ASSESSMENT OF RESTORED AND UNRESTORED OYSTER REEFS AND THE PRESENCE OF PERKINSUS MARINUS IN ESTUARINE WATERS OF ALABAMA

Except where reference is made to the work of others, the work described in this thesis is my own or was done in collaboration with my advisory committee.

John Dunne

Certificate of Approval:

Yolanda J. Brady
Associate Professor
Fisheries and Allied Aquacultures

David B. Rouse, Chair
Professor
Fisheries and Allied Aquacultures

Richard K. Wallace
Associate Professor
Fisheries and Allied Aquacultures

Stephen L. McFarland
Dean
Graduate School

ASSESSMENT OF RESTORED AND UNRESTORED OYSTER REEFS AND THE PRESENCE OF PERKINSUS MARINUS IN ESTUARINE WATERS OF ALABAMA

John Dunne

A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of Master of Science

Auburn, Alabama

December 15, 2001

ASSESSMENT OF RESTORED AND UNRESTORED OYSTER REEFS AND THE PRESENCE OF PERKINSUS MARINUS IN ESTUARINE WATERS OF ALABAMA

John Dunne

Permission is granted to Auburn University to make copies of this thesis at its discretion, upon request of individuals or institutions and at their expense. The author reserves all publication rights.

Signature of Author

Date

Copy sent to:

Name Date

VITA

John Dunne was born May 8, 1971 in Smithtown, New York. He was reared in Vermont from the age of 6 and graduated from the University of Vermont with a Bachelor of Science degree in Fisheries and Wildlife Biology in 1995. He then married Ruth White of Bridgehampton, New York. Together they served as Peace Corps volunteers in Solomon Islands, South Pacific from 1996 – 1999. In September 1999, he began graduate studies in Auburn University's Department of Fisheries and Allied Aquacultures.

THESIS ABSTRACT

ASSESSMENT OF RESTORED AND UNRESTORED OYSTER REEFS AND THE PRESENCE OF PERKINSUS MARINUS IN ESTUARINE WATERS OF ALABAMA

John Dunne

Master of Science, December 15, 2001
(B.S. University of Vermont, 1995)

91 Typed Pages

Directed by David B. Rouse

Reef assessments were completed on Whitehouse Reef and Klondike Reef in Mobile Bay. A portion of Whitehouse Reef has recently been restored with oyster shell cultch. Assessments of the restored area compared to the unrestored area showed that the reef restoration effort was successful in increasing mean oyster density more than eight-fold (to 49.93 oysters/m^2 from 5.87 oysters/m^2). However, adverse conditions persist on the reef, as demonstrated by a follow-up assessment, which revealed mean oyster density declined to near pre-restoration levels. Production on the unrestored area of the reef has remained consistent with historical records. An assessment of Klondike Reef showed the oyster resource is minimal, mean oyster density was 0.16/m^2 compared to 0.85/m^2 in

1969, and mean weight of exposed cultch was 0.03 kg/m^2 in 2000 compared to 0.39 kg/m^2 in 1969.

Prevalence and intensity of Perkinsus marinus ("dermo"), the primary oyster disease of concern in the region, was analyzed at Whitehouse Reef and Cedar Point Reef during summer, 2000. Hemolymph assay technique with Ray's Fluid Thioglycollate Medium (RFTM) was used to detect disease in oysters obtained monthly, from each site. Whitehouse Reef oysters did not appear to be threatened by the disease. However, it is important to note that young oysters were sampled from Whitehouse Reef and "dermo" does not generally establish itself in oysters before their second year of growth. Results from Cedar Point Reef showed the oyster population is at critical level of disease intensity. Weighted incidence of "dermo" was 1.61 and prevalence 94.0% at Cedar Point Reef.

Further studies involving constant water quality measurements and periodic "dermo" analysis are recommended for both Whitehouse Reef and Cedar Point Reef in order to continue to work toward understanding the enigma of oyster production in Alabama.

ACKNOWLEDGEMENTS

I would like to thank all of the staff at the Auburn University Marine Extension and Research Center, especially Captain Blan Page and Scott Rikard, for their help on this project, as well as their friendship and hospitality; Dr. Len Rodgers for his computer related aid; Dr. Anthony Guarino for his assistance with statistics; Stuart Goong for his intelligence at editing; and Drs. Yolanda Brady, and Richard Wallace for all of their advice. I would especially like to thank my wife, Ruth, for her help in recording, double- and triple-checking data, and all of her support throughout the pursuit of this degree. And finally, a special thanks to Dr. David Rouse for being a patient and respectful advisor.

Style manual or Journal used: Journal of the World Aquaculture Society

Computer software used: Microsoft® Word 2000, Microsoft® Excel 2000, Microsoft® Power Point 2000, SPSS® 10.0 for Windows

TABLE OF CONTENTS

LIST OF TABLES..... xi

LIST OF FIGURES..... xii

CHAPTER I. OYSTER REEF ASSESSMENTS.

Abstract..... 1

Introduction..... 3

Description of study site..... 4

Alabama oyster harvesting rules and regulations..... 7

Factors that affect oyster production and survival in Alabama..... 8

Oyster reef restoration..... 19

Alabama oyster reef surveys and assessments..... 22

Alabama reef rehabilitation efforts..... 23

Cultch material..... 25

Materials and Methods..... 27

Reef assessments..... 27

Reef survey..... 29

Data analysis..... 29

Results and Discussion..... 31

Whitehouse Reef..... 31

Klondike Reef..... 36

CHAPTER II. PRESENCE OF PERKINSUS MARINUS.

Abstract..... 46

Introduction..... 47

Disease history & taxonomy..... 47

Disease distribution..... 49

Effect on oysters..... 50

Diagnosis of disease prevalence and intensity..... 51

Transmission of disease..... 52

Environmental parameters and seasonality..... 54

Management/control strategies..... 55

Materials and Methods..... 58

Results and Discussion..... 60

LITERATURE CITED..... 66

LIST OF TABLES

Chapter I.

Table 1. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Whitehouse Reef in 1969[1], 1998[2], and 2000 (unrestored area). Different superscripts in the same row indicate a significant difference, $p \leq 0.05$. Statistical tests were conducted on 1998 and 2000 data only. 39

Table 2. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Whitehouse Reef in 2000, unrestored area and restored area. Different superscripts in the same row indicate a significant difference, $p \leq 0.05$. 40

Table 3. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Whitehouse Reef in 2000, first and second assessments of restored area. Different superscripts in the same row indicate a significant difference, $p \leq 0.05$. 41

Table 4. Klondike Reef boundary coordinates from a survey completed during summer, 2000. 42

Table 5. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Klondike Reef in 1969[1] and 2000. No statistical tests were conducted on these data. 43

LIST OF FIGURES

Chapter I.

