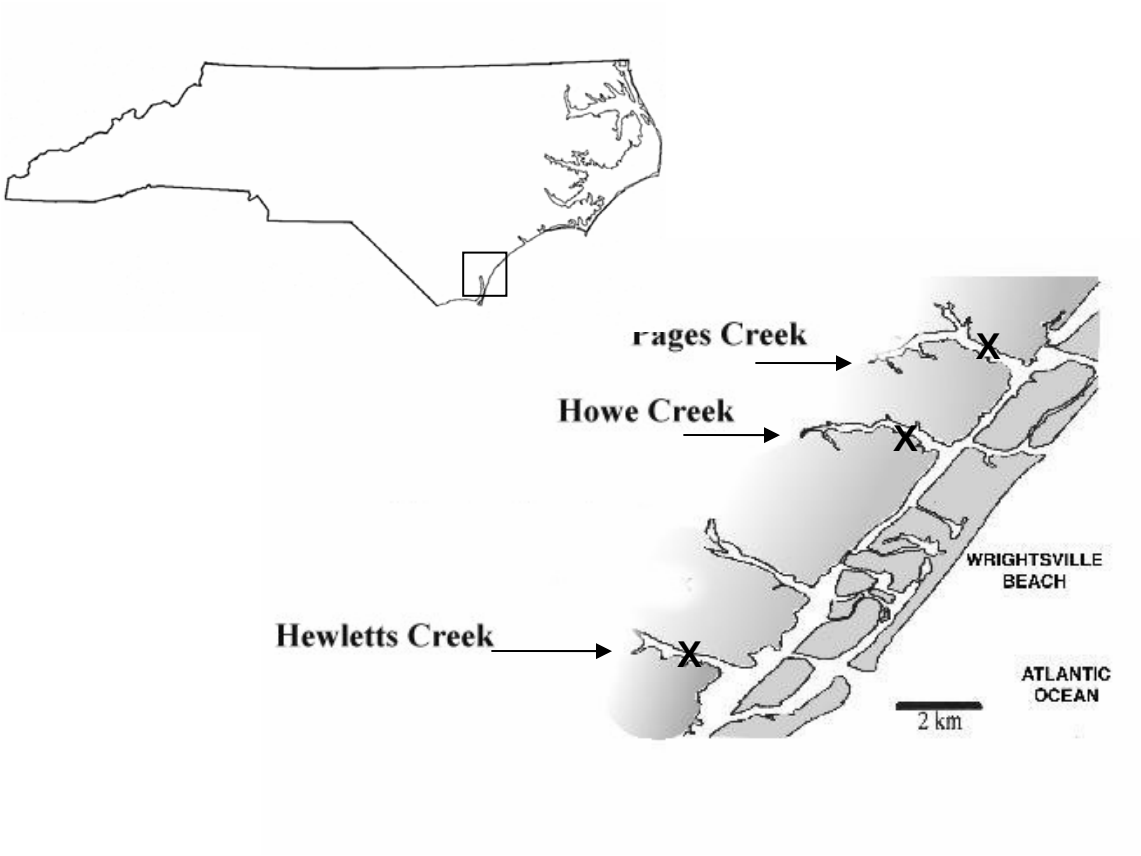

Figure 1. Location of tidal creeks used in study in southeastern North Carolina. Study areas in the mid-section of each creek are indicated with an "X." Salinity (28ppt-36ppt) and tidal regime (-0.6ft.-1.2ft.) were consistent for all creeks and all sampling sites. Wrightsville Beach is indicated for reference.

Table 1. Background landscape and water quality parameters for Hewletts, Howe, and Pages Creeks. Area, population, and impervious surface data are from Mallin et al. 2000. Percent development data are from Mallin et al. 1998. Chlorophyll *a* represent monthly means (extremes) for 1994-1997 from 3-4 stations per creeks (Mallin et al. 1998). Mean (extreme) turbidity and mean fecal coliform data are from Mallin et al. 1998.

	Creek		
Parameter	Hewletts	Howe	Pages
area (ha)	2,393	1,210	1,230
human population	13,000	3,937	4,185
percent development	81	51	56
percent impervious surface	18	13.9	8.7
average chl *a*(µg/L)	11.9 (203.8)	9.4 (88.4)	2.8 (40.7)
average nitrate (µM-N)	6.18 (41.54)	1.64 (26.52)	1.31 (7.59)
fecal coliform (CFU/ 100mL)	55	5	4
average turbidity (NTU)	N/A	6.5 (18.7)	4.9 (14.1)

Hewletts Creek and Howe Creek than in Pages Creek (Mallin et al. 1998, Mallin et al. 2003, Mallin et al.2005) (Table 1). However, while the three creeks differ in historic conditions, none of the creeks are severely impacted.

Oyster Reef Characteristics

Since water quality may affect oyster condition (i.e. oyster health), oyster populations and reef characteristics may also be impacted. Natural reef sites in each creek (R1, R2, and R3) were characterized in July/August 2005 using percent shell cover, live oyster density, oyster size, reef height, and rugosity. Each reef was sampled at random using a 50cm x 50cm square quadrat. Percent shell cover was determined by noting the material (i.e. live oyster, shell hash, or mud) under the intersection points of a 16 point grid system as well as estimated visually (total % of area covered by shell hash and live oysters). Oyster density was recorded as the number of live oysters within a quadrat and sizes (shell height in mm- umbo to edge of shell) of a random subset of 20 live oysters were measured. The average height of a reef was determined by measuring the highest point in each quadrat, from the sediment to the tip of the tallest shell. Rugosity was randomly sampled at five locations per reef. Rugosity is a measure of reef complexity and was measured by draping a 100cm chain across the reef in a straight line following the contour of the shell (Alphin and Posey 2006). The ratio of the conformed chain length (end to end straight measurement) to the total chain length is a measure of relative rugosity (0-1) with a lower number indicating greater vertical complexity.

Water Quality Measurements

Background data on water quality conditions for the mid-sections of the three creeks was obtained from studies previously conducted as part of the New Hanover County Tidal Creeks Program and from ongoing monthly sampling (Mallin et al. 1998, Mallin et al. 2005, Mallin et al. 2006). Because of their potential impact (direct or indirect) on oysters, emphasis was on salinity, temperature, turbidity, DO, and chorophyll *a*. In addition to the data from the Tidal Creeks Program, total suspended solids (TSS) and percent organics, were measured as part of this study. These two parameters were not specifically assessed by other programs, and it was thought that they may be important factors affecting oysters. Data was collected weekly from August 2006-January 2007 during spring tides (high flow) and neap tides (low flow) as well as following rain events. No data was collected the last two weeks of November due to a sewage spill which elevated fecal coliform counts to unsafe levels. While this sampling did not correspond to disease testing periods, it provided data on current conditions in the three creeks.

Triplicate one liter water samples were collected in subtidal channels upstream from all sampling sites in each creek. The samples were collected on the incoming tide, approximately 1.5 hours after low tide. Total suspended solids (TSS) for each site were determined by filtering 500ml of the water sample through a sterile glass fiber filter (0.45μm pore size, 47mm diameter). The filter was dried in a combustion oven for 30 minutes at 250°C in order to remove any moisture and weighed prior to filtration. Once the water samples were filtered, the filter containing the solids was placed in a drying oven for 1 hour at 70°C and then weighed again with TSS levels indicated by weight

differences (APHA 1998). In order to determine the amount of organics present in the water samples, the filters were placed in a combustion oven at 430°C for four hours. The filters were then weighed to obtain a post-combustion weight (g). Percent organics and organic concentration (mg/L) were determined based on weight differences. Salinity (ppt) was determined for each creek using a refractometer.

Preliminary Testing

Preliminary testing was conducted on oysters from each of the three tidal creeks during June/July 2005 in order to assess the infection levels in each creek prior to the start of the study and to examine size effects. Testing was performed on two size classes of oysters- first year oysters ranging from 40mm-50mm and second year oysters greater than 75mm. *P. marinus* infection was measured for 15 oysters from each of the size classes. The results of this testing indicated that a larger size class of oysters (60-80mm) would be the most appropriate size class for future sampling. The goal was to use oysters that had resided in the creeks long enough to respond to creek conditions. Parasite loads were also measured in oysters from a natural population in Stump Sound, North Carolina- the same area where the hatchery stock oysters were reared.

An initial assessment of *P. marinus* infection was also performed on a group of 20 hatchery-raised oysters in August 2005 before any of the outplants were deployed to establish a baseline infection level. It was not required that infection be absent in the hatchery oysters. Rather, infection was measured in these oysters to ensure that the hatchery stock had uniform and low background infection levels. Oyster tissue condition was also assessed for all oysters during preliminary testing.

Oyster Sampling

Oysters were sampled from the creeks four times during the course of this project: September 2005, November 2005, February 2006, and June 2006. September and November correspond to the period when prevalence and intensity of infection are thought to be at their highest. February is a time when infection levels are thought to decrease due to dormancy of the *P. marinus* parasite (Crosby and Roberts 1990, Ewart and Ford 1993, Ragone Calvo and Burreson 1994, Burreson and Ragone-Calvo 1996, Ford and Tripp 1996). Air and water temperature and salinity were measured during each sampling.

Twenty oysters from natural, intertidal populations were collected per sampling period at each reef site (R1, R2, R3). The oysters were sampled from various areas on the reefs at random using a 50cm x 50cm quadrat. The natural oysters sampled ranged in size from 60mm-80mm and were estimated to be at least one year old. This size class was chosen based on past work (Burreson and Ragone Calvo 1996, Ford and Tripp 1996, Volety et al. 2000) and preliminary data, indicating exposure to creek conditions long enough to show a response. Preliminary data indicated a size: infection relationship, with larger oysters having greater infection levels.

An equivalent number of deployed hatchery-raised oysters were also sampled from the creeks during each sampling period. The hatchery oysters were of the same size class as the natural oysters (approximately 60mm-80mm) at the start of the project. However, these oysters were less than a year in age (approximately 9 months old). The hatchery oysters were obtained in August 2005 from J&B Oyster AquaFood in Stump Sound, North Carolina with the brood stock originating from Louisiana. The hatchery-

raised oysters were placed in cages and deployed on mudflats at three sites (C1, C2, C3) adjacent to the natural reefs in each creek. The cages were elevated and secured approximately 10cm above the creek bottom using PVC/rebar racks. The hatchery oysters were caged to ensure recovery. The hatchery stock outplants were deployed in the tidal creeks the first week of August 2005, one month prior to the first sampling period (September 2005). The outplants consisted of 60 hatchery oysters per cage with three cages per site. The number of oysters included in the outplants was considerably larger than the sample size of 20 oysters to take into account the potential mortality that may occur during the first month.

Diagnosis of *Perkinsus marinus* infection

For all of the oysters sampled, natural and hatchery-raised, prevalence and intensity of *P. marinus* infection was measured using Ray's fluid thioglycollate medium (RFTM) tissue assay (Ray 1952, 1966). This is a semi-quantitative assay in that it gives numerical values for infection intensity while not requiring actual counts of *Perkinsus* spp. cells present in the sample. It is the standard method for monitoring Dermo disease at the population level (Ray 1966, Bushek et al. 1994).

Following collection, each oyster was labeled and shucked. Rectal tissue and a piece of mantle tissue (approximately 5mm x 5mm) over the labial palps were then excised from the oysters. Rectal tissue was chosen since *P. marinus* infection tends to target tissues of the oyster digestive system. It is also the tissue most commonly used for disease monitoring. Mantle tissue was used to measure infection in another region of the body. The location from which the mantle tissue was removed was chosen because of the

labial palps role in feeding (Ford and Tripp 1996). Sterile instruments were used between tissues and between oysters to prevent cross-contamination. The mantle and rectal tissues were placed in individual test tubes containing 9.5ml of RFTM. The tissues were then incubated for five to seven days in the dark at room temperature. The medium in each test tube was supplemented with 1ml of an antibiotic (Penicillin G) and antimycotic (Streptomycin) solution to reduce contamination. After the incubation period, the tissue samples were placed on a microscope slide, teased apart to ensure even staining, and stained using a 5:1 aqueous dilution of Lugol's iodine solution. The stained tissues were then pressed with a cover slip and examined microscopically at low magnification (40 to 100x). The slides were analyzed for *P. marinus* infection levels within 24 hours of staining so that stain did not fade and the parasite cells were easily visible. Those tissues not able to be examined immediately after the 5-7 day incubation period were stored in the dark at 4°C until analysis. Tissues can be stored for three months without deteriorating if the culture is kept refrigerated (Ashton-Alcox et al. 2006).

For each individual oyster, intensity of *P. marinus* infection was determined using a variation of the Mackin scale (Craig et al. 1989, Mackin 1962), which rates infection from 0 to 5 (negative to heavy) based on the density of parasites in the oyster tissue. According to this scale, each oyster is categorized and assigned a numerical value of infection intensity (e.g. negative= 0, very light=0.33, light negative=0.67, light=1, light positive=1.33, light/moderate negative=1.67, light/moderate=2, etc.) depending on the number of parasites in the tissue sample or percent coverage by the *P. marinus* cells. *P. marinus* cells are only counted for "light" or lower levels of infection. For heavier

infections, infection intensity is determined by the percentage of the tissue occupied by the parasite. Overall prevalence and intensity of infection was calculated for each sample of 20 oysters. Prevalence indicates the proportion of individuals in each sample that were infected. The intensity of infection was determined by taking the average of the infection intensity scores for all oysters in the sample.