Figure 1. Alabama's oyster producing waters. A: Cedar Point Reef, B: Whitehouse Reef, C: Klondike Reef, D: Fish River Reef, E: Shellbank Reef. 5

Figure 2. Mean density (#/m^2) of oysters sampled on Whitehouse Reef, per size class, 1998 and 2000 (unrestored area). 44

Figure 3. Mean density (#/m^2) of oysters sampled on Whitehouse Reef, per size class, unrestored and restored areas, 2000. 44

Figure 4. Mean density (#/m^2) of oysters sampled on Whitehouse Reef, per size class, first and second assessments, 2000. 45

Figure 5. Mean density (#/m^2) of oysters sampled on Klondike Reef, per size class, 2000. 45

Chapter II.

Figure 1. Prevalence (%) of "dermo" infection at Whitehouse Reef and Cedar Point Reef, June through September, 2000 sampling periods. 64

Figure 2. Weighted incidence (WI) of "dermo" infection at Cedar Point Reef, June through September, 2000 sampling periods. 64

Figure 3. Water temperature (°C) at Bon Secour Bay, Alabama, June through September, 2000 sampling periods. 65

Figure 4. Mean height (mm) of oysters sampled for "dermo" from Whitehouse Reef and Cedar Point Reef, August and September, 2000. 65

CHAPTER I. OYSTER REEF ASSESSMENTS

ABSTRACT

Much of Alabama's oyster production occurs on one reef, Cedar Point Reef in Mississippi Sound, while many other reefs remain marginally productive to unproductive. Whitehouse Reef, a marginally productive reef in Mobile Bay, received additional oyster shell cultch material during oyster restoration efforts in 1999. During summer, 2000, Whitehouse Reef was assessed in order to determine the efficacy of the restoration effort and to quantify the status of the unrestored oyster population as compared to a pre-restoration assessment completed in 1998. A second assessment of Whitehouse Reef was conducted during summer, 2000 to quantify the effect of a suspected oyster die-off. Results of the first assessment showed the restoration effort was successful at increasing mean oyster density on the reef to $49.93/m^2$ on the restored reef area compared to $5.87/m^2$ on the unrestored area. However, the second assessment verified that there had been a substantial oyster die-off on the reef, which decreased oyster density on the restored area to a near pre-restoration level of $8.40/m^2$. Mean oyster density on the unrestored area of the reef was similar to oyster densities found in 1998 ($7.21/m^2$ in 1998 and $5.87/m^2$ in 2000). Klondike Reef, an unproductive reef in eastern Mobile Bay, was surveyed and assessed during summer, 2000. Results of the assessment revealed that the oyster resource on Klondike Reef is minimal, and the amount of cultch material available for increasing oyster production is very low. A further understanding of adverse factors

affecting recruitment and survival of oysters on unproductive reefs in Alabama is essential before embarking on further restoration efforts.

INTRODUCTION

The Gulf of Mexico is the leading oyster producing region in the U.S. and production has remained stable for the past several decades (Ogle and Beaugez 1988; Leard *et al.* 1999). Alabama contains several species of oyster, but only the eastern oyster (Crassostrea virginica, Gmelin 1791) is commercially important (Wallace 1993). Alabama's annual oyster landings have averaged approximately 453 metric tons (MT) since the 1880's (Wallace 1993). Although Alabama remains consistent with recent trends, representing 3 – 4% of the total Gulf catch, oyster landings have been declining since the 1960's (Berrigan *et al.* 1991). Average annual landings in the 1960's were 440 MT ($400,054 value) and in the 1990's 268 MT ($996,673 value) (personal communication, National Marine Fisheries Service, Fisheries Statistics and Economics Division). Annual harvests vary due to environmental factors and the natural cyclical nature of oyster populations (Swingle 1977; Eckmayer 1979). For instance, the lowest annual landing for the period from 1990 – 1999 occurred in 1990, at 37.9 MT, and the highest in 1992 at 545.1 MT (personal communication, National Marine Fisheries Service, Fisheries Statistics and Economics Division). Processing and related businesses add an economic multiplier of 3 – 4 times the landings value, resulting in substantial economic value for coastal Alabama (May 1971; Wallace 1993).

Description of study site

There are 977 km of tidal shoreline and 1,528 square km of estuarine water in Alabama (May 1971). These waters have an average diurnal tidal range of 0.4 to 0.6 meters (May 1969; Bault 1972).

Alabama's coast is dominated by Mobile Bay (Fig. 1), a combination drowned river valley, bar-built (Schroeder and Wiseman 1986), positive estuary with a counter-clockwise flow pattern in which flood tides circulate up the eastern side of the bay and ebb tides circulate down the western side of the bay. Due to this flow pattern, natural oyster reefs exist more northerly on the eastern side of the bay than on the western side (Hoese *et al.* 1972; Lee 1979). Mobile Bay, the second largest estuarine system and the largest open water bay on the Gulf Coast, is approximately 50 km long, about 14 km wide at the upper portion and about 34 km wide at the lower portion (McClellan 1965; Hoese *et al.* 1972; Schroeder and Wiseman 1986). The average depth of the bay is about 3.2 m with the exception of a ship channel that is 120 m wide and 12 m deep and maintained as such by dredging (May 1973; Schroeder *et al.* 1990). The entrance to the Bay, between Mobile and Pelican Points, known as Main Pass, is about 5.4 km wide (Wiseman *et al.* 1988). Main Pass is responsible for 85% of the water exchange between the Bay and the Gulf of Mexico while the remaining 15% passes through Pass aux Herons, between Cedar Point and Dauphin Island, to Mississippi Sound (Wiseman *et al.* 1988). Ninety-five percent of the freshwater introduced into the Bay comes from the Mobile River system in the north (Schroeder *et al.* 1990).

Alabama's oyster producing areas are divided into three distinct regions, consisting of Mississippi Sound on the west (including Cedar Point Reef) (Fig. 1A),

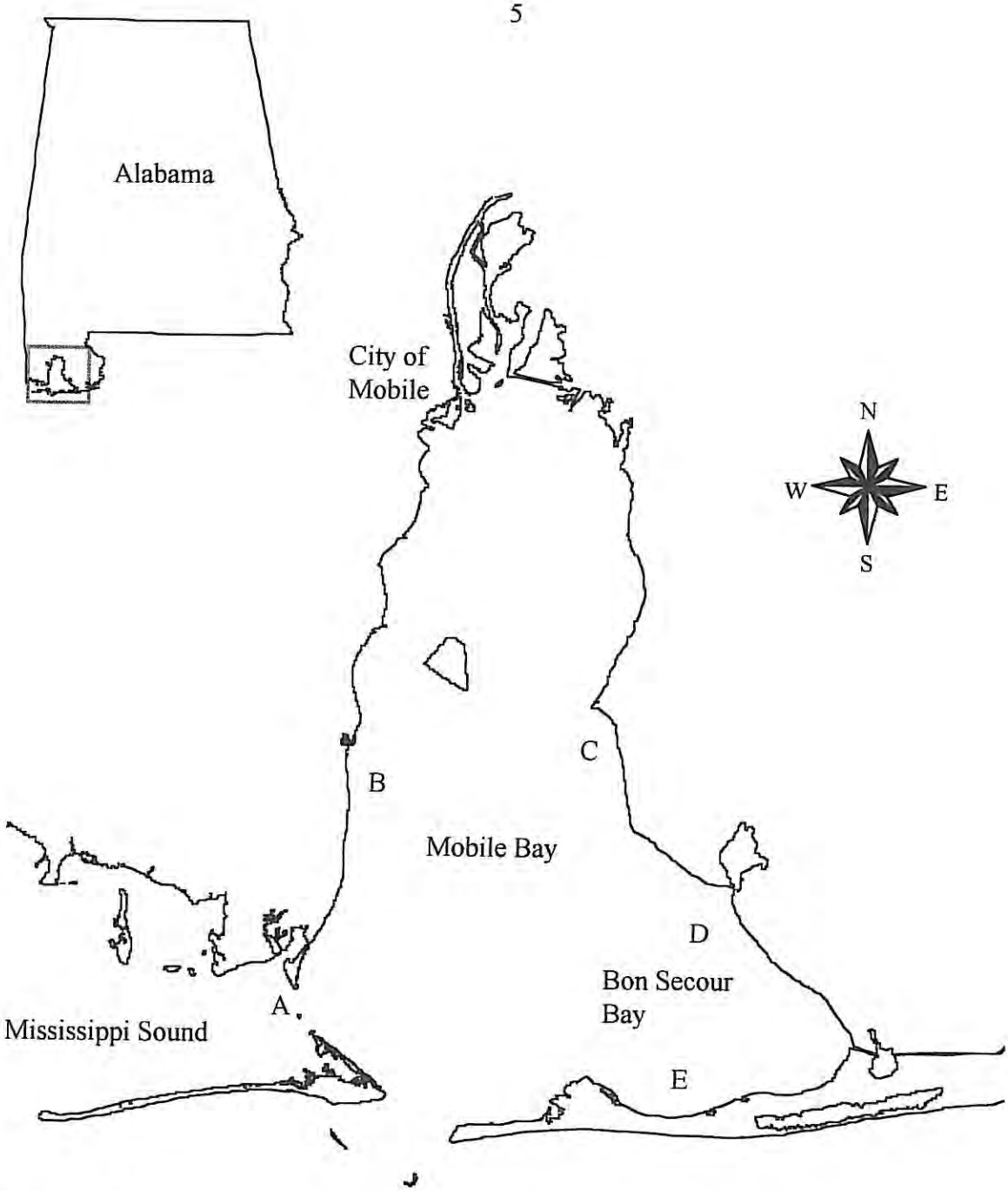

Figure 1. Alabama's oyster producing waters. Point A: Cedar Point Reef; B: Whitehouse Reef; C: Klondike Reef; D: Fish River Reef; E: Shellbank Reef.

Mobile Bay (including Whitehouse Reef and Klondike Reef) (Fig. 1B and C), and Bon Secour Bay on the east (including Fish River Reef, and Shellbank Reef) (Fig. 1D and E) (Engle 1945). Cedar Point Reef, Alabama's most productive oyster reef, producing approximately 90% of Alabama's oyster landings, yields market-sized oysters in about two years (Engle 1945; Eckmayer 1979). The two main reefs of interest to this study include Whitehouse Reef and Klondike Reef. Whitehouse Reef is a large reef system located in western Mobile Bay. The entire system is comprised of many patch reefs ranging in area from approximately 0.1 – 56.5 ha (Tatum *et al.* 1995). One of these reefs, Whitehouse C (30° 24.856' N, 88° 04.007' W), has been designated as a dual-purpose reef, serving as an experimental oyster rehabilitation area and an artificial fishing reef. Klondike Reef (30° 27.460' N, 87° 55.940' W) is an unproductive reef that has not received management attention since the 1940's.

Bottom salinity in Mobile Bay ranges from 0 – 30‰ (May 1969; Saoud 2000) with an average of 14.2‰ (Bault 1972). Mobile Bay is a positive estuary with a decreasing salinity gradient from the mouth of the estuary in the south to the head of the estuary in the north (Lee 1979). Salinity also tends to decrease from east to west, which is intuitive considering the counter-clockwise flow pattern in the Bay (Engle 1945; Lee 1979).

Temperature in Mobile Bay ranges from 5 – 32 °C, with an average of 20.4 °C (Bault 1972). Temperature tends to remain relatively uniform Bay wide (Bault 1972; May 1973; Lee 1979).

Dissolved oxygen (D.O.) averages 7.4 ppm and periodically reaches hypoxic and anoxic conditions in the Bay (Bault 1972; May 1972; Saoud *et al.* 2000). Anoxic conditions are achieved by the accumulation and oxidation of organic matter and biological respiration (Lund 1957; May 1973). Poor water circulation further exacerbates anoxic conditions by keeping bay waters stratified. Lack of water circulation, due to normal, low tidal change and low winds (< 10 knots) is a common contributor to oxygen depletion in estuaries, especially Mobile Bay (May 1973).

A local phenomenon known as a "jubilee" commonly occurs on the eastern side of Mobile Bay as a result of low D.O. These jubilees cause demersal fishes and crustaceans to come ashore in an effort to escape wind-driven low D.O. waters. Jubilees generally occur in summer, usually during an easterly wind, and their effect on oysters is unreported. Jubilee events average about 5 per year and were first recorded in 1867 (May 1973)

Alabama oyster harvesting rules and regulations

Oyster harvesting is permanently closed in a 29,780 ha area of Alabama waters due to industrial and domestic pollution (May 1971). Most of the closed area is comprised of upper Mobile Bay from a point at the mouth of East Fowl River on the west, extending east to the ship channel and northeast to Daphne. This area of the Bay, however, includes only 5.3 ha of oyster beds. Dauphin Island Bay, much of Portersville Bay, Oyster Bay, the Fairhope sewage outfall and the Grand Hotel sewage outfall are also closed to oyster harvesting (Eckmayer 1979; Alabama Marine Resources Division (MRD) 2000).

Due to high fecal coliform counts, all of Alabama's oyster producing areas are periodically closed to harvesting by the Alabama Department of Public Health. Closures are always a result of low salinity periods due to high discharge levels from the Mobile River system (May 1971; Eckmayer 1979). Closures can sometimes result in a significant amount of lost oyster harvesting time, thus valuable income for oyster harvesters.

Commercial oyster harvesters must purchase an annual license for the fee of $26. Oyster sacks taken by commercial oyster harvesters must be tagged with an oyster tag ($0.25 each) that designates the harvester's license number, date, and location of harvest. Oysters may be taken from public reefs and bottoms by hand tonging only, Monday through Friday, during times set by the Marine Resources Division and/or Alabama Department of Public Health. The daily sack limit is set at 8 sacks per harvester and the legal harvest size of oysters is 76.2 mm (3 inches) shell height, with an undersize allowance of equal to or less than 5% (72.4 mm). Culling of undersized oysters must occur on the reef from which they were taken and by the time of daily reef closure. Oysters must also be sacked and tagged by the time of daily reef closure or by the time of departure from the reef at which they were harvested (Alabama MRD 2000).

Factors that affect oyster production and survival in Alabama

Reproduction and spat set

Eastern oysters become reproductively viable as early as the first month of development (Berrigan *et al.* 1991). They are protandric species, first developing as males and possibly changing to females within the first year (Thompson *et al.* 1996).