Physiological Condition Measurements

Condition index (CI), a ratio of soft tissue dry weight to internal shell volume (Lawerence and Scott 1982, Abbe and Albright 2003), was determined for all natural and hatchery stock oysters sampled during the four sampling periods and for preliminary background measures. This is a measure of soft tissue growth and is considered to be an indicator of oyster health (Austin et al. 1993). Condition index was calculated for each oyster using a variation of Hopkin's formula (Lawerence and Scott 1982, Austin et al. 1993):

$$CI = [(\text{dry weight of tissue}) * 100] / \text{internal shell volume}$$

In order to determine the internal shell volume, a water displacement method was used comparing water displaced by the closed/intact, whole oyster to the water displaced by the empty oyster shell (Abbe and Albright 2003). Wet tissue weight was determined for each oyster. Dry tissue weight was obtained once the oyster tissues had dried for 24 hours at 70°C in a drying oven. CI was also assessed visually, using the standardized method of Quick and Mackin (1971). This rating system ranks oyster condition on a scale of 1 to 9 based on the appearance of its tissues, with 1 being the best (a plump,

opaque oyster) and 9 being the poorest (a watery, transparent oyster) condition. This is the method used by state and management agencies and is standard protocol. The background oysters from the three creeks during June/July 2005 were only assessed using the visual CI.

In addition to CI, shell growth was assessed for a group of 90 hatchery oysters that were tagged and measured prior to deployment. A single cage of these tagged hatchery oysters was deployed in each creek. Growth measurements were taken during the second sampling period (November 2005) and again during the fourth sampling period (June 2006). Measurements were made in two dimensions- shell height and width (mm) using calipers (Morales-Alamo and Mann 1989, Encomio et al. 2005). Shell height was taken to be the long axis of the oyster from the umbo to the outer edge of the shell whereas shell width was taken at the widest part of the shell perpendicular to shell height. The purpose of these measurements was to estimate the growth rate of the hatchery population deployed in a particular creek and to compare growth of this stock among the creeks. Mortality counts (i.e. number of gaping, articulated shells) were made for the hatchery oysters sampled from all sites during each of the four sampling periods and were also taken when hatchery oyster growth was assessed in the creeks.

Test For Potential Caging Effects

Since all hatchery oysters were caged and were being compared to uncaged natural oysters throughout the course of the study, it was necessary to test for potential caging effects (e.g. the effect of cage enclosure and oyster density on growth rate, mortality, and potentially on *P. marinus* infection levels). To do this, natural oysters

were deployed in cages on mudflats and uncaged areas on reefs in Hewletts Creek. Oysters were collected from all three reef sites in Hewletts Creek in December 2005. The oysters were separated from one another, tagged, and measured (shell height and width) prior to deployment. All cages contained the same density of oysters (60 oysters per cage) as the hatchery oysters used for disease testing and were placed in the same manner at the caging sites (C1, C2, and C3). The oysters deployed on the reefs were in the same density as average live oyster density on the reefs (50 oysters), determined from quadrat sampling. They were deployed in a marked 50cm x 50cm square area on the reefs, cleared of all other live oysters. The caged and uncaged natural oysters were measured monthly from January 2006- September 2006 to assess growth rates between the different site types. Oyster mortality was also assessed at the caged and uncaged sites

Statistical Analysis

All statistics were run on PC-SAS, version 9.1.3 (SAS Institute, Cary NC). Initial analysis using three-way Analysis of Variance (ANOVA) indicated interaction among variables necessitating the use of two-way ANOVAs to examine main effects. Two-way ANOVA was used to determine differences in infection, condition, tissue volume, and tissue wet weight between sampling period and creek, sampling period and oyster type (i.e. hatchery or natural) and creek and oyster type as well as to test for interaction between those factors. When interactions were found, 1-way ANOVAs were used to separate the effects of the two factors. The Student-Newman-Keul (SNK) test was used to conduct pair-wise comparisons and determine differences where ANOVAs were

significant. Two-way ANOVAs were also used to test for statistical differences in TSS and % organics among the creeks and between spring and neap tides.

One-way ANOVAs were used to assess differences in oyster reef characteristics (shell cover, oyster density, oyster size, reef height, rugosity) among the creeks. Tukey tests were used to determine differences among creeks where ANOVAs were significant. The shell cover and rugosity data were arcsine-sqrt transformed before analysis. Live count data were log transformed as it did not meet homogeneous variance requirements. Background water quality data for each creek was qualitatively compared to reef metrics to observe potential relationships between these variables.

Growth of tagged hatchery oysters and of natural oysters deployed in Hewletts Creek, was analyzed using the Mixed Procedure in SAS. This procedure generated 2-way ANOVA output comparing the effects of time (months deployed) and creek on changes in shell height and width as well as interactive effects of these factors on oyster growth. For the natural oysters deployed in Hewletts Creek to assess potential caging effects, the effects of time (months deployed) and caging treatment (caged vs. uncaged) on changes in shell height and width were compared as well interactive effects of these factors on growth. All growth analysis was adjusted using the Bonferroni method. This method adjusts the significance level based on the number of pair-wise comparisons in the analysis.

Mortality counts for the hatchery oysters were assessed using the GENMOD procedure in SAS to compare percent oyster mortality between sampling periods and among creeks and interactions among these factors. Mortality counts for the natural oysters in Hewletts Creek were assessed in the same manner, comparing differences in

percent oyster mortality over time and between caged and uncaged treatments and interactions between these factors. All mortality data was log transformed, and the output generated estimates of the log odds (i.e. probability) of mortality occurring. Chi-square (goodness-of-fit test) was used to estimate how closely oyster mortality matched expected mortality (i.e. probability of constant mortality).

Water quality data (i.e. total suspended solids and salinity) collected as part of this study was compared to the New Hanover County Tidal Creek Program's 2006-2007 data (i.e. turbidity and salinity) for the same time period. Comparisons were made using the Correlation procedure in SAS (Spearman's rank correlation- a non-parametric analysis). Background data (2005-2006) from the Tidal Creeks Program (i.e. salinity, temperature, turbidity, dissolved oxygen, chlorophyll *a*) was correlated to oyster disease and condition data for the same time period. Water quality parameters for the 3 months prior to each sampling period were compared to infection intensity, condition, and reef metrics for each sampling to relate water quality to oyster health. Correlation was used to determine the relation between *P. marinus* infection levels in mantle and rectal tissues as well as to compare the two condition indices (calculated condition and visual condition). Infection levels and oyster condition were also correlated.

RESULTS

Preliminary Testing

Preliminary disease testing of natural oysters during June/July 2005 showed no significant difference for *P. marinus* infection intensity in either tissue (mantle or rectal) among the three tidal creeks. However, infection intensity was significantly lower in the

natural oysters sampled from Stump Sound than it was in any of the three creeks- rectal infection ($F=13.07$, $p<0.0001$), mantle infection ($F=10.86$, $p<.0001$). The rectal and mantle tissues from Stump Sound had mean infections intensities of 0.17 and 0.18 respectively (Note: these values correspond to less than very light infections on Mackin scale). Infection was detected in only 32% of the Stump Sound oysters whereas infection was 90-100% in the oysters sampled from the three tidal creeks (Table 2).

Infection intensity varied between small (40-50mm) and large (>75mm) size classes of oysters from the tidal creeks for both mantle and rectal tissues. Infection ($F=4.18$, $p=0.0434$) was significantly greater in large oysters than small oysters (infection values of 1.31 and 0.70 respectively) for rectal tissue. A similar size class relationship was seen for mantle tissue infection. For both rectal and mantle tissue there was no interaction between size class and creek ($F=0.72$, $p=0.3979$ and $F=1.84$, $p=0.1781$ respectively). Visually assessed CI did not vary among the size classes ($F=0.05$, $p=0.8269$) or among creeks ($F=1.26$, $p=0.2930$) during preliminary testing (Table 2). However, an interaction ($F=7.40$, $p=0.0078$) was present for size class and creek with small oysters having lower condition in Hewletts Creek while large oysters had lower condition in Howe Creek.

Infection intensity was significantly lower in the subset of hatchery oysters tested in August 2005 prior to deployment than in the natural oysters from the creeks sampled during June/July 2005. This was true for both mantle tissue ($F=11.60$, $p=0.0009$) and rectal tissue ($F=11.99$, $p=0.0007$). Infection prevalence was also lower in hatchery oysters than in natural oysters from the tidal creeks (Table 2).

Table 2. *Perkinsus marinus* infection levels and oyster condition data for preliminary testing (June/July 2005 for natural oysters from tidal creeks and Stump Sound; August 2005 for hatchery stock from J&B). Size class data includes tidal creek oysters only. Data presented as mean (±SE). RV= rectal infection intensity; MV= mantle infection intensity; CIV= visually assessed condition; N= natural; H= hatchery.

	Prevalence	**RV**	**MV**	**CIV**
Origin				
Hewletts Creek	100%	1.04 (0.19)	1.05 (0.18)	5.67 (0.32)
Howe Creek	100%	1.14 (0.08)	1.16 (0.07)	5.88 (0.21)
Pages Creek	90%	1.31 (0.16)	1.29 (0.12)	5.71 (0.29)
Stump Sound (N)	32%	0.17 (0.07)	0.18 (0.09)	5 (0.44)
J&B AquaFood (H)	75%	0.88 (0.15)	0.90 (0.14)	5 (0.29)
Oyster Size				
Small (40-50mm)	93%	0.70 (0.08)	0.86 (0.08)	5.68 (0.19)
Large (+75mm)	97.50%	1.31 (0.15)	1.21 (0.13)	5.61 (0.25)

Infection Prevalence

No significant differences were found for infection prevalence during September 2005-June 2006, either temporally or among creeks. Prevalence of *P. marinus* infection was 95%-100% for all sampling periods and creeks with the only exception being February 2006 for natural oysters in Howe creek. No significant differences were seen for infection prevalence between natural and hatchery oysters (nearly 100% in both).

Infection Intensity

Tissue Type Differences

Infection intensity was consistently higher in rectal tissue than in mantle tissue for both oyster types, among all creeks, and for all sampling periods. However, there was a strong positive correlation ($r=0.7815$, $p<0.0001$) between the two tissue types. Because of this correlation between mantle and rectal infection levels, the remainder of the results will only use rectal tissue infection values. Mantle tissue infection values will only be presented when mantle tissue represents a different pattern than rectal tissue.

Rectal Tissue

Rectal infection intensity varied across sampling periods ($F=31.61$, $p<0.0001$) with the highest infection occurring in November 2005 (Mackin scale: 1.57) and the lowest infection occurring in February 2006 (0.94). September 2005 and June 2006 did not differ significantly from each other, but differed from both November 2005 and February 2006. There was an interaction present between sampling period and oyster type ($F=13.79$, $p<0.0001$), but not between sampling period and creek ($F=1.94$,

p=0.0721). For hatchery oysters, November 2005 infection levels were significantly higher than during all other sampling periods. For natural oysters, infection levels during all other sampling periods were significantly higher than February 2006 infection levels (Figures 2-3, Appendix A, D).

Rectal tissue infection intensity also varied among creeks (F=3.59, p=0.0278) with Howe Creek having a higher intensity (1.40) than either Pages or Hewletts Creeks (1.30 and 1.27 respectively). No interactions were present between creek and oyster type (F=0.19, p=0.8240) or between creek and sampling period (F=1.94, p=0.0721) for rectal tissue infections (Appendix A, D).

Overall, infection intensity varied between hatchery and natural oysters for rectal tissue (F=34.99, p<0.0001) with hatchery oysters (1.46) having significantly higher infections than natural oysters (1.20). No interaction was detected between oyster type and creek (F=0.19, p=0.8240). There was an interaction present between oyster type and sampling period (F=13.79, p<0.0001). Rectal infection was significantly higher in hatchery oysters than in natural oysters during November 2005 and February 2006 but not during the sampling periods of September 2005 and June 2006 (Figures 2-3; Appendix A, D).

Mantle Tissue

Mantle tissue infection intensity also varied across sampling periods (F=39.06, p<0.0001), following the same pattern as rectal infection intensity. There was also an interaction between sampling period and oyster type (F=7.52, p<0.0001) similar to the pattern seen for rectal tissue infection. However, for mantle tissue infection there was

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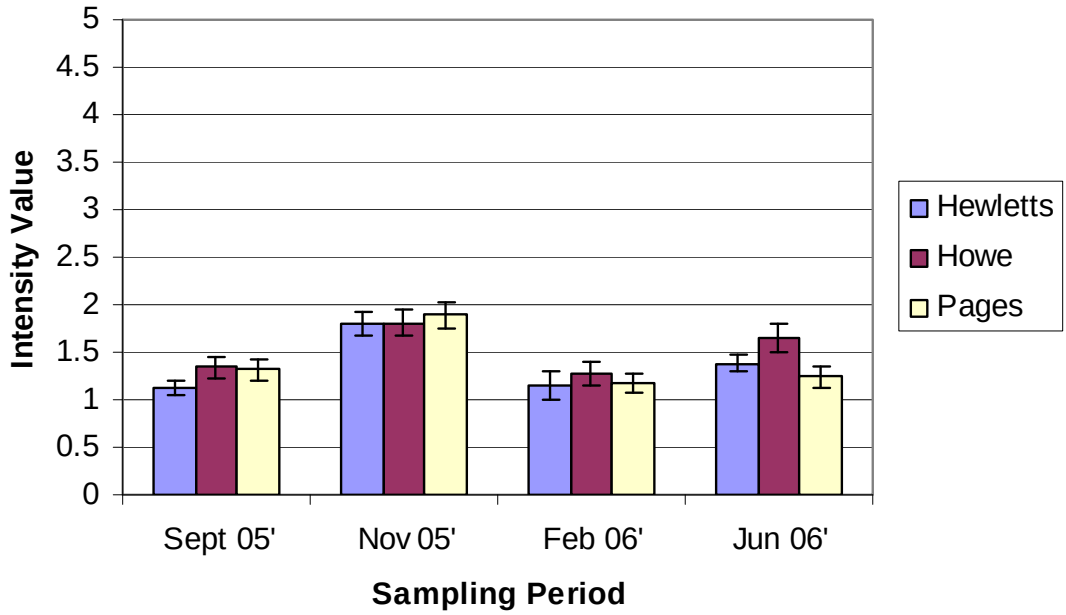