According to Butler (1949), gametogenesis generally occurs at salinities ≥ 6‰. Annual oyster spawning events are triggered by optimum environmental conditions, such as temperature and planktonic food availability, which will enable larvae to survive and grow quickly. Spawning is ultimately synchronized by environmental cues and the presence of species-specific gametes in the water (Ingle 1951; Thompson *et al.* 1996). Eastern oyster reproduction is non-incubatory and eggs and sperm are discharged directly into the water column (Galtsoff 1964). Development of fertilized eggs requires salinities ≥ 14 – 15‰ with an upper limit of 39‰ and an optimum salinity range of 23.0 – 29.0‰ (Davis 1958). According to Ingle (1951), isolated spawning of eastern oysters in Apalachicola Bay, FL did not occur at temperatures below 22.5 °C while mass spawning occurs at temperatures ≥ 26 °C. Males commonly spawn before females (Ingle 1951; Quayle and Newkirk 1989). The oyster's larval period, which is affected by temperature and food supply, lasts for 14 – 21 days, after which time larvae set on available cultch material (Ingle 1951; Quayle and Newkirk 1989; Kennedy 1996). Mackenzie (1981) reported that oyster larvae survive best at temperatures of 27.5 – 32.5 °C with a lower lethal limit of 17.5 °C. Prytherch (1934, as cited in Davis 1958) observed larvae attach to substrate in salinities ranging from 5.6 – 32.2‰ and proposed an optimum salinity for setting of 16.0 – 18.0‰. Salinity requirements for best growth of larvae are 12.5 – 27‰ with a lower limit, usually resulting in poor survival and/or slow growth, of 7.5‰ (MacKenzie 1981).

Spat set in Alabama occurs from May to October with two peaks, from May – June and September – October, with a greater set occurring during the fall peak (Hoese *et al.* 1972; May 1972; Lee 1979; Saoud 2000). Rates of spat set in Alabama have been

reported as zero spat per shell at an unproductive reef in Bon Secour Bay, Fish River Reef, to 7 – 18 spat per shell at the most productive reef, Cedar Point Reef (Saoud 2000). During a study of oyster spat setting in Alabama, Lee (1979) found setting rates ranging from 0.2/m^2/day near Whitehouse Reef to 78/m^2/day near Cedar Point Reef with an average set of 15/m^2/day at Cedar Point Reef. Saoud (personal communication, Auburn University Department of Fisheries and Allied Aquacultures) periodically found setting rates to be similar between Whitehouse Reef and Cedar Point Reef. It is important to note, however, that due to the "intensification effect" described by Pineda and Caswell (1997), caution must be taken when interpreting results of setting studies when setting substrate is limited.

Reported causes of larval mortality include ingestion of larvae by benthic invertebrates such as oysters, blue mussels (Mytilus edulis) and hard clams (Mercenaria mercenaria) (MacKenzie 1981). These animals, and other benthic invertebrates, have been shown to ingest oyster larvae but excrete them in pseudofeces where they are sometimes able to escape given adequate current to scatter pseudofeces (MacKenzie 1981). Barnacles (Balanus sp.) and tube-worms (Polydora ligni) have been shown to ingest larvae but release them within a few minutes (MacKenzie 1981).

Temperature

The eastern oyster is commonly found in waters ranging from -2 to 36 °C with an upper thermal mortality limit of 48.5 °C (Shumway 1996). Andrews (1982) noted that oysters in Virginia entered a state of dormancy as temperature remained ≤ 5 °C and

resumed feeding at approximately 10 °C. Temperature is an important factor when coupled with the effects of adverse conditions such as burial, low salinity, and low D.O.

Salinity

Generally, the optimum salinity range for C. virginica is 14 to 28‰ while it occurs in waters with salinity ranging from 0 to 40‰ (Shumway 1996; Saoud 2000). May (1971) stated that the optimal salinity range for oysters in Alabama is 10 – 20‰. There are differing reports with regard to salinity limits of the species. Researchers have reported varying minimum values for indefinite survival ranging from 4 to 7.5‰ (Shumway 1996). Gunter (1950, 1953, as cited in Shumway 1996) reported that oysters are tolerant of salinities as low as 2‰ for up to a month. Oysters in Mobile Bay that were exposed to salinities approximating 1‰ for four weeks survived, while high mortalities resulted under the same condition for 7 weeks (May 1972). Andrews (1982) noted that oysters in Virginia entered a dormant state when salinity was ≤ 5‰ and resumed feeding when salinities exceeded 5‰.

Dissolved oxygen

Eckmayer (1979) stated that low D.O. is the most limiting factor for all aquatic life in Mobile Bay. Dissolved oxygen levels have been shown to affect oyster larval settlement, growth, and survival. Baker and Mann (1992) found that over a 24-hour period 4% of larvae settled in an anoxic environment (< 1% saturation), 18% in a hypoxic environment (20% saturation), and 38% in a normoxic environment (100% saturation). Settled larvae in an anoxic environment exhibited no growth while larvae in a hypoxic

environment grew at approximately 30% the rate of larvae in a normoxic environment. Juvenile spat in anoxic conditions had a median mortality time of 3.5 days and those in a hypoxic condition died in approximately 5.5 days (Baker and Mann 1992). Tolerance of larvae to anoxic conditions increases with size and development (Widdows *et al.* 1989).

According to Eckmayer (1979) oysters survived up to 5 days in a low D.O. event of < 1.0 ppm, temperature of 17 °C, and salinity of 25.0‰. Saoud *et al.* (2000) reported total mortality of oysters when D.O. remained at 0 ppm for 6 consecutive days at a temperature of 28 °C.

Turbidity

Turbidity, caused by silt and other suspended solids, can have a detrimental effect on oyster recruitment by causing mortalities while larvae are in the water column, and making settling substrate inadequate for spat set (Brodtmann 1991).

Growth

Oysters in Alabama are capable of reaching harvest size (76.2 mm) in as little as 16 months in culture situations and 24 months on reefs (Wallace 1993; Wallace *et al.* 1999). This relatively quick growth rate has distinct biological advantages in that southern oysters are susceptible to predation and disease for a shorter time than their northern counterparts, thus resulting in higher production (Engle 1945). Saoud (2000) reported findings of oysters reaching 55 mm in one year at an unproductive reef in Bon Secour Bay. Adult eastern oysters vary in shell height from 5.0 cm to 35.6 cm (Galtsoff 1964).

Predation

Oyster drills (Thais haemostoma), also referred to as conches, whelks, borers, and snails, are often identified as the most important predator of oysters in Alabama (Engle 1945, Eckmayer 1979). According to May and Bland (1970, as cited in Lee 1979) distribution of oyster drills is regulated by salinity. Engle (1945) stated drills are found throughout the reefs and bay bottoms of Alabama. According to McClellan (1965), drills favor an average salinity of approximately 15‰, while drill mortality occurs and activity decreases at salinities ≤ 10‰. However, Menzel *et al.* (1966) stated an average salinity of 15‰ limits distribution of drills. Archer (1947) found drills in just 5 of 12 dredge hauls in a study of oyster reefs after a hurricane.

May (1968) blamed oyster drills for mortalities amounting to 80 – 95% of oyster spat less than 50 mm in shell height. However, he did not report how oyster drill predation was proven as the source of mortality. The fact that these high mortalities occurred during the warm spring and summer months also raises suspicion of "dermo", a common warm water oyster disease, as being a factor. Moore (1913) also stated most oysters killed by drills are less than 50 mm in height. Density of oyster drills on reefs, sometimes surpassing that of oysters, ranges from $0/m^2$ in low salinity areas to approximately $0.5/m^2$ in high salinity areas (Eckmayer 1979).

Control of oyster drills is difficult as their larvae are planktonic and they are predatory immediately after settlement and metamorphosis (McClellan 1965; Eckmayer 1979). Utilizing the common sense technique involving disposal of drills while culling oysters would have positive effects on reducing oyster mortalities due to drill predation (Engle 1945). McClellan (1965), and others, described a control technique that takes

advantage of the drills' tendency to exhibit negative geotactism. During the breeding season of April – June, females tend toward raised areas to lay their eggs (Engle 1945). Hatching takes about two weeks. By placing stakes equipped with egg-laying substrate, such as palmetto leaves and twigs, on the reef, oyster drill egg cases can be periodically removed by pulling stakes and removing and destroying eggs. However, due to high fecundity of drills, this is an expensive and labor-intensive measure. The best control measure involves freshets and simply removing oyster drills from the reef while culling, and disposing of them on dry land (Engle 1945; McClellan 1965; Eckmayer 1979).

Crabs also cause considerable damage to oysters. Menzel and Hopkins (1955), through studies done in Louisiana, claim the blue crab (Callinectes sapidus) is more detrimental to oyster populations than the oyster drill. According to McClellan (1965) and Lee (1979) blue crabs occur over Alabama's entire oyster producing area. Many researchers speculate that only young oysters (3 – 40 mm) are susceptible to predation by blue crabs. Menzel and Hopkins (1955) noted blue crabs conducted a cursory "test" of an oyster and if it did not yield to the first attack, the crab moved from oyster to oyster until a weak oyster was found. At that point, the oyster was attacked with full force and eaten within a few minutes. Menzel and Hopkins (1955) speculated that blue crabs kill few healthy oysters. Blue crab predation can have a substantial effect on oyster populations as one crab is capable of killing up to 19 spat per day (Menzel and Hopkins 1955). Krantz and Chamberlin (1978) observed complete ingestion of cultchless spat and shell measuring up to 15 mm shell height. They noted blue crabs up to 80 mm carapace width were unable to kill cultchless oysters greater than 25 mm shell height and endured great

difficulty in manipulating oysters set on cultch. Ogle and Beaugez (1988) observed blue crabs were capable of opening oysters up to 40% their size.

Stone crabs (Menippe mercenaria) are a serious oyster predator in the Gulf (Krantz and Chamberlin 1978), each crab capable of killing an average of 0.6 oysters/day (Menzel and Hopkins 1955). Eckmayer (1979) stated stone crabs can kill up to 15 times more oysters than an oyster drill. Similar to the oyster drill, the stone crab's distribution is limited by salinity. In a study done by Hoese *et al.* (1972) they noted juvenile stone crabs occurred at two of the high salinity sites in eastern Mississippi Sound, and adults were found at one higher salinity site than juveniles.

Mud crabs (Eurypanopeus depressus) also occur in Mobile Bay (Archer 1947; Engle 1948; Lee 1979; Eckmayer 1979). Archer (1947) found mud crabs in 100% of dredge hauls completed while evaluating the effects of a 1947 hurricane, one month after the storm. Engle (1948) also found mud crabs to be numerous three months after the same storm. In areas of oyster production where oyster drills are lacking, the mud crab is a primary predator (Krantz and Chamberlin 1978). However, Archer (1947) described mud crabs as scavengers. Mud crabs are non-migratory, and active throughout the year (MacKenzie 1981).

The black drum (Pogonias cromis) is often implicated as the main fish predator of oysters in the Gulf. It is a schooling, migratory fish found throughout Alabama's oyster producing waters, in all levels of salinity (Moore 1913; Engle 1945). Moore (1913) and Engle (1945) noted the drum prefers culled oysters and oysters isolated from clusters. They are not able to attack oysters in clusters with sharp edges as they destroy oysters by

crushing them with their grinding teeth (Moore 1913). Wire mesh fencing has been shown to be effective in keeping black drum off of oyster reefs.

Flatworms (Stylochus sp.) occur in Mobile Bay and adjacent waters (Menzel *et al.* 1966; Hoese *et al.* 1972) and are a well-known predator of oyster spat and barnacles (Menzel and Hopkins 1955; MacKenzie 1981). They have also been implicated as substantial enemies to bivalve spat in other parts of the world (personal observation). According to Pearse and Wharton (1937, as cited in Menzel *et al.* 1966), flatworms, when in high densities, are capable of causing considerable damage to oyster populations. Menzel *et al.* (1966) found Stylochus sp. at densities as high as $50/m^2$ in Apalachicola Bay, Florida.

Fouling and competition

Hooked mussels (Ischadium recurvum) are common bivalves associated with oyster reefs in Alabama (Moore 1913; Engle 1945; McClellan 1965). Hooked mussels compete with oysters for food and space and cause economic problems with regard to processing. Due to the consequence of strong byssal thread attachment by mussels to oysters, increased processing is required to remove both unsightly mussels and byssal threads from oysters for the half shell trade (Moore 1913; McClellan 1965). Moore (1913) and Ingle (1951) stated hooked mussels grow at such a rate that they are capable of engulfing and smothering oysters. Contrary to many of the above-mentioned predators, hooked mussels flourish in low salinity waters and are inhibited by high salinity (Moore 1913; Menzel *et al.* 1966).

Barnacles (Balanus sp.) are the most abundant fouling organism in Alabama, setting at rates up to 78 times greater than oysters (Hoese *et al.* 1972). Barnacles cause a reduction in growth of adjacent oysters through competition for food and space. Although they compete for substrate space, their presence has been shown to increase oyster settlement rates (Ingle 1951; Osman *et al.* 1989). Oyster larvae will settle on both live and dead barnacles, and often move to open spaces after settlement to attach to clean substrate (Osman *et al.* 1989). Barnacles have been shown to cause some oyster mortality by undercutting oysters and pushing their upper valve up, thus interfering with valve closure (MacKenzie 1981).

Bryozoans (Membranipora sp.) are the second most abundant fouling organism in Alabama (Hoese *et al.* 1972). Unlike barnacles, oysters are unable to settle on bryozoans, however, the presence of bryozoans has been shown to have a positive influence on oyster settlement rates on adjacent cultch material (Osman *et al.* 1989). The cause of this influence is unknown and could be due to several factors, including the disruption of currents over cultch material by the occupancy and feeding of residents, or the presence of some sort of chemical attractant to oyster larvae (Osman *et al.* 1989).