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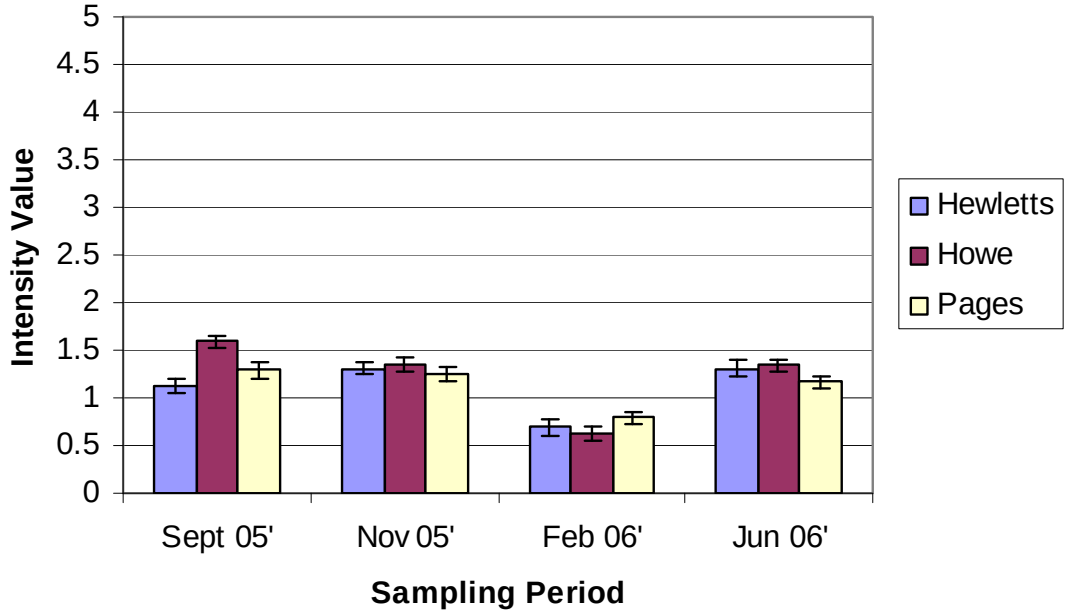
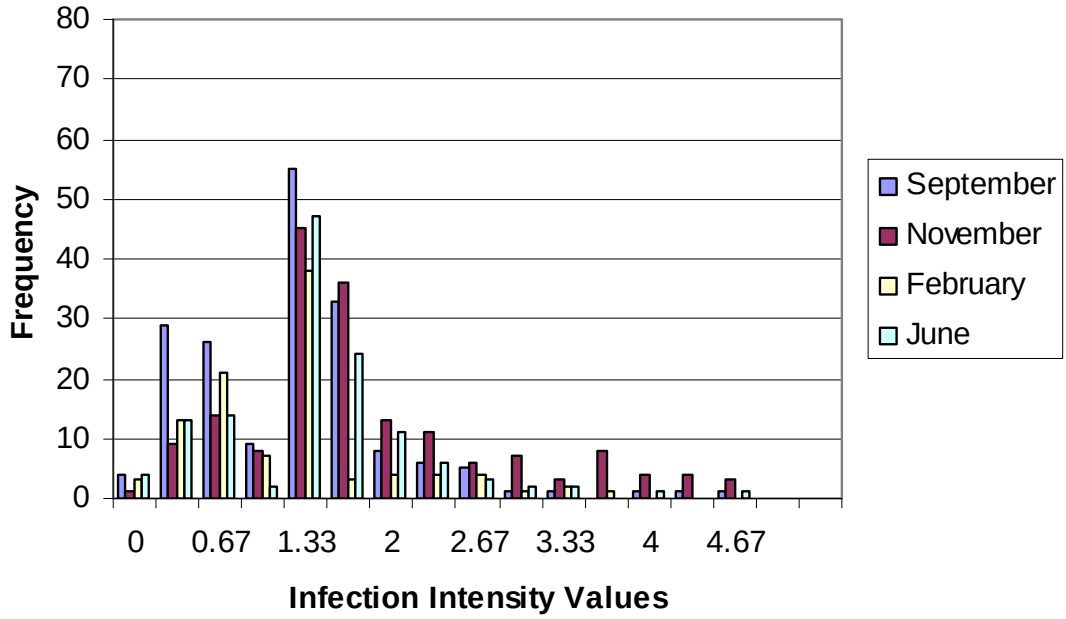


Figure 2. a.) Mean rectal infection intensity across sampling periods by creek for hatchery oysters. b.) Mean rectal infection intensity across sampling periods by creek for natural oysters. Bars indicate mean intensity values (±SE) for each sampling period in each creek. Infection intensity is ranked 0-5 (negative to heavy) using a variation of the Mackin scale (Craig et al. 1989, Mackin 1962).

a.)



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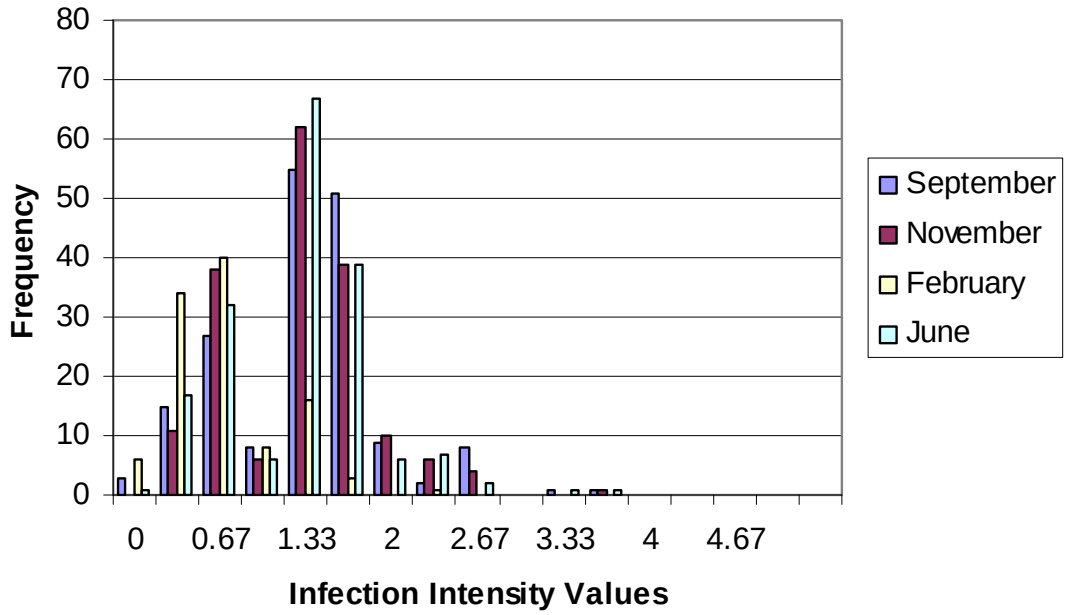

Figure 3. a.) Frequency distribution of rectal infection intensity values by sampling period for hatchery oysters. b.) Frequency distribution of rectal infection intensity values by sampling period for natural oysters. Infection intensity is ranked 0-5 (negative to heavy) using a variation of the Mackin scale (Craig et al. 1989, Mackin 1962).

also an interaction present between sampling period and creek ($F=3.02$, $p=0.0062$). Mantle intensity followed the overall sampling period pattern for Hewletts Creek (highest in November 2005 and lowest in February 2006). However, in Pages Creek mantle infection was significantly higher in November 2005 than in all other sampling periods. In Howe Creek mantle infection was significantly lower in February 2006 than in all other sampling periods (Appendix A, D).

Mantle tissue infection intensity varied among creeks ($F=3.04$, $p=0.0482$) with higher infection in Howe Creek (Mackin scale: 1.19) than in Hewletts Creek (1.07). However, mantle infection in Pages creek did not differ from either of the other two creeks. As with rectal infection there was no interaction present between creek and oyster type ($F=0.24$, $p=0.7881$). Infection was significantly higher in hatchery oysters than natural oysters in all creeks. There was an interactive effect between creek and sampling period ($F=3.02$, $p=0.0062$). During September 2005, mantle infection in Howe Creek was significantly higher than in either of the other two creeks, but no creek differences were seen in the other three sampling periods (Appendix A, D).

Overall, infection intensity varied between hatchery and natural oysters ($F=52.84$, $p<0.0001$) with hatchery oysters (1.30) having significantly higher infections than natural oysters (0.99). No interaction was present between oyster type and creek ($F=0.24$, $p=0.7881$). An interaction was present between oyster type and sampling period ($F=7.52$, $p<0.0001$) similar to that seen for rectal infection (Appendix A, D).

Initial vs. Final Infection Intensities

Rectal infection intensity in natural oysters sampled during preliminary testing in June/July 2005 was similar to infection intensity during the final sampling period in June 2006. Infection intensity in hatchery oysters tested in August 2005 prior to deployment was also similar to infection intensity in June 2006 (Table 3). Preliminary infection intensity for both natural and hatchery oysters differed significantly from the infection intensity during November 2005 and February 2006.

Physiological Condition

Calculated Condition Index

Calculated CI was measured quantitatively as a ratio of soft tissue dry weigh to internal shell volume. A higher value indicates better oyster condition. Calculated CI varied among sampling periods ($F=28.00$, $p=<0.0001$). The lowest condition occurred in November 2005 (calculated condition value: 3.89) with highest condition in February 2006 (6.12) and intermediate condition in September 2005 and June 2006. This pattern was the inverse of the seasonal trend for infection intensity. There were interactions present between sampling period and creek ($F=4.70$, $p<0.0001$) and between sampling period and oyster type ($F=15.27$, $p<.0001$) for calculated CI. Condition peaked in February 2006 for Hewletts and Pages Creek and in June for Howe Creek. However, June 2006 condition did not differ significantly from February 2006 condition in Howe Creek. For hatchery oysters, November 2006 condition was significantly lower than during all other sampling periods. For natural oysters, condition was significantly lower during all other sampling periods than it was during February 2006. While natural oyster condition

Table 3. Summer 2005 vs. Summer 2006 *Perkinsus marinus* infection levels and oyster condition data for hatchery and natural oysters in all three tidal creeks (June/July 2005 vs. June 2006 for natural oysters; August 2005 vs. June 2006 for hatchery oysters). Data presented as mean (±SE). RV= rectal infection intensity; MV= mantle infection intensity; CIV= visually assessed condition.

Hatchery Oysters	RV	MV	CIV
Hewletts Creek			
Initial (Aug. 05')	0.88 (0.15)	0.90 (0.14)	5 (0.29)
Final (June 06')	1.38 (0.09)	1.12 (0.08)	5.52 (0.24)
Howe Creek			
Initial (Aug. 05')	0.88 (0.15)	0.90 (0.14)	5 (0.29)
Final (June 06')	1.65 (0.15)	1.47 (0.13)	6.23 (0.21)
Pages Creek			
Initial (Aug. 05')	0.88 (0.15)	0.90 (0.14)	5 (0.29)
Final (June 06')	1.24 (0.11)	1.15 (0.11)	6.44 (0.20)

Natural Oysters	RV	MV	CIV
Hewletts Creek			
Initial (Jun./Jul. 05')	1.31 (0.37)	1.21 (0.32)	5 (0.51)
Final (June 06')	1.31 (0.09)	1.12 (0.08)	5.77 (0.15)
Howe Creek			
Initial (Jun./Jul. 05')	1.30 (0.14)	1.07 (0.17)	6.4 (0.34)
Final (June 06')	1.34 (0.07)	1.05 (0.06)	5.92 (0.11)
Pages Creek			
Initial (Jun./Jul. 05')	1.31 (0.16)	1.29 (0.12)	5.71 (0.29)
Final (June 06')	1.17 (0.06)	1.04 (0.06)	5.38 (0.13)

peaked in February 2006, hatchery oyster condition peaked in June 2006 (Figures 4-5; Appendix A, D).

Calculated CI did not vary among tidal creeks ($F=0.60$, $p=0.5486$). There was no interactive effect for condition between creek and oyster type ($F=2.67$, $p=0.0699$), but an interaction was detected between creek and sampling period ($F=4.70$. $p<0.0001$). In November 2005, oyster condition in Howe Creek was significantly higher than in Hewletts Creek, in February 2006, condition in Hewletts Creek was significantly higher than in the other two creeks, and in June 2006, Howe Creek oysters had significantly higher condition than in the other two creeks (Appendix A, D).

Overall ($F=234.99$, $p<0.0001$) hatchery oysters (3.51) had significantly lower condition than natural oysters (5.94). Hatchery oyster condition was significantly lower than natural oyster condition for all sampling periods and among all three creeks (Figures 4-5; Appendix A, D).

Oyster tissue volume varied among sampling period, creek, and oyster type. However, there were no interactions among any of these factors. Oyster volume was significantly lower ($F=15.95$, $p<0.0001$) in February 2006 than during any other sampling period. Oysters in Pages Creek had significantly higher ($F=4.93$, $p=0.0074$) tissue volume than either of the other two creeks. Hatchery oyster tissue volume was significantly greater ($F=967.24$, $p<0.0001$) than natural oyster tissue volume (Appendix A, D).