Disease

The main oyster disease of concern in Alabama is due to a warm season, protozoan pathogen caused by the etiological agent commonly known as "dermo". "Dermo" has caused mass mortality of oysters in some areas of the Gulf as well as Chesapeake Bay. The primary mode of infection is derived from the ingestion of waterborne "dermo" cells. The effect of "dermo" on oysters includes lysis of tissues and,

ultimately, failure of tissue and organ function. The quick growth rate of oysters in the Gulf often allows them to escape mortality caused by the disease as they are usually harvested before mortality occurs (usually during their second year). Infection of "dermo" is related to salinity and temperature. "Dermo" prefers temperatures above 20 °C and salinity above 12 – 15‰ with salinity below 10‰ resulting in decreased development.

Silt/sedimentation

Silt and sedimentation inhibit oyster recruitment by making settling substrate inadequate or unavailable for larval settlement and attachment. Excessive silt and sedimentation caused by storms, run-off, channel dredging, harvesting, and shell planting can reduce growth or cause mortalities in oyster populations (Dunnington 1968; MacKenzie 1981). Dunnington (1968) found survival time of oysters buried in 76 mm of mixed sand and mud was directly correlated to temperature. Buried oysters died quicker in summer, starting at 2 days, than in winter, starting at 5 weeks. Dunnington (1968) found that oysters buried up to 12 mm deep were able to clear their shells of sediment as long as water temperature was conducive to pumping. May (1971) reported that, in some areas of Mobile Bay, substrate covered with a light layer of silt and sedimentation is periodically cleaned by currents and wave action, the same modes of silt and sedimentation delivery.

According to Ryan (1969, as cited in Saoud 2000), the annual load of suspended sediment entering Mobile Bay is 4.2 million MT of which 3.0 million MT is deposited in the Bay. According to Saoud (2000) the rate of sediment accretion in Alabama varies

from reef to reef. He found sediment deposited at rates of 0.53 – 0.89 mL/m/day on Fish River Reef in Bon Secour Bay and 0.14 – 0.27 mL/m/day on Cedar Point Reef in Mississippi Sound. Current is important in keeping shells clean and substrate available for spat set (Engle 1945).

Oyster reef restoration

Oyster reef restoration stems from a desire to increase, sustain or revitalize production, enhance ecosystem services provided by oysters and oyster reefs, and realize their use as keystone species and their role as essential fish habitat (Leard *et al.* 1999; Luckenbach *et al.* 1999; Wallace *et al.* 1999). Oyster larvae require clean, hard substrate (cultch) upon which to settle and grow. It is this concept upon which many restoration efforts are based (MacKenzie 1989; Berrigan 1990). If suitable substrate is not available during critical settling times, larvae may settle on unsuitable bottom and die. Most U.S. states from Connecticut to Texas are involved in some sort of oyster restoration effort due to reduced oyster populations and harvests as a result of overfishing, disease, pollution, habitat destruction, and other natural disasters. In Alabama, the issues are similar and many reefs require rehabilitation due to overharvesting, oyster population reduction due to adverse environmental events such as low D.O. and freshets, low spat setting rates, lack of shellplanting to replace lost or removed cultch, and low oyster density (May 1971; Eckmayer 1979; Saoud *et al* 2000). May (1973) suggested that low D.O. events, which destroyed many oyster populations in the past, and poor setting rates, were the main causes of insufficient oyster production on some reefs in Bon Secour and Mobile Bays.

Oyster population management began in the 1800's. According to Ogle and Beaugez (1988), the first oyster transplantation in the U.S. occurred in 1810 and the first shell planting occurred in 1855. Recent efforts of oyster population restoration primarily involve planting shell cultch or oyster seed on previously productive reefs or other hard bottoms. Where spat set is adequate, cultch is placed. Commercially suitable spat set yields 1.8 – 2.5 spat per shell of cultch (MacKenzie 1981; Morales-Alamo and Mann 1990). Planting cultch is probably the most effective oyster reef restoration method (Leard *et al.* 1999). Where spat set is not optimum but oyster growth is good, restoration involves planting seed. Seed planting is also a method of hastening the recovery of oyster populations and increasing harvests sooner than planting cultch material (Engle 1945).

Suitability of underlying bottoms is an important factor to consider when restoring oyster reefs. Due to the sheer weight of cultch material and the tendency of non-oyster grounds to consist of mud or sand substrate, the success of cultch material in not becoming buried is important. If cultch is placed on mud bottoms it may sink into the mud, or siltation may cause reduced spat set, survival and growth. Sandy bottoms, although relatively hard, are usually inadequate due to the fluidized nature of sand during storm and wave action, causing constructed beds to sink or become buried in sand. Factors including oyster growth and recruitment must also be considered. Sites of historically successful oyster recruitment and growth should be the focus of restoration efforts. Historical records and local knowledge are good resources to use in order to understand areas of adequate spat set and growth. Sampling the water column for oyster

food availability will also enhance knowledge of where to place cultch or seed oysters (Kennedy and Sanford 1999).

Rates at which shell cultch should be planted vary from 200 – 12,000 bushels/ha, depending on bottom type (Engle 1945; Kennedy and Sanford 1999). Softer bottoms would require more cultch material. However, a shell planting rate of 200 bushels/ha was deemed inadequately thin for Alabama's reef restoration efforts and Engle (1945) suggested approximately 810 bushels/ha be planted. If resident adult oysters are lacking in the area to be planted with cultch material, it is recommended that 1 bushel of adults be planted for every 5 – 10 bushels of cultch in order to enhance spat set (Ingersoll 1881, as cited in Kennedy and Sanford 1999). Seed oyster planting should approximate 1200 – 2000 bushels/ha (Kennedy and Sanford 1999).

It is commonly recommended to plant cultch material just before or during peaks of spat set in order to reduce the chance of silt/sedimentation fouling cultch material. Preferably, shell cultch should be planted in areas with adequate current that will enable spat set to spread over a wide area and avoid localization of set (Kennedy and Sanford 1999). Current has been reported to keep cultch clean of silt/sedimentation thus enhancing spat set and survival (Engle 1945).

Shell cultch planting has proven successful in most oyster restoration efforts. In Connecticut, harvests increased from 300,000 bushels to 1.5 million bushels eight years after planting (Kennedy and Sanford 1999). Berrigan (1990) reported an increase in oyster density from near zero to $160/m^2$ in one year. In Florida, restoration costs were recouped in one year following a rehabilitation effort caused by the effects of a hurricane (Berrigan 1990). Maryland estimated a ratio of 1:2, planted shell to young oysters, as a

result of it's planting efforts. Butler (1954, as cited in Kennedy and Sanford 1999) speculated that in conditions made optimal by restoration efforts, annual per-hectare production in the Gulf of Mexico could amount to approximately 2,200 bushels of oysters. Cost:benefit ratios have been reported as 1:2.3 to 1:3.5, two years after restoration efforts in Florida and 1:20.7, ten years after clam shell cultch was planted in Apalachicola Bay, FL (Leard *et al.* 1999).