Tissue wet weight varied among sampling periods and for oyster type. Wet weight was significantly lower in November 2005 ($F=13.75$, $p<0.0001$) than in any other sampling period. Hatchery oyster wet weight was significantly greater ($F=259.44$,

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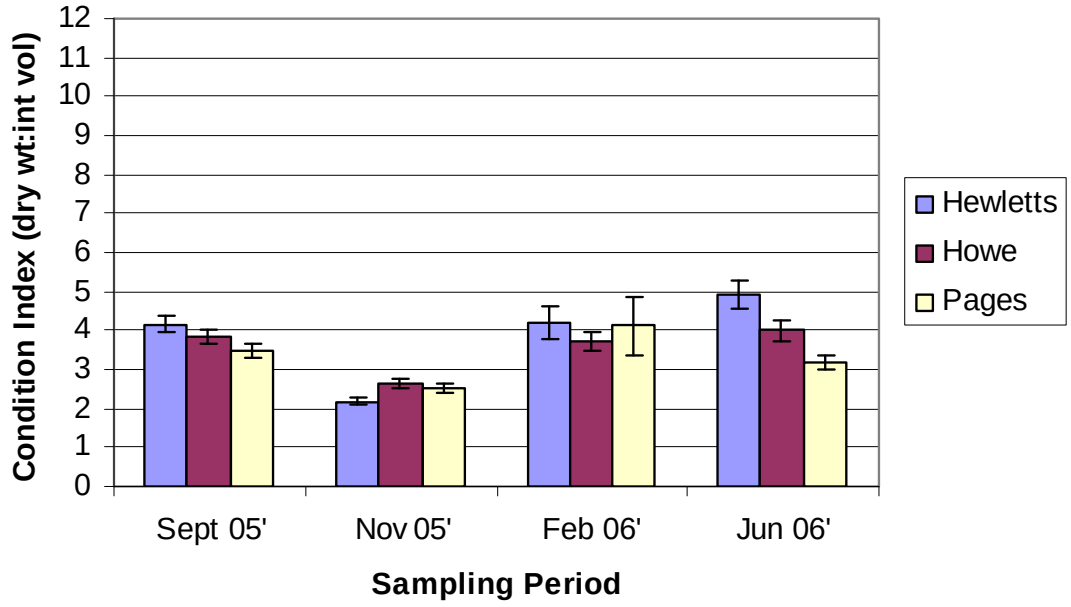

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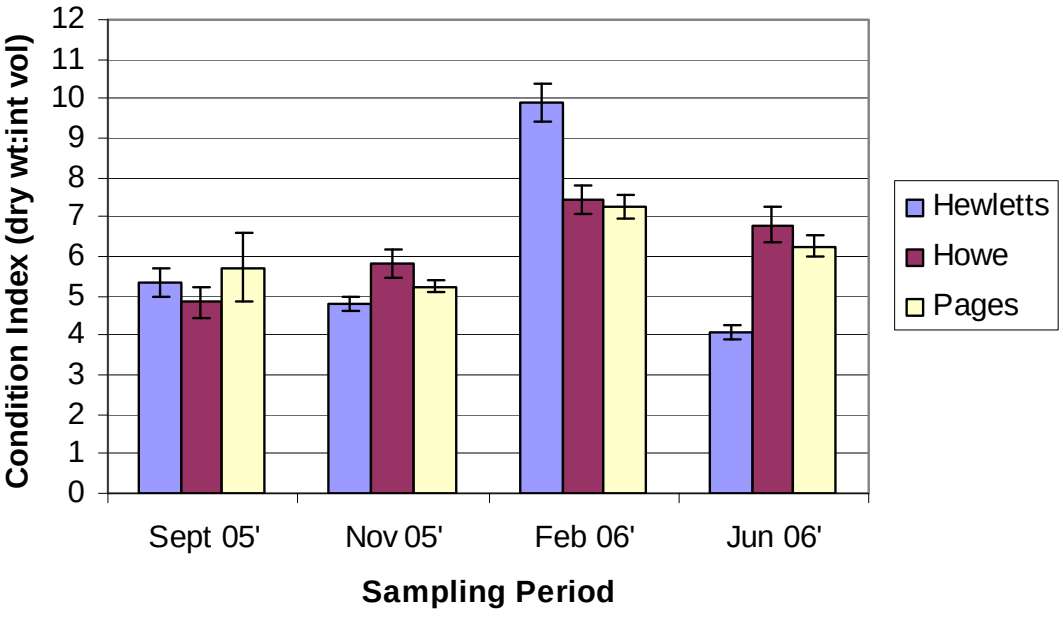
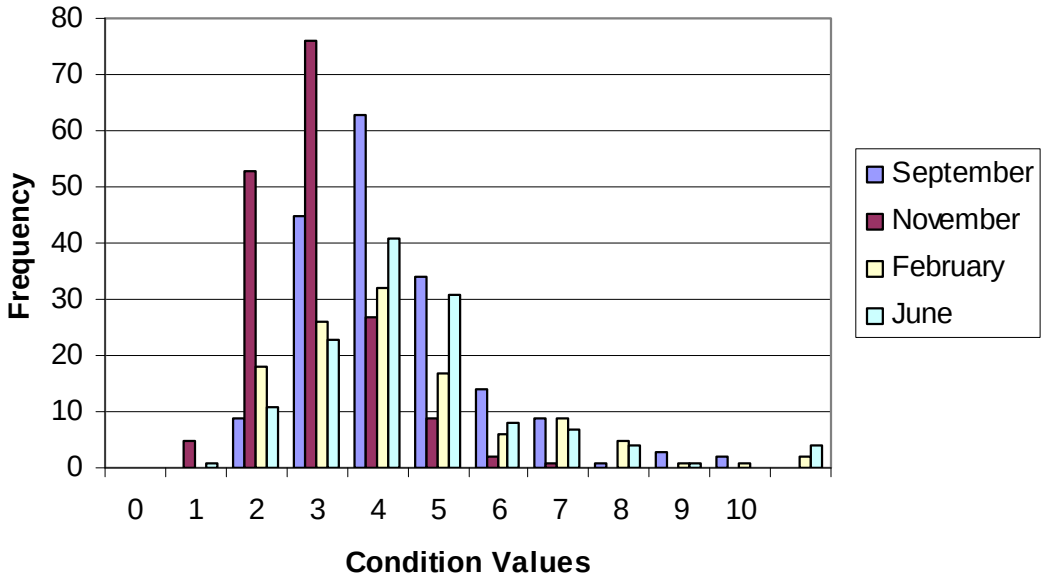


Figure 4. a.) Mean calculated condition index across sampling periods by creek for hatchery oysters. b.) Mean calculated condition index across sampling periods by creek for natural oysters. Bars indicate mean condition values (±SE) for each sampling period in each creek. Condition is a ratio of dry tissue weight to internal shell volume. Higher values indicate better tissue condition.

a.)



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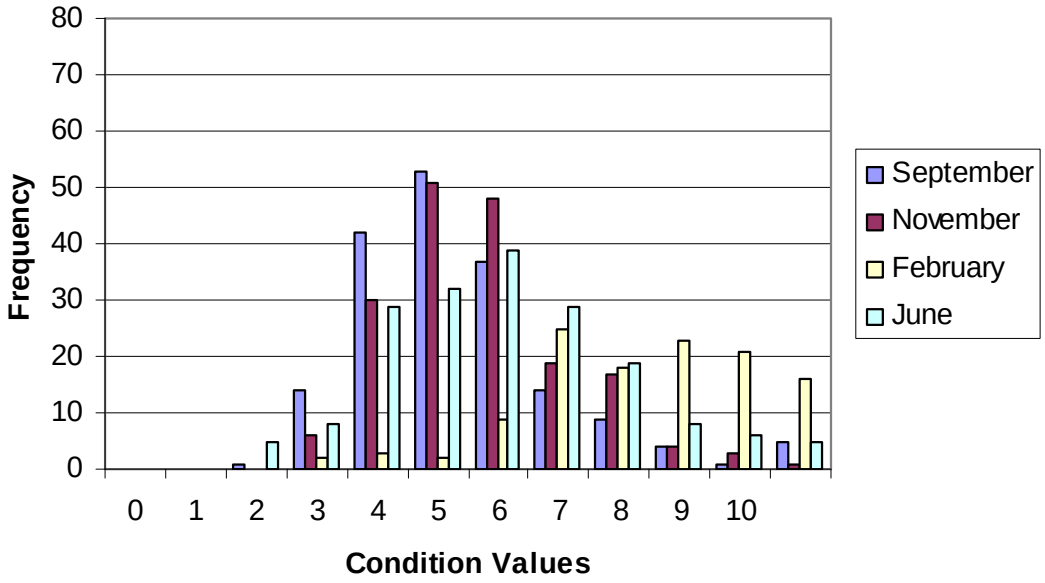

Figure 5. a.) Frequency distribution of calculated condition index by sampling period for hatchery oysters. b.) Frequency distribution of calculated condition index by sampling period for natural oysters. Condition is a ratio off dry tissue weight to internal shell volume. Higher values indicate better tissue condition.

$p<0.0001$) than natural oyster wet weight. Tissue wet weight did not differ among creeks. There were interactive effects among sampling period and creek ($F=3.70$, $p=0.0012$), sampling period and oyster type ($F=35.83$, $p<0.0001$), and creek and oyster type ($F=5.06$, $p=0.0065$) (Appendix A, D).

Visual Condition Index

Higher visual condition values indicate poorer condition (Note: this is the reverse of calculated CI). Visually assessed CI varied among sampling periods ($F=25.90$, $p<0.0001$), but did not follow the same pattern as calculated condition. The lowest condition (highest visual CI value) occurred during September 2005 (visual condition value, Quick and Mackin: 6.44) and November 2005 (6.36) and highest condition occurred during February 2006 (5.61). There was an interactive effect for visual CI between sampling period and creek ($F=2.15$, $p=0.0453$) and between sampling period and oyster type ($F=15.16$, $p<0.0001$). For Pages Creek condition peaked in June, while peak condition occurred in February for Howe and Hewletts Creeks. For hatchery oysters, condition peaked in June and was lowest in November. Condition peaked in February and was lowest in September for natural oysters (Appendix A, D).

Visually assessed condition did not vary among tidal creeks ($F=0.69$, $p=0.4997$). However, there was an interaction present between creek and sampling period ($F=2.15$, $p=0.0453$) as well as between creek and oyster type ($F=4.29$, $p=0.0139$). For natural oysters, condition was significantly higher in Pages Creek than in Howe Creek. Condition in Hewletts Creek did not differ from the other two creeks. Condition did not differ among creeks for hatchery oysters (Appendix A, D).

Overall, ($F=113.39$, $p<0.0001$) hatchery oysters (6.54) had significantly lower condition than natural oysters (5.73). Hatchery oyster visual condition was significantly lower than natural oyster visual condition for all sampling periods and for all creeks (Appendix A, D).

Condition Index Comparison

A negative correlation ($r= -0.4900$, $p<0.0001$) was present between calculated oyster condition and visually assessed oyster condition (reflecting the inverse nature of those scales) (Figure 6). Analysis showed a weak positive correlation ($r=0.0701$, $p=0.0129$) between calculated CI and tissue wet weight while a negative correlation was present between calculated CI and oyster tissue volume ($r=-0.6889$, $p<0.0001$).

Relationship Between Infection and Oyster Condition

The temporal trend seen for calculated condition was the inverse of the pattern seen for infection intensities. As infection intensity increased over time, oyster condition decreased and vice versa. There was a significant negative correlation between rectal infection and calculated CI ($r=-0.2515$, $p<0.0001$) (Figure 7) and a significant positive correlation between rectal infection and visually assessed CI ($r=0.1984$, $p<0.0001$). The same pattern was seen when comparing mantle infection intensities to the condition indices.

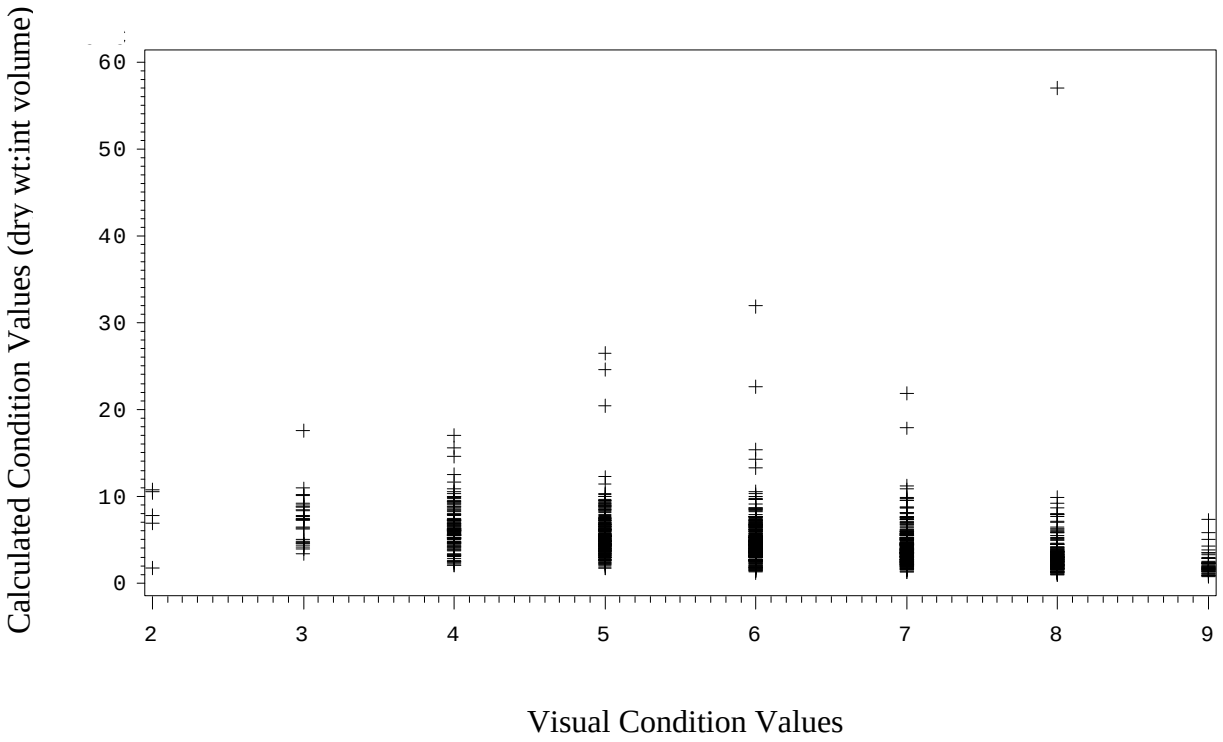

Figure 6. Scatterplot showing correlation between calculated condition index and visual condition index. Calculated condition is a ratio of dry tissue weight to internal shell volume. Higher values indicate better tissue condition. Visual condition is ranked based on appearance of oyster tissues (Quick and Mackin 1971). Higher values indicate poorer condition.

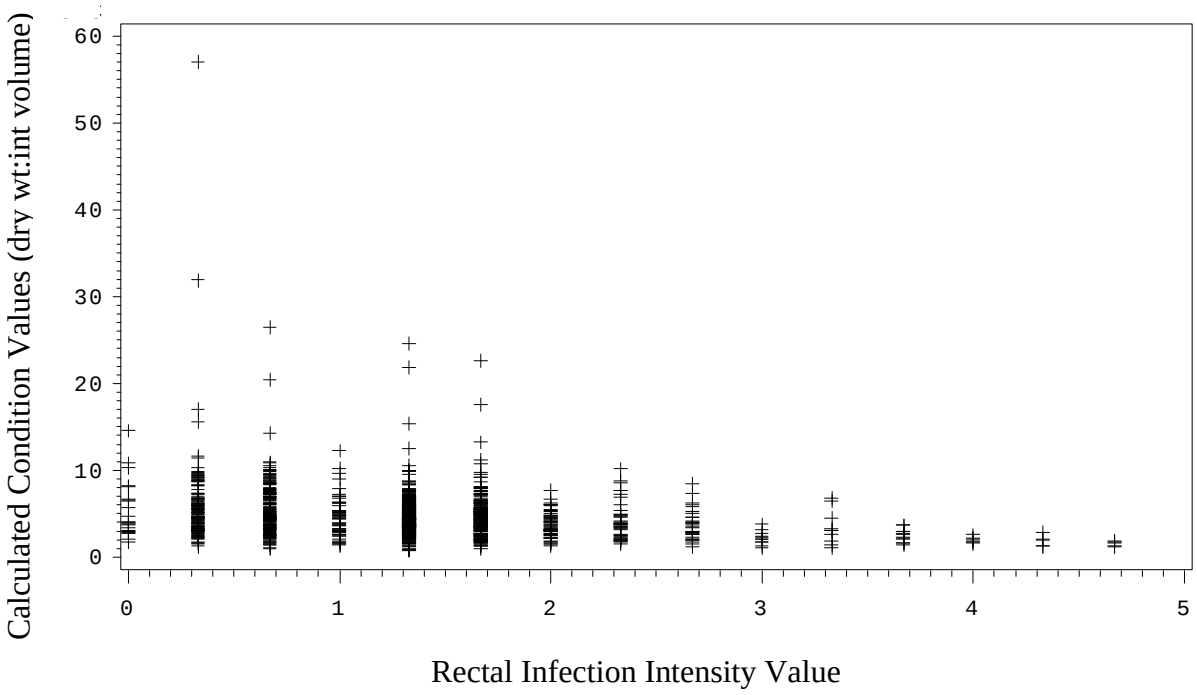

Figure 7. Scatterplot showing correlation between calculated condition index and rectal infection intensity. Calculated condition is a ratio of dry tissue weight to internal shell volume. Higher values indicate better tissue condition. Infection intensity is ranked 0-5 (negative to heavy) using a variation of the Mackin scale (Craig et al. 1989, Mackin 1962).

Growth

Hatchery oyster growth varied over time ($F=50.70$, $p<0.0001$) and among creeks ($F=14.26$, $p<0.0001$) for shell height. An interaction was present for hatchery oyster height between time and creek ($F=8.57$, $p<0.0001$; Bonferroni adjusted alpha=0.0028). In Hewletts Creek growth was significantly different between the initial August 2005 measurement and the final measurement in June 2006 as well as from November 2005 to June 2006. For the hatchery oysters deployed in Howe Creek, height changes were significantly different for all time intervals. In Pages Creek growth was significantly different from August 2005 to November, but not for any other time interval. There were no initial height differences among the creeks in August 2005. However, height was significantly greater in Howe Creek in November 2005 and June 2006 (Figure 8; Appendix B).

Hatchery oyster growth also varied over time for shell width ($F=10.16$, $p<0.0001$). There was no significant width change among creeks ($F=0.90$, $p=0.4070$). However, an interaction was present for hatchery oyster width between time and creek ($F=3.34$, $p=0.0133$). For Hewletts Creek and Pages Creek growth was not significant over time. For Howe creek the only significant change in width was from August 2005 to November 2005. There were no initial width differences among the creeks in August 2005 or at any other time (Appendix B).

Mortality

For the tagged hatchery oysters used for growth assessment, mortality did not vary over time ($F=0.46$, $p=0.4994$) or among creeks ($F=5.22$, $p<0.0737$). However, an

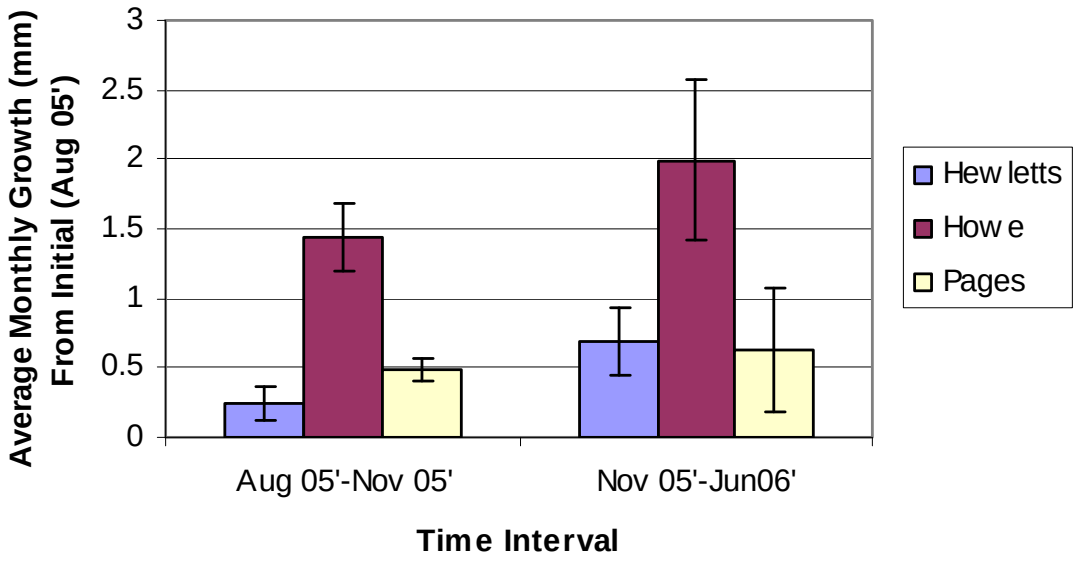

Figure 8. Average monthly growth (mm) of hatchery oysters from initial measurements in August 2005 (n=30). Bars indicate mean change in shell height (±SE) for oysters measured during a given sampling period in each creek.

interaction was present for oyster mortality between time and creek ($F=9.43$, $p<0.0090$; Bonferroni adjusted alpha=0.0056). For Hewletts Creek and Howe Creek, mortality was significantly lower in June 2006 than in November 2005. In June 2006 mortality in Howe Creek was significantly lower than mortality in the other two creeks (Appendix B).

For the hatchery oysters sampled from the cages used for disease testing, mortality varied among sampling periods ($F=586.72$, $p<0.0001$) but did not vary among creeks ($F=4.00$, $p=0.1351$). An interaction was detected for mortality between sampling period and creek ($F=45.82$, $p<0.0001$; Bonferroni adjusted alpha=0.0027 for sampling period and 0.004 for creek). Oyster mortality was greatest in November 2005 for Hewletts Creek and Pages Creek and in September 2005 for Howe Creek. The lowest mortality occurred in February 2006 for Howe Creek and Pages Creek and in September 2005 for Hewletts Creek. Mortality in Howe Creek was significantly greater in September 2005 than in either of the other two creeks. In February 2006, mortality was greater in Hewletts Creek than in Pages Creek. In June 2006, Hewletts Creek and Pages Creek had greater mortality than Howe Creek (Figure 9, Appendix B).

Test for Potential Caging Effects

For the natural oyster deployed in Hewletts Creek to assess potential caging effects, growth varied over time ($F=72.63$, $p<0.0001$) and between caged and uncaged treatments ($F=24.49$, $p<0.0001$) for shell height. An interaction was detected for oyster height between time and treatment ($F=21.47$, $p<0.0001$; Bonferroni adjusted alpha=0.0008). A significant height increase was observed between the initial measurements in December 2005 to the final measurements in September 2006 for caged

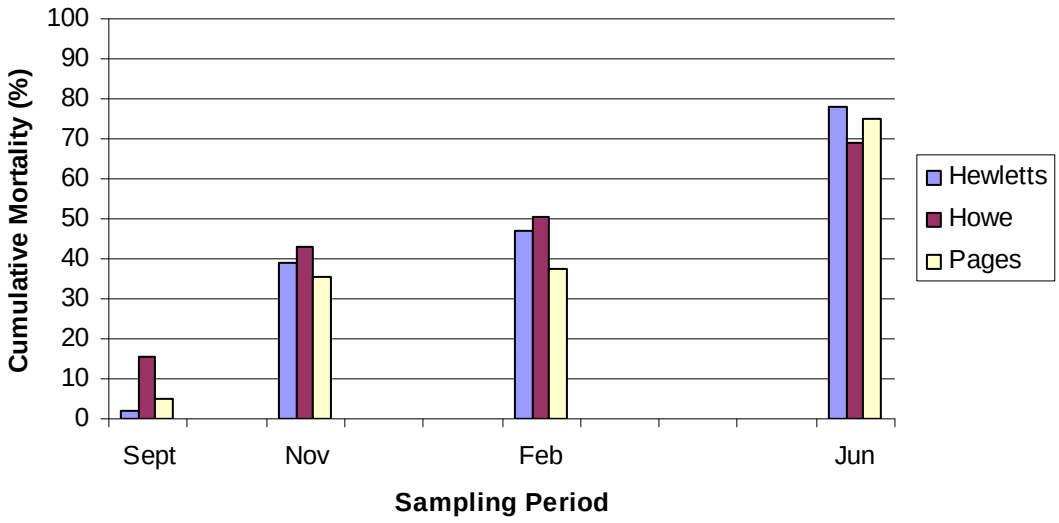

Figure 9. Cumulative mortality of hatchery oysters sampled for disease testing. Bars indicate percent mortality from initial deployment to a given sampling period in each creek (September and November: n=180, all creeks; February: n= 116 for Hewletts, 110 for Howe, 120 for Pages; June: n=180 for Hewletts and Pages, 120 for Howe). The x-axis is adjusted to account for different time intervals.

and uncaged oysters. Differences in height changes between the caged and uncaged oysters were highly significant every month beginning in February 2006. Caged oysters grew at a faster rate than uncaged oysters, indicating potential caging effects (Figure 10; Appendix C). The same overall pattern was seen for width changes in the caged and uncaged oysters (Appendix C).

For natural oysters, mortality varied over time ($F=47.41$, $p<0.0001$) but did not vary among caged and uncaged treatments ($F=2.61$, $p=0.1064$). An interaction was present between time and treatment ($F=14.29$, $p=0.0265$; Bonferroni adjusted alpha=0.0011 for time and 0.0063 for treatment). Mortality was not significantly different over time for the caged sites. For the uncaged oysters mortality was significantly lower in January 2006 and February 2006 than it was for any other month. Mortality was significantly greater at the caged sites than at the uncaged sites only in January 2006 and February 2006. Thus, caging effects existed for mortality only for the first two months following oyster deployment (Figure 11, Appendix C).

Oyster Reef Characteristics

Calculated and visually assessed percent shell cover varied significantly from each other and among the creeks ($F=20.80$, $p<0.0001$). Pages Creek had the greatest calculated shell cover (93.7%) while Howe Creek had the least (69.6%). Live oyster density also varied among the creeks ($F=8.93$, $p=0.0003$) with Howe Creek having significantly higher oyster density than either of the other two creeks. Oyster size differed among the creeks ($F=10.64$, $p<0.0001$) with oysters in Pages Creek being

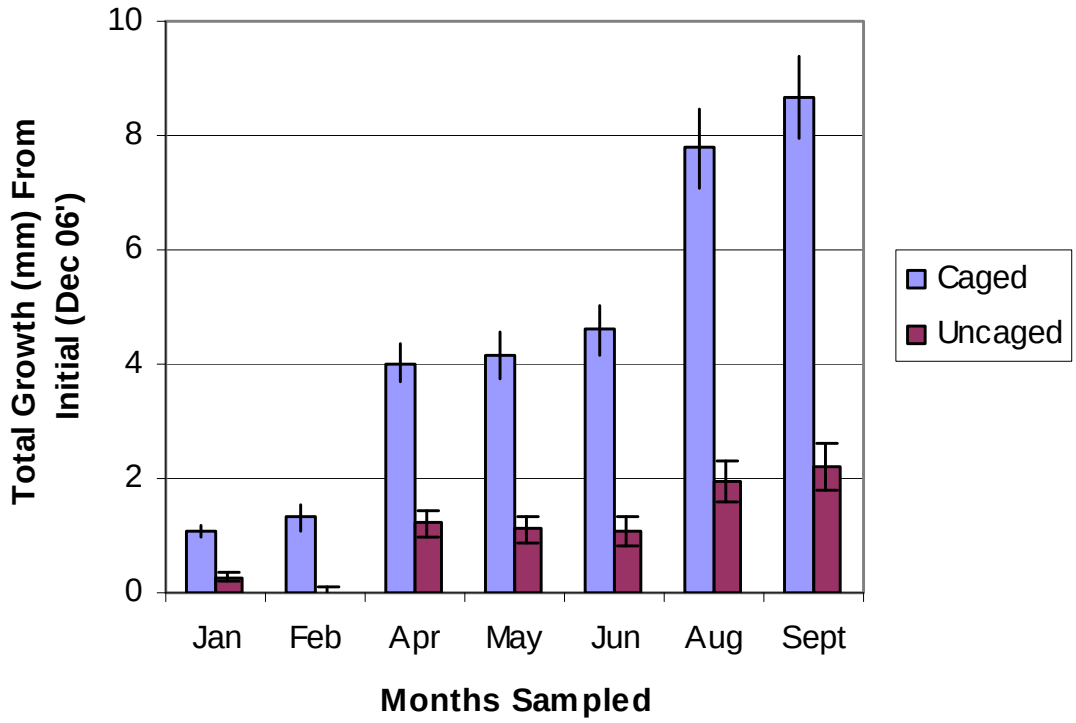

Figure 10. Total growth (mm) from initial measurements in December 2005 of natural oysters deployed in Hewletts Creek to assess potential caging effects (n=30). Bars indicate mean cumulative change in shell height (±SE) for each month for each treatment (caged vs. uncaged).