Alabama oyster reef surveys and assessments

Mapping and surveying efforts in Alabama were first conducted in the 1800's. The first hydrographic survey was conducted in 1847 and a chart of Alabama's estuarine waters was printed in 1851. Specific work on mapping oyster reefs and studying the condition and population of reefs were completed by Ritter in the late 1800's, Moore in 1911, Bell in the 1950's, and May in 1969 (Moore 1913; May 1971; Tatum *et al.* 1995). The most recent report by May (1971) noted 1,240 ha of public oyster bottom in Alabama. This is slightly different than previous reports showing areas of oyster beds in Alabama consisting of 1,256 ha and 1,622 ha by Ritter and Moore, respectively (May 1971). Discrepancies in these reports may exist due to differing methods of study and definitions of oyster bottom. Due to the amount of freshwater influx into the Bay by the fourth largest river discharge in the United States, the Mobile River system, natural oyster reefs are restricted to the lower portion of the Bay (Engle 1945; McClellan 1965; May 1971; Hoese *et al.* 1972). However, May (1971) also attributed oyster drill predation as being a major limiting factor in oyster distribution and abundance in Alabama.

Galtsoff in the 1920's and Engle in the 1940's assessed Alabama's oyster reefs but these studies were confined to understanding the status of the oyster population (Tatum *et al.* 1995). In 1995 reefs in the Dauphin Island/Cedar Point area were surveyed in order to understand the extent of reef systems as various development efforts and storm events had occurred since the last major survey in 1969 (Tatum *et al.* 1995). Recent efforts include an assessment of three unproductive oyster reefs: Klondike Reef, Whitehouse Reef, and Shellbank Reef; and the Bay's main productive reef, Cedar Point Reef by Smith in 1998. This work will report the results of assessments of oyster resources present on Whitehouse Reef and Klondike Reef, and a boundary survey of Klondike Reef in 2000.

Alabama reef rehabilitation efforts

Although various shell and seed oyster plantings occurred throughout Alabama's oyster bearing waters beginning in 1909, more productive efforts began in the 1940's as a result of a survey by Engle (Swingle and Hughes 1976). Engle (1945) proposed daily collections and plantings of oyster shell on Alabama's oyster reefs. Daily collections and plantings of shells obtained from processors would allow the undersized, attached spat on processed oysters to remain viable and complete their life cycle on reefs (Alabama Department of Conservation 1943; Engle 1945). In 1944, per Engle's recommendation, more than 90,000 barrels (1 barrel = 0.12 m^3) of shell were planted at a rate of approximately 740 barrels/ha on various productive and potentially productive reefs in Alabama (Alabama Department of Conservation 1944). Three new oyster beds: Little Point Clear, Grand Bay, and Marsh Island Reefs, were created during this shell planting.

During 1944 shell planting, Klondike Reef received 2,217.2 barrels (Alabama Department of Conservation 1945).

More than 15,000 barrels of seed oysters were dredged from various locations in western Mobile Bay (Whitehouse Reef, Birmingham Buoy, and Kings Bayou) and used to seed reefs in Bon Secour Bay and other areas of Mobile Bay and Mississippi Sound in 1947 (Alabama Department of Conservation 1947). This work was recognized as the first large-scale effort to restore the Alabama oyster industry by planting seed oysters.

In 1947 a hurricane struck the region and did some damage to Alabama's oyster reefs due to siltation. Reefs adjacent to soft bottoms were especially affected by siltation while reefs adjacent to hard bottoms sustained relatively little damage (Archer 1948). Berrigan (1990) reported reefs adjacent to sand bottoms in Apalachicola Bay, FL were most affected by "depositional degradation" due to a hurricane. Archer (1948) suggested that water movement would remove siltation caused by hurricanes, thus allowing reefs to return to their normal state of health. In 1948, 40,000 barrels of seed oysters were planted throughout Mobile Bay and Bon Secour Bay and 25,000 barrels of shell cultch material were planted in eastern and western Mobile Bay. After receiving shell for rehabilitation efforts, reefs remained closed to harvesting until reopened due to future regulation (Alabama Department of Conservation 1948). During the years of rehabilitation efforts, State reefs were surveyed periodically to assess the status of oyster populations.

According to Tatum *et al.* (1995) the 1980's represented the period of the most intense shell planting in Alabama. They reported no volumes of shell planted for much of the 1980's but stated volumes planted from 1989 to 1994 totaling 25,231 m^3. No

details were given as to which reefs, in particular, received shell cultch material. In 1999, Whitehouse Reef was planted with 7,645 m^3 oyster shell cultch material (personal communication, M. Van Hoose, AL Marine Resources Division).

Cultch material

Prior to the 1970's, oyster shell was used as cultch material for shell planting efforts. During the cultch planting efforts of the 1970's and 1980's, however, clam shell was used due to economics and availability of material (Tatum *et al.* 1995). Much of the clam shell used for cultch was derived from dredging. Due to environmental concerns, clam shell cultch was abandoned and the use of oyster shell resumed (Brodtmann 1991; Leard *et al.* 1999).

Oyster shell is abundant in Alabama because nearly 20% of oysters harvested in the Gulf are processed in Alabama. By law, Alabama processors are required to plant at least half of all Alabama shell that they process. There are three ways in which processors can meet this requirement: 1) they may plant half of the shell they processed, at their expense, 2) if they are located adjacent to a barge loading site, they may give ¾ of the shell they processed to the planting effort and transport and planting will be done at the State's expense, or 3) regardless of proximity to a barge site, they may give all of their shell (including other Gulf state's shell) to the planting effort and it will be hauled away at the State's expense (Tatum *et al.* 1995). Most processors choose the third option (Tatum *et al.* 1995).

Other possible cultch materials include archaeological coral (Tatum *et al.* 1995; Wallace *et al.* 1999), limestone, concrete, dredged oyster shell, cement, scallop shell (FL

only), coal fly ash, and porcelain (Soniat *et al.* 1991; Leard *et al.* 1999). Although oyster larvae will set on a variety of other materials, including gypsum (Soniat *et al.* 1991), gypment (cement stabilized gypsum) (Haywood and Soniat 1992), masonite (Marshall 1954) and asbestos plates (Hoese *et al.* 1972; Saoud 2000), many of these materials are used for experimental purposes and would prove economically impractical for use as planted cultch material.

MATERIALS AND METHODS

Two naturally occurring oyster reefs in Alabama were studied during the summer, 2000 (Fig. 1). Whitehouse Reef C (36.4 ha) (also known as Denton Reef), consisting of an unrestored natural reef area (12.1 ha) and a restored reef area surrounded by concrete rubble (24.3 ha) was assessed twice for oyster resources. Klondike Reef (64.9 ha) was surveyed and assessed for oyster resources prior to restoration. Neither of these reefs has recently been commercially harvested due to low oyster densities (Smith 1999). Whitehouse Reef is the most northerly reef on the west side of Mobile Bay that produces oysters, while Klondike Reef is the most northerly reef on the eastern side (Engle 1945).

Reef surveys and assessments were completed with assistance from the research staff, captain, research vessel, and field and laboratory equipment at Auburn University's Marine Extension and Research Center in Mobile, Alabama.