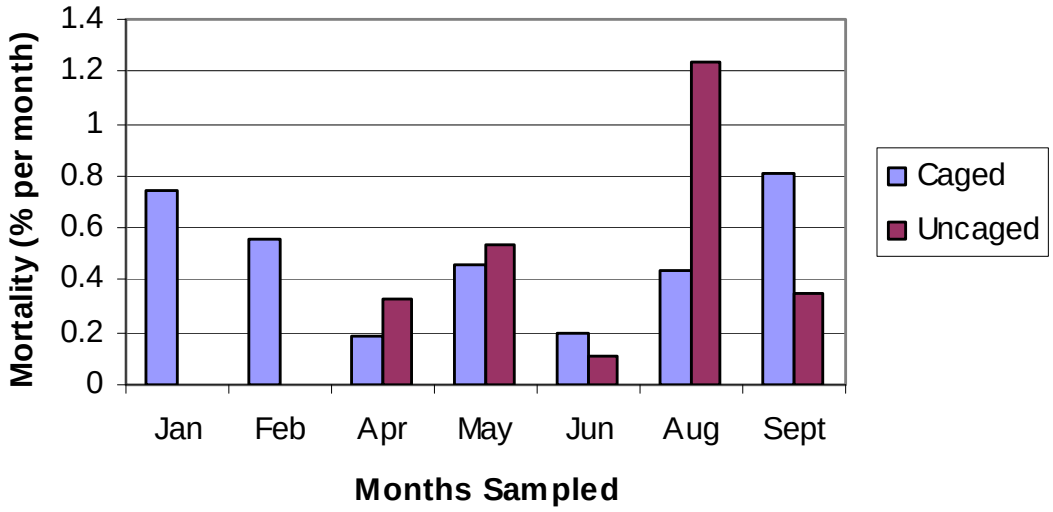

Figure 11. Average mortality per month of natural oysters deployed in Hewletts Creek to assess potential caging effects (Jan: n=270 caged, 150 uncaged; Feb: n=268 caged, 150 uncaged; Apr: n=266 caged, 150 uncaged; May: n=264 caged, 148 uncaged; Jun: n= 258 caged, 144 uncaged; Aug: n=255 caged, 143 uncaged; Sept: n=246 caged, 129 uncaged). Mortality data adjusted for number of months deployed. Bars indicate mean percent mortality each month for each treatment (caged vs. uncaged).

significantly larger than oysters in the other two creeks. Reef height was significantly greater in Hewletts Creek than in Pages Creek ($F=6.36$, $p=0.0022$) while reef rugosity did not differ among creeks ($F=0.77$, $p=0.4643$) (Table 4).

When oyster reef metrics were compared to water quality parameters sampled during the same time period correlations were detected. A negative correlation was present between live oyster density and turbidity ($r=-0.4397$, $p=0.0461$) while a positive correlation existed between live oyster density and salinity ($r=0.5252$, $p=0.0144$). Percent shell cover was positively correlated with turbidity ($r=0.6716$, $p=0.0009$) and negatively correlated with salinity ($r=-0.6179$, $p=0.0028$). When comparisons were made by creek, correlations between metrics and water quality were only present in Hewletts Creek. The same pattern was present for shell cover and turbidity and shell cover and salinity as in the overall comparisons. In Hewletts Creek reef height was negatively correlated with turbidity ($r=-0.7410$, $p=0.0224$) and positively correlated with salinity ($r=0.7379$, $p=0.0232$).

Water Quality

Total suspended solids (TSS) varied among the creeks ($F=7.05$, $p=0.0012$) but not between tides ($F=0.00$, $p=0.9837$). TSS was significantly higher in Hewletts Creek (27.90 mg/L) and Howe Creek (25.20 mg/L) than in Pages Creek (16.13 mg/L) (Table 5). Percent organics varied among the creeks ($F=7.70$, $p=0.0006$) but did not vary with tide ($F=3.19$, $p=0.0778$). Percent organics were higher in Hewletts Creek (25.6%) and Pages Creek (25.1%) than in Howe Creek (21.2%) (Table 5). TSS and organic levels indicate a trend toward Hewletts Creek having lesser water quality. When TSS data collected

Table 4: Oyster reef characteristics data for July/August 2005. Measurements were taken using 50 x 50cm square quadrats. Data is presented as means for each creek. Oyster size= shell height (mm); density= number of live oysters; %cover= percent live oyster and shell hash; rugosity= reef vertical complexity (cm), higher value is more complex; reef height= highest point (cm) sediment to shell.

	Characteristics				
Creek	Oyster Size	Density	% Cover	Rugosity	Reef Height
Hewletts	54.6	51.3	82.8	69	15.4
Howe	54.8	68.1	64.9	61.2	13.5
Pages	58.5	42.2	91.8	66.2	12.5

Table 5. Water quality data collected weekly from August 2006 to January 2007. Data presented as means (extremes) for each creek. TSS= total suspended solids (µg/ml); % org= percent organics; org con= organic concentration (µg/ml); salinity (ppt).

	Parameters			
Creek	TSS	% Org	Org Con	Salinity
Hewletts	27.90 (50.2)	25.6 (53.1)	6.9 (12.4)	21.3 (36)
Howe	25.2 (61.8)	21.2 (31.4)	5.4 (13.9)	23.9 (35)
Pages	16.13 (36.8)	25.1 (55.6)	4.0 (9.6)	34.5 (40)

during this study was compared to turbidity data from the Tidal Creeks Program collected over the same time period there was no significant correlation between the two parameters ($r=0.2776$, $p=0.2808$).

Relationship Between Water Quality and Oyster Disease/ Condition

There were significant correlations between infection intensity and oyster condition were compared to water quality parameters in the creeks three months prior to the sampling periods (data from the Tidal Creeks Program 2005-2006). Positive correlations were observed for rectal infection intensity vs. temperature and turbidity ($r=0.1925$, $p<0.0001$ and $r=0.0757$, $p=0.0080$ respectively). Rectal infection was negatively correlated with dissolved oxygen (DO) ($r=-0.1648$, $p<0.0001$). No relationship was apparent between rectal infection intensity and salinity or chlorophyll *a*. Calculated CI was positively correlated with salinity and DO ($r=0.0806$, $p=0.0042$ and $r=0.2392$, $p<0.0001$ respectively) and negatively correlated with temperature, turbidity, and chlorophyll *a* ($r=-0.2198$, $p<0.0001$ $r=-0.1369$, $p<0.0001$ and $r=-0.0886$, $p=0.0017$ respectively).

DISCUSSION

Primary objectives of this study were to determine whether *Perkinsus marinus* infection levels in oysters and oyster condition varied among tidal creek estuaries that differed in historical water quality. *A priori* expectations were that oysters in historically more impacted creeks would have higher infection prevalence and intensity (Hewletts >Howe > Pages) and lower condition (Hewletts< Howe< Pages). However, infection

was nearly 100% prevalent in all three creeks regardless of historic water quality conditions. Moreover, natural oysters in Howe Creek, the intermediate creek in terms of historic water quality parameters, had the highest intensity of *P. marinus* infection. There were no creek differences in infection levels for hatchery oysters. There were no differences among creeks for visually assessed condition for either oyster type (i.e. natural or hatchery) while Howe Creek had the highest calculated condition index for two of the sampling periods for the natural oysters. Neither growth nor mortality followed patterns among creeks consistent with past water quality though growth was greater in Howe Creek during August 2005-November 2005 and November 2005-June 2006. Oyster mortality was higher in Hewletts and Pages creeks at the conclusion of the study in June 2006.

One possible explanation why infection and oyster condition did not follow expected patterns among the creeks may be that water quality conditions in this system are not as different as they once were. In recent years Howe Creek has experienced problems with increased sedimentation and nutrient inputs as a result of development in its watershed (Alphin and Posey 2006), and Pages Creek appears to be undergoing increased development, possibly exceeding that of the other two creeks. This recent development in Howe Creek and Pages Creek may have altered water quality patterns among the creeks. Drought during 2005 (NOAA-National Weather Service, 2006), may also have led to more similar water quality among the three creeks because of regionally lower storm-water runoff into the creeks.

Increasing similarity in chlorophyll *a* and average turbidity among the three creeks is supported by the 2005-2006 New Hanover County Tidal Creeks Program report

(for the same time period that this study was conducted) (Mallin et al. 2006). TSS and % organics sampled as part of this study also indicate that the creeks are now more similar in water quality conditions. While there was a trend toward higher TSS and organic levels in Hewletts Creek, TSS in Howe Creek and % organics and Pages Creek did not differ significantly from Hewletts. Oyster reef characteristics (e.g. rugosity, oyster density, shell cover) also were similar among the creeks, or at least did not follow historic water quality patterns.

While historically different, the tidal creeks used in this study are not severely impacted by anthropogenic inputs. Lack of infection and condition differences among the creeks may reflect common extreme conditions in the intertidal environment (e.g. temperature and hypoxia due to aerial exposure at low tide) compared to impacts of the modest level of human influence in the tidal creeks. Intertidal conditions may not only physiologically affect the oyster host, but also the parasite. Elevated temperatures such as those experienced at low tide have been shown to increase the metabolism and significantly decrease the growth rate of *Perkinsus* (Milardo, 2006). Thus, impacts of the intertidal environment may have overwhelmed influences of human inputs in the tidal creek system.

Despite the lack of predicted creek differences there were significant relationships between infection and oyster condition data relative to water quality parameters in the creeks 3 months prior to the sampling. Infection intensity increased with temperature and turbidity and decreased with increasing DO concentration. Calculated tissue condition increased with salinity and DO concentration. Tissue condition decreased with increases in temperature, turbidity, and chlorophyll *a*. These patterns underscore the potential

relationships, both direct and indirect, of environmental conditions on oyster health and susceptibility to *P. marinus* infection in intertidal habitats.

The 100% prevalence across all creeks may be due to the uniform water quality in the system. This high infection prevalence is consistent with prevalence seen in other systems (e.g. Chesapeake Bay, Gulf of Mexico) (Ford and Tripp 1996). A study conducted in South Carolina by Crosby and Roberts (1990) showed only four months of the year when infection prevalence fell below 100%. The North Carolina Division of Marine Fisheries has shown near 100% prevalence in the state since testing started in the early 1990's. In this study, prevalence remained near 100% even in February when intensity declined. This may be the result of warmer temperatures in the region, which allows more parasites to survive the winter months (Burreson and Ragone Calvo 1996). The warmer winters also may allow longer *P. marinus* infective periods (Ewart and Ford 1993, Ford and Tripp 1996). The high infection prevalence seen in this study may also be the result of the size class (60-80mm) of oysters sampled. These oysters are in their second year (when infections generally increase, Paynter and Burreson 1991, Burreson and Ragone Calvo 1996, Volety et al. 2000) and filter a larger volume of water thereby increasing contact with parasites (Ford and Tripp 1996, Volety et al. 2000).

Despite high prevalence, overall, *P. marinus* infection intensities were low throughout the study. The limited studies done on intertidal oysters also show the high prevalence but low intensity pattern observed in this study (Crosby and Roberts 1990, Bobo et al. 1996). Most infection intensities fell into the light to light-moderate categories (0.33 to 1.67 on the ranking scale). It is possible that conditions within the study area are not favorable for further proliferation of the *P. marinus* parasite.

Conditions associated with aerial exposure at low tide can inhibit heavy infection. Under conditions of high temperature and hypoxia, *P. marinus* increases it aerobic metabolism which subsequently reduces its ability to proliferate (Milardo 2006). Thus, summer conditions in an intertidal habitat may keep infection at sublethal levels. Given the size of the oyster sampled, the oysters were likely in their second year of infection. Oysters with heavier infections may have already died and thus were not among the oysters sampled, contributing to low average intensity.

Overall, oyster condition was relatively low throughout the study. Based on the Quick and Mackin scale (1971) for visual condition, most oysters had condition values between 5 and 7 (i.e. tissues were gray in color, transparent, and did not fill the shell cavity). The tissue conditions observed in oysters from the tidal creeks are consistent with condition studies conducted in other areas (both subtidal and intertidal) (e.g. Lawrence and Scott 1982, Austin et al. 1993) and may reflect declining habitat value in many portions of their range.

While infection prevalence did not vary, *P. marinus* infection intensity and oyster condition did vary across sampling periods. Overall, infection intensities were at their highest in November and at their lowest in February. This pattern is consistent with studies on the influence of temperature on *P. marinus* infection, which indicate that infection intensity increases at higher temperatures (Chu and Green 1989, Fisher et al. 1992, Chu and LaPeyre 1993, Ewart and Ford 1993, Ford and Tripp 1996). Hatchery oyster mortality also showed a temporal trend, with highest mortality in November. Disease-mediated mortality follows a seasonal pattern with high moralities of infected oysters occurring during warm summer months (Ford and Tripp 1996).