Reef assessments

Reef assessments were conducted by random sampling at a rate of 1 m^2/0.4047 ha (1 acre), using similar methods previously described by May (1971) and Smith (1999). Samples were completed with the use of a 150-meter transect line, marked at 1 meter intervals and 10 meter intervals, labeled white mesh sample bags (80 cm x 55 cm with mesh approximately 5 mm x 5 mm), 3.8-L Ziploc® subsample bags, and 1-m^2 quadrats

with a 0.04-m^2 subsample area. Quadrats were fabricated out of 25.4-cm PVC pipe with holes drilled in the corners to allow it to fill with water and sink, and two corners of the quadrat were outfitted with bolts in order to fix it to the reef bottom while sampling. Each end of the transect line was equipped with anchors and floats in order to anchor the end of the line to its proper location, and locate the line when deploying divers. Starting point position and orientation of the transect line on the reef, and placement of sample bags within 10 meter intervals on the transect line (resulting in 15 samples per transect line) were chosen randomly. Once randomly located on the transect line, sample bags were attached to the line with metal clips at their corresponding meter marks. One end of the line was submerged at the pre-determined starting point on the reef. The line was then drawn off the stern of the boat in the pre-determined random direction and guided as such with a Garmin® Differential Global Positioning System (DGPS). The line was kept taut in order to insure that it was straight when laid down on the reef. One SCUBA diver, equipped with a dive flag, quadrat, cable ties, and subsample bags, was deployed at each end of the transect line. Divers swam the length of the line until encountering a sample bag. The quadrat was placed on the reef surface to delineate the sample area and all surficial bottom material was gathered and deposited in the sample bag. Bottom material in the 0.04-m^2 subsample was taken at each sample site and placed in the Ziploc® bag. The subsample bag was placed in the mesh bag, which was cable-tied shut and left attached to the line. The diver then swam the line to the next sample bag and repeated this procedure until both divers met in the middle of the transect line. Due to poor visibility conditions common in the Bay, much of the sampling work was done by feel.

When sampling was complete, the line and sample bags were pulled into the boat, sample bags removed from the line and placed in coolers or under wet burlap bags. Samples were brought to the lab and analyzed for cultch weight and oyster weight of each sample and subsample using a digital (to the nearest 0.1 g) or spring scale (to the nearest 10 g) depending on sample size; shell height of all living oysters with a digital caliper (to the nearest 0.1 mm), number of oyster drills per sample, and number of mussels and total mussel weight (to the nearest 0.1 g) per subsample. If it was not possible to analyze samples immediately, they were placed in a freezer and thawed for several hours, at ambient temperature, before analyzing.

Reef survey

The boundary of Klondike Reef, delineated by the gradient between shell bottom and soft/sand bottom, was determined by conducting soundings of the Bay bottom with a cane pole. Soundings were taken every 250 decimal minutes latitude by sounding the Bay bottom along said latitude until the reef boundary was determined. If 250 decimal minutes seemed to give inadequate detail, intermediate soundings were completed. Precise locations of the reef boundary were recorded with a DGPS.

Data analysis

Data were analyzed using the SPSS® 10.0 statistical program. In order to evaluate the differences in means, independent t-tests were conducted on mean height of oysters, mean density of oysters, mean weight of exposed cultch, mean density of oyster drills,

and mean density of hooked mussels between reef assessments. Differences were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

Whitehouse Reef

Two reef assessments were conducted on Whitehouse Reef C during summer, 2000. The first assessment (completed between 3 and 11 August) consisted of 6 transects (90 samples). Four transects were completed within the restored reef area and the remaining two were completed on the unrestored reef area. The first assessment was conducted in order to understand the efficacy of the reef restoration effort completed by the Alabama Marine Resources Division in fall, 1999, which involved the planting of 7,645 m^3 oyster shell cultch (personal communication, M. Van Hoose, AL MRD). This study also allowed a comparison of the oyster population as if it were not restored (unrestored area transects) by comparing these results with an assessment completed in 1998 by Smith (1999).

The second assessment was completed (on 24 August and 15 September) in order to quantify an oyster die-off that occurred shortly after the first assessment was completed. The second assessment consisted of four transects (60 samples) conducted on the restored reef area only.

1998 and 2000 (unrestored area) assessments

Mean density of oysters in 2000, 5.87/m^2 (S.E. = 2.19, range = 0 – 53), was not significantly less than the mean oyster density found in 1998 (7.21/m^2, S.E. = 0.66, range = 0 – 25) (Table 1). Mean height of oysters was 41.74 mm (S.E. = 3.00, range = 7.3 – 76.2 mm) in 2000, and 35.57 mm (S.E. = 0.88, range = 9.0 – 137.0 mm) in 1998 (Table 1). Mean weight of exposed cultch was 0.93 kg/m^2 (S.E. = 0.37) in 2000, and 0.20 kg/m^2 (S.E. = 0.02) in 1998 (Table 1). Mean weight of total available cultch (weight of exposed cultch + weight of oysters) was 1.04 kg/m^2 (S.E. = 0.40) in 2000, and 0.46 kg/m^2 (S.E. = 0.03) in 1998. Mean density of oyster drills was significantly greater in 2000 (0.33/m^2, S.E. = 0.12) than in 1998 (0.00/m^2) (Table 1). Aside from a significant increase in the density of oyster drills on Whitehouse Reef between 1998 and 2000, the oyster population on the unrestored area remained unchanged between the two years.

First assessment, restored area and unrestored area

In 2000, after restoration efforts, mean density of oysters was significantly greater on the restored reef area, 49.93/m^2 (S.E. = 5.98, range = 0 – 207), than the unrestored reef area, 5.87/m^2 (S.E. = 2.19, range = 0 – 53) (Table 2). Mean height of oysters on the first assessment was not significantly different on the restored reef area (38.74 mm, S.E. = 1.31, range = 2.5 – 83.7 mm) than the unrestored reef area (41.74 mm, S.E. = 3.00, range = 7.3 – 76.2 mm) (Table 2). Mean weight of exposed cultch was significantly greater on the restored reef area (7.22 kg/m^2, S.E. = 0.75) than the unrestored reef area (0.93 kg/m^2, S.E. = 0.37) (Table 2). Mean weight of total available cultch was 8.13 kg/m^2 (S.E. =

0.85) on the restored area and 1.04 kg/m^2 (S.E. = 0.40) on the unrestored area. Mean density of oyster drills was significantly different between the two assessments, 0.73/m^2 (S.E. = 0.14) on the restored area and 0.33/m^2 (S.E. = 0.12) on the unrestored area (Table 2). Mean density of hooked mussels was significantly greater on the restored area (870.42/m^2 S.E. = 137.66) than the unrestored area (145.83/m^2, S.E. = 104.68) (Table 2).

Second assessment of restored area

Mean density of oysters was 49.93/m^2 (S.E. = 5.98, range = 0 – 207) on the first assessment, significantly more than the second assessment (8.40/m^2, S.E. = 1.15, range = 0 – 42) (Table 3). Mean height of oysters on the restored reef area on the first assessment