In northern areas (e.g. Chesapeake Bay) prevalence and intensity of *P. marinus* infection begins to increase in June as water temperatures increase above 20°C. Infection prevalence and intensity are at their highest in September/October following maximum summer temperatures (Burreson and Ragone Calvo 1996). Prevalence and intensity decrease dramatically over the winter and spring as temperature declines, with minimum values occurring in late spring (Ewart and Ford 1993, Ford and Tripp 1996, Burreson and Ragone Calvo 1996). Disease related mortality peaks between September and October (Ewart and Ford 1993, Burreson and Ragone Calvo 1996, Ford and Tripp 1996). In more southern areas, infection and mortality cycles are not as discrete, most likely because the temperature rarely drops low enough to suppress the metabolic activity of the parasite or the oyster host (Ford and Tripp 1996). Warmer spring and autumn temperatures extend the period when oysters can become infected and milder winters allow more of the parasites to survive (Ewart and Ford 1993, Ford and Tripp 1996, Villalba et al. 2004). In the southeastern United States and Gulf of Mexico, *P. marinus* prevalence and intensity are generally lowest from January to May and highest from August to November (Ray 1954, Crosby and Roberts 1990). Disease related mortalities in southern regions are greatest from July to November, with mortality later in this period further south in the range (Quick and Mackin 1971, Ford and Tripp 1996).

In this study, the highest infection intensities were observed during the November sampling with increases over winter values being observed by June. Highest infections may have occurred in November rather than September because high air temperatures experienced by oysters at low tide during summer months can actually inhibit *P. marinus* proliferation. Under such conditions the parasite becomes stresses and exhausts it

metabolic capacity (Milardo 2006). The cooler temperatures in November were likely more conducive to *P. marinus* proliferation. While hatchery oyster mortality followed a temporal pattern similar to *P. marinus* infection, it is unlikely that this mortality was disease related. *P. marinus* is known to cause mortality in oysters (Fisher et al. 1992, Chu and LaPeyre 1993, Burreson and Ragone Calvo 1996, Ford and Tripp 1996), however mortality usually does not occur until infection becomes advanced (Paynter and Burreson 1991, Burreson and Ragone Calvo 1996, Volety et al. 2000). Overall infection intensity in this study was low so oyster mortality is likely the result other factors.

Oyster tissue condition and shell growth also showed a temporal pattern. Overall, condition was at its highest in February and at its lowest in November, inverse of the pattern seen for *P. marinus* infection. Condition index of oysters has often been negatively correlated with *P. marinus* infection (Lawrence and Scott 1982, Craig et al. 1989 Crosby and Roberts 1990, Paynter and Burreson 1991, Ford and Tripp 1996, Kennedy 1996). The negative effect of infection on tissue condition has been attributed to a decrease in the amount of energy available to infected oysters (Villalba et al. 2004). Choi et al. (1989) found that the energy consumed by the parasites can exceed what an oyster needs for its own metabolic demands. However, most adverse effects (e.g. depletion of glycogen reserves, disruption in feeding) are observed in oysters with advanced infections (Ford and Tripp 1996, Dittman et al 2001). The relatively low infection intensities seen in this study suggest it is unlikely that the parasite is responsible for changes observed in oyster condition over time, and the correlation may reflect common indirect influences on both condition and intensity.

Many environmental and physiological factors influence oyster tissue condition. Condition is often high in winter and early spring as oysters accumulate glycogen and nutrients for gonadal development/ gametogenesis (Austin et al. 1993, Shumway 1996), coincident with the time of the year when *P. marinus* infection is at its lowest level. Tissue condition declines once spawning has occurred. In southern areas spawning takes place in the spring and fall with minor spawning throughout the summer (Austin et al. 1993, Shumway 1996, Villalba et al. 2004). Thus, post-spawning condition coincides with the times of highest infection intensity. In the summer, oysters are exposed to low DO, high temperatures, and higher salinity (Paynter 1996, Shumway 1996, Lenihan et al. 1999), especially during aerial exposure at low tide, which may impact condition.

Overall, growth rate (for shell height) was greater from November 2005-June 2006 than it was from August 2005-November 2005. Thus, shell growth was greater at times of the year corresponding to lower infection intensity and higher condition. *P. marinus* infection is known to reduce shell growth (Burreson 1991, Paynter and Burreson 1991, Ford and Tripp 1996). Even oysters with light infections have been shown to deposit shell at a slower rate than uninfected oysters (Burreson 1991). The fact that the hatchery oysters deposited less shell during the warmer months may be due to the higher *P. marinus* levels at that time, combined with temperature and other stressors present during the summer in intertidal habitats. This is contrary to the fact that growth is thought to be most rapid in the summer (Shumway 1996) when metabolic activity is higher and oysters feed more often, which has been shown in studies conducted in subtidal areas.

While infection prevalence did not differ between hatchery and natural oysters, there were marked differences in infection intensity and tissue condition between the oyster types. Hatchery oysters consistently had higher infection intensities and lower condition than the natural oysters. One possible explanation for hatchery vs. natural oyster response may be transplant effects. Hatchery individuals were acclimated to conditions in Stump Sound, NC (the location of the hatchery where they were reared), a more subtidal/ lower intertidal area and a system characterized by lower salinity. When these oysters were deployed in the tidal creeks, they were exposed to different conditions than they had previously experienced. Stress from this transplant may have had long term impacts on both condition and infection intensity of the hatchery oysters.

Hatchery oysters may have had less exposure to the pathogen then oysters in the tidal creeks, possibly making them more susceptible to the parasite when exposed to it at higher levels. Higher prevalence and intensity of P. marinus infection occur at higher salinity (Chu and Greene 1989, Paynter and Burreson 1991, Ford and Tripp 1996). Thus, the parasite may have been less abundant in Stump Sound than in the tidal creeks. This is supported by preliminary prevalence data- infection 32% prevalent in Stump Sound oysters, 75% prevalent in hatchery oysters prior to deployment. Moreover, though hatchery oysters were of the same size class as the natural tidal creek oysters, the hatchery oysters were younger (approximately 9 months old) than the natural oysters (estimated close to 2 years old). Thus, natural tidal creek oysters had twice as long to acquire infection.

The differences between the two oyster types may also reflect stock parentage. Transplant studies have suggested that oysters from different regions are physiologically

distinct from one another (Loosanof and Nomejko 1951, Gaffney 1996, Dittman et al. 1998), especially those from the Gulf vs. mid-Atlantic areas. The hatchery brood stock for oysters used in this study originated from the Gulf of Mexico (i.e. Louisiana). Thus, there may be a physiological basis for the differences in infection intensity and condition between the hatchery and natural oysters. Oysters from the Gulf of Mexico, which have a longer history of exposure to P. marinus, are less susceptible to infection than oysters from the Atlantic coast. Gulf coast oysters may have been naturally selected for resistance (Ford and Tripp 1996, Gaffney and Bushek 1996). Given that the hatchery oyster brood stock originated from Louisiana, it might have been expected to be more tolerant to infection. However, this did not appear to be the case.

Caging artifacts should also be considered in comparing hatchery and natural oysters. Natural oysters were deployed on reefs and in cages on mudflats in Hewletts Creek to test for potential caging effects. Growth rate (shell height) was greater in caged oysters than in uncaged oysters for all months except January and February. Oyster mortality varied over time for uncaged oysters but not for caged oysters, with greater mortality occurring during warmer months of the year. Caging effects were only present for mortality during January and February, with greater mortality occurring for the caged treatment, possibly reflecting immediate transplant effects. Even though mortality differences existed, percent mortality was low for both treatments. Greater growth of oysters in cages may reflect reduced heat stress due to shading (Bartol et al. 1999) or increased filtering efficiency with baffling, of otherwise relatively fast-moderate tidal currents (Grizzle et al. 1992).

A significant (although weak) relationship existed between calculated condition index and visually assessed condition index. Because it is inexpensive and easy to do, visual assessment of condition is widespread and often used by government agencies (Abbe and Albright 2003, Ashton-Alcox et al. 2006, NCDMF-personal communication) to estimate oyster tissue quality. However, this index is subjective and not necessarily a good estimate of oyster health. Due to its subjectivity, potential problems arise if more than one person does the visual assessments. The method for calculating condition, however, is a quantitative measure and therefore much less subjective. The numbers provide a more accurate assessment of oyster health. The two methods did show some differences in patterns in this study.

There was a trend of rectal tissue infections having consistently higher intensities than mantle tissue infections. This was expected given that the earliest tissue lesions are found in the epithelial tissue of the stomach and small intestine (Mackin 1962, Ford and Tripp 1996). However, infection can also occur through the gill or mantle epithelium and is common in these tissues (Ford and Tripp 1996). A significant positive relationship was present between infection levels in the two tissue types indicating that both tissues provide reliable measures of infection in this study.

The 100% infection levels and overall poor condition of oyster tissues in all three creeks indicate that the oysters in these tidal creeks are stressed. Many areas in the tidal creeks have been closed to shellfishing for health reasons (i.e. fecal coliform levels). However, these areas were also assumed to act as oyster sanctuaries, with plans for large oysters in these areas to serve as a larval source for other areas. The low condition of these oysters may reflect poorer source stocks than previously thought. The declining

water quality in the tidal creeks is likely a contributor to oyster stress. This calls for a reassessment of land use practices in the tidal creek watersheds and of using closed areas as sanctuaries for replenishment purposes. The trend of declining water quality needs to be slowed/ stopped to prevent further detriment to resident oyster populations.

The seasonal/ temporal patterns of *P. marinus* infection suggest a need to re-evaluate the timing of oyster outplants. It has been suggested that uninfected seed should be planted as late in the (growing) season as possible to avoid prime infectious periods (Ewart and Ford 1993, Burreson and Ragone Calvo 1996). The results of this study support an opposite strategy. Since infections and mortality were highest in November (late fall), oyster seed should be planted as early as possible (i.e. April/May). The oysters would have time to grow before the onset of disease and high infection levels later in the year. It is possible that the oysters could be harvested before their second summer of infection.

The differences in infection levels between hatchery and natural oysters in this study indicated the importance of stock origin for restoration purposes. Uninfected oysters seed or seed from areas with less exposure to the *P. marinus* pathogen should not be planted near sources of infection (e.g. native populations). Infection would be easily transferred to the planted oysters and reduce performance. Deploying oyster seed from local brood stocks may also reduce the impact of the parasite.

Since *P. marinus* infection is among the factors contributing to the decline of oyster populations, understanding the factors that affect the onset and progression of infection is essential to restoring and conserving oyster populations. This study has implications for understanding biology of oysters and parasites, especially in the

intertidal environment. The results suggest that conditions associated with the intertidal environment may not only affect oysters but also the *P. marinus* parasite. Conditions of the intertidal habitat also appear to impact seasonal cycles of *P. marinus* infection in the southeastern United States. Overall the results of this study underscore the idea that a variety of factors likely interact to influence *P. marinus* infection and oyster health.

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APPENDIX

Appendix A: a.) Results of 2-way ANOVA for the effects of sampling period, creek, and interactions on *Perkinsus marinus* infection levels and oyster tissue condition. b.) Results of 2-way ANOVA for the effects of sampling period, oyster type, and interactions on *Perkinsus marinus* infection levels and oyster tissue condition. c.) Results of 2-way ANOVA for the effects of creek, oyster type, and interactions on *Perkinsus marinus* infection levels and oyster tissue condition. Shown are F-values (p-values). When differences are present, SNK rankings are shown in decreasing order. Different superscript letters indicate significant differences ($p<0.05$). RV= rectal infection intensity; MV= mantle infection intensity; CIC= calculated condition; CIV= visually assessed condition; TV= tissue volume; WW= tissue wet weight; H= hatchery; N= natural

a.

Variable	Sampling Period	Creek	Sampling Period*Creek
RV	31.96 (<0.0001) Nov^{a}, Jun^{b}, Sept^{b}, Feb^{c}	3.59 (0.0278) How^{a}, Pag^{b}, Hew^{b}	1.94 NS
MV	39.06 (<0.0001) Nov^{a}, Jun^{b}, Sept^{b}, Feb^{c}	3.04 (0.0482) How^{a}, Pag^{ab}, Hew^{b}	3.02 (0.0062) **Sept**: How^{a}, Pag^{b}, Hew^{b} **Hew**: Nov^{a}, Jun^{b}, Sept^{b}, Feb^{c} **Nov**: NS **How**: Nov^{a}, Sept^{a}, Jun^{a}, Feb^{b} **Feb**: NS **Pag**: Nov^{a}, Jun^{b}, Sept^{b}, Feb^{b} **Jun**: NS
CIC	28.00 (<0.0001) Feb^{a}, Jun^{b}, Sept^{b}, Nov^{c}	0.60 NS	4.70 (<0.0001) **Sept**: NS **Hew**: Feb^{a}, Sept^{b}, Jun^{b}, Nov^{c} **Nov**: How^{a}, Pag^{ab}, Hew^{b} **How**: Jun^{a}, Feb^{a}, Sept^{b}, Nov^{b} **Feb**: Hew^{a}, Pag^{b}, How^{b} **Pag**: Feb^{a}, Juna^{b}, Sept^{ab}, Nov^{b} **Jun**: How^{a}, Pag^{b}, Hew^{b}

CIV	25.90 (<0.0001) Sept[a], Nov[a], Jun[b], Feb[c]	0.69 NS		2.15 (0.0453)
			Sept: NS	**Hew**: Nov[a]. Sept[a], Jun[b], Feb[b]
			Nov: NS	**How**: Sept[a], Nov[ab], Jun[b], Feb[c]
			Feb: NS	**Pag**: Sept[a], Nov[ab], Feb[b], Jun[b]
			Jun: NS	
TV	15.95 (<0.0001) Sept[a], Jun[a], Nov[a], Feb[b]	4.93 (0.0074) Pag[a], How[b], Hew[b]		1.16 NS
WW	13.75 (<0.0001) Jun[a], Feb[a], Sept[a], Nov[b]	0.98 NS		3.70 (0.0012)
			Sept: NS	**Hew:** Feb[a], Jun[a], Sept[a], Nov[b]
			Nov: Pag[a], How[a], Hew[b]	**How:** NS
			Feb: NS	**Pag:** Jun[a], Sept[b], Feb[b], Nov[b]
			Jun: How[a], Pag[b], Hew[b]	

b.

Variable	Sampling Period	Oyster Type	Sampling Period*Oyster Type	
RV	31.61 (<0.0001) Nov[a], Jun[b], Sept[b], Feb[c]	34.75 (<0.0001) H[a], N[b]		13.79 (<0.0001)
			Sept: NS	**H**: Nov[a], Jun[b], Sept[b], Feb[b]
			Nov: H[a], N[b]	**N**: Sept[a], Nov[a], Jun[a], Feb[b]
			Feb: H[a], N[b]	
			Jun: NS	

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MV	39.06 (<0.0001) Nov[a], Jun[b], Sept[b], Feb[c]	52.59 (<0.0001) H[a], N[b]		7.52 (<0.0001)
			Sept: NS **Nov**: H[a], N[b] **Feb**: H[a], N[b] **Jun**: H[a], N[b]	**H**: Nov[a], Jun[b], Sept[b], Feb[b] **N**: Nov[a], Jun[a], Sept[a], Feb[b]
CIC	28.00 (<0.0001) Feb[a], Jun[b], Sept[b], Nov[c]	234.90 (<0.0001) N[a], H[b]		15.27 (<0.0001)
			Sept: N[a], H[b] **Nov**: N[a], H[b] **Feb**: N[a], H[b] **Jun**: N[a], H[b]	**H**: Jun[a], Feb[a], Sept[a], Nov[b] **N**: Feb[a], Jun[b], Sept[b], Nov[b]
CIV	25.90 (<0.0001) Sept[a], Nov[a], Jun[b], Feb[c]	113.39 (<0.0001) H[a], N[b]		15.16 (<0.0001)
			Sept: H[a], N[b] **Nov**: H[a], N[b] **Feb**: H[a], N[b] **Jun**: H[a], N[b]	**H**: Nov[a], Sept[b], Feb[bc], Jun[c] **N**: Sept[a], Nov[b], Jun[b], Feb[c]
TV	34.52 (<0.0001) Sept[a], Jun[a], Nov[a], Feb[b]	967.24 (<0.0001) H[a], N[b]		2.29 NS
WW	13.75 (<0.0001) Jun[a], Feb[a], Sept[a], Nov[b]	259.44 (<0.0001) H[a], N[b]		35.83 (<0.0001)
			Sept: H[a], N[b] **Nov**: H[a], N[b] **Feb**: H[a], N[b] **Jun**: H[a], N[b]	**H**: Jun[a], Sept[a], Feb[b], Nov[c] **N**: Feb[a], Jun[b], Nov[b], Sept[c]

c.

Variable	Creek	Oyster Type	Creek*Oyster Type	
RV	3.75 (0.0237) How[a], Pag[b], Hew[b]	34.99 (<0.0001) H[a], N[b]	0.19 NS	
MV	3.26 (0.0388) How[a], Pag[ab], Hew[b]	52.84 (<0.0001) H[a], N[b]	0.24 NS	
CIC	0.47 NS	234.99 (<0.0001) N[a], H[b]	2.67 NS	
CIV	0.82 NS	113.59 (<0.0001) H[a], N[b]	4.29 (0.0139) **Hew**: H[a], N[b] **How**: H[a], N[b] **Pag**: H[a], N[b]	**H**: NS **N**: How[a], Hew[ab], Pag[b]
TV	7.4 (0.0006) Pag[a], How[b], Hew[b]	932.64 (<0.0001) H[a], N[b]	2.28 NS	
WW	0.76 NS	261.25 (<0.0001) H[a], N[b]	5.06 (0.0065) **Hew:** H[a], N[b] **How:** H[a], N[b] **Pag:** H[a], N[b]	**H**: NS **N**: Pag[a], Hew[b], How[b]

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Appendix B: a.) Results of 2-way ANOVA for the effects of month, creek, and interactions on shell growth and oyster mortality for hatchery oysters tagged for growth assessment. b.) Results of 2-way ANOVA for the effects of sampling period, creek, and interactions on oyster mortality for hatchery oysters sampled from cages for disease testing. Shown are F-values (p-values). When differences are present, SNK rankings are shown in decreasing order. Different superscript letters indicate significant differences ($p<0.05$). Aug= August 2005; Nov= November 2005; Jun= June 2006.

a.

Variable	Month	Creek	Month*Creek	
Shell Height	50.7	14.26	8.57	
	(<0.0001)	(<0.0001)	(<0.0001)	
	Jun^{a}, Nov^{b}, Aug^{c}	How^{a}, Hew^{b}, Pag^{b}	**Hew**: Jun^{a}, Nov^{bc}, Aug^{c}	**Aug**: NS
			How: Jun^{a}, Nov^{b}, Aug^{c}	**Nov**: How^{a}, Pag^{b}, Hew^{b}
			Pag: Jun^{a}, Nov^{a}, Aug^{b}	**Jun**: How^{a}, Hew^{b}, Pag^{b}
Shell Width	10.16	0.9	3.34	
	(0.0001)	NS	(0.0133)	
	Jun^{a}, Nov^{b}, Aug^{b}		**Hew**: NS	**Aug**: NS
			How: Nov^{a}, Aug^{ab}, Jun^{b}	**Nov**: NS
			Pag: NS	**Jun**: NS
Mortality	0.46	5.22	9.43	
	NS	NS	(0.0090)	
			Hew: Nov^{a}, Jun^{b}	**Nov**: NS
			How: Nov^{a}, Jun^{b}	**Jun**: Hew^{a}, Pag^{a}, How^{b}
			Pag: NS	

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b.

Variable	Sampling Period	Creek	Sampling Period*Creek
Mortality	586.72	4	45.82
	(<0.0001)	NS	(<0.0001)
	Nov[a], Jun[b], Sept[b], Feb[c]		**Sept**: How[a], Pag[b], Hew[b] **Hew**: Nov[a], Jun[b], Feb[b], Sept[c]
			Nov: NS **How**: Sept[a], Nov[a], Jun[b], Feb[b]
			Feb: Hew[a], How[ab], Pag[b] **Pag**: Nov[a], Jun[a], Sept[b], Feb[b]
			Jun: Hew[a], Pag[a], How[b]

Appendix C: Results of 2-way ANOVA for the effects of month, site, and interactions on shell growth and oyster mortality for natural oysters deployed to assess potential caging effects. Shown are F-values (p-values). When differences are present, SNK rankings are shown in decreasing order. Different superscript letters indicate significant differences ($p<0.05$). Months 1-8= Dec., Jan., Feb., Apr., May, Jun.; Aug., Sept respectively; C= caged; R= reef (uncaged).

Variable	Month	Site		Month*Site
Shell Height	72.63	24.49		21.47
	(<0.0001)	(<0.0001)		(<0.0001)
	$8^a, 7^a, 6^b, 5^b, 4^b, 2^a, 3^a, 1^a$	C^a, R^b	**1**: NS	**C**: $8^a, 7^a, 6^b, 5^b, 4^b, 3^c, 2^c\ 1^c$
			2: NS	**R**: $8^a, 7^a, 4^b, 5^b, 6^b, 2^c, 3^c, 1^c$
			3: C^a, R^b	
			4: C^a, R^b	
			5: C^a, R^b	
			6: C^a, R^b	
			7: C^a, R^b	
			8: C^a, R^b	
Shell Width	79.11	64.42		24.02
	(<0.0001)	(<0.0001)		(<0.0001)
	$7^a, 8^a, 6^b, 4^b, 5^b, 3^a, 2^a, 1^a$	C^a, R^b	**1**: NS	**C**: $7^a, 8^a, 6^b, 4^b, 5^b, 3^c, 2^a, 1^a$
			2: NS	**R**: $8^a, 7^a, 6^a, 5^a, 4^a, 2^b, 1^b, 3^b$
			3: C^a, R^b	
			4: C^a, R^b	
			5: C^a, R^b	
			6: C^a, R^b	
			7: C^a, R^b	
			8: C^a, R^b	

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Mortality	47.41 (<0.0001) 6^a, 7^a, 4^b, 1^b, 2^b, 3^c, 5^c	2.61 NS		14.29 (0.0265)
			1: C^a, R^b	**C**: NS
			2: C^a, R^b	**R**: 6^a, 4^b, 3^b, 7^b, 5^b, 1^c, 2^c
			3: NS	
			4: NS	
			5: NS	
			6: NS	
			7: NS	

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Appendix D: a.) Infection intensity and condition data by sampling period, creek, and oyster type. Data are presented as means (±SE). b.) Infection intensity and condition data for hatchery and natural oysters for each sampling period in each creek. Data are presented as means (±SE). RV= rectal infection intensity; MV= mantle infection intensity; CIC= calculated condition; CIV= visually assessed condition; TV= tissue volume; WW= tissue wet weight.

a.

	RV	MV	CIC	CIV	TV	WW
Sampling Period						
September 05'	1.30 (0.04)	1.10 (0.04)	4.56 (0.18)	6.44 (0.06)	16.4 (0.39)	3.93 (0.08)
November 05'	1.57 (0.05)	1.41 (0.04)	3.89 (0.11)	6.36 (0.08)	15.5 (0.33)	3.46 (0.06)
February 06'	0.94 (0.05)	0.74 (0.05)	6.12 (0.24)	5.61 (0.10)	12.9 (0.40)	3.96 (0.09)
June 06'	1.33 (0.04)	1.14 (0.04)	5.01 (0.15)	5.84 (0.07)	16.3 (0.40)	4.15 (0.09)
Creek						
Hewletts	1.27 (0.04)	1.07 (0.04)	4.76 (0.14)	6.07 (0.07)	15.0 (0.34)	3.85 (0.08)
Howe	1.40 (0.04)	1.19 (0.04)	4.91 (0.14)	6.18 (0.07)	15.1 (0.34)	3.79 (0.07)
Pages	1.30 (0.04)	1.15 (0.04)	4.68 (0.17)	6.09 (0.07)	16.3 (0.33)	3.93 (0.07)
Oyster Type						
Hatchery	1.46 (0.04)	1.30 (0.04)	3.51 (0.08)	6.54 (0.06)	20.2 (0.25)	4.50 (0.07)
Natural	1.20 (0.02)	0.99 (0.02)	5.94 (0.13)	5.73 (0.05)	11.2 (0.16)	3.28 (0.04)

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b.

Hatchery Oysters	RV	MV	CIC	CIV	TV	WW
Hewletts Creek						
September 05'	1.12 (0.07)	1 (0.09)	4.17 (0.22)	6.65 (0.16)	21.2 (1.08)	5.33 (0.22)
November 05'	1.8 (0.13)	1.66 (0.13)	2.2 (0.10)	7.02 (0.16)	20.6 (0.67)	3.37 (0.15)
February 06'	1.16 (0.15)	0.87 (0.14)	4.19 (0.40)	6.32 (0.33)	15.5 (0.85)	4.45 (0.32)
June 06'	1.38 (0.09)	1.12 (0.08)	4.91 (0.35)	5.52 (0.24)	18.1 (0.77)	5.33 (0.24)
Howe Creek						
September 05'	1.34 (0.11)	1.31 (0.12)	3.85 (0.16)	6.45 (0.16)	20.3 (0.75)	4.92 (0.22)
November 05'	1.81 (0.14)	1.58 (0.14)	2.64 (0.13)	7.02 (0.20)	19.7 (0.81)	3.92 (0.16)
February 06'	1.28 (0.13)	1.01 (0.13)	3.73 (0.25)	6.13 (0.22)	18 (1.01)	4.40 (0.25)
June 06'	1.65 (0.15)	1.47 (0.13)	4.01 (0.27)	6.23 (0.21)	23 (0.84)	4.79 (0.24)
Pages Creek						
September 05'	1.32 (0.11)	1.16 (0.11)	3.45 (0.18)	6.72 (0.15)	22.5 (0.66)	4.53 (0.16)
November 05'	1.89 (0.13)	1.71 (0.13)	2.53 (0.13)	6.8 (0.21)	19.5 (0.66)	3.72 (0.20)
February 06'	1.18 (0.10)	1.22 (0.15)	4.11 (0.75)	6.65 (0.20)	16.8 (0.91)	3.88 (0.18)
June 06'	1.24 (0.11)	1.15 (0.11)	3.17 (0.16)	6.44 (0.20)	24.5 (0.79)	5.53 (0.27)

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Natural Oysters	RV	MV	CIC	CIV	TV	WW
Hewletts Creek						
September 05'	1.12 (0.08)	0.9 (0.08)	5.33 (0.36)	6.12 (0.15)	10 (0.49)	2.69 (0.09)
November 05'	1.31 (0.07)	1.08 (0.07)	4.8 (0.18)	6.07 (0.17)	10.6 (0.37)	2.99 (0.09)
February 06'	0.69 (0.08)	0.48 (0.05)	9.89 (0.49)	4.58 (0.21)	7.7 (0.51)	3.96 (0.18)
June 06'	1.31 (0.09)	1.12 (0.08)	4.09 (0.18)	5.77 (0.15)	15 (0.52)	3.3 (0.12)
Howe Creek						
September 05'	1.59 (0.06)	1.34 (0.08)	4.85 (0.37)	6.77 (0.13)	12.2 (0.56)	2.99 (0.10)
November 05'	1.35 (0.07)	1.15 (0.06)	5.81 (0.37)	5.65 (0.16)	10.3 (0.44)	3.17 (0.12)
February 06'	0.62 (0.08)	0.43 (0.04)	7.45 (0.35)	4.98 (0.14)	8.99 (0.53)	3.48 (0.20)
June 06'	1.34 (0.07)	1.05 (0.06)	6.81 (0.47)	5.92 (0.11)	10.1 (0.47)	3.09 (0.09)
Pages Creek						
September 05'	1.29 (0.08)	0.9 (0.07)	5.72 (0.88)	5.97 (0.16)	12.3 (0.46)	3.12 (0.11)
November 05'	1.25 (0.08)	1.33 (0.08)	5.25 (0.18)	5.67 (0.14)	13 (0.46)	3.61 (0.13)
February 06'	0.79 (0.06)	0.48 (0.06)	7.25 (0.28)	5.05 (0.15)	10.3 (0.53)	3.64 (0.18)
June 06'	1.17 (0.06)	1.04 (0.06)	6.27 (0.25)	5.38 (0.13)	11.8 (0.62)	3.69 (0.14)

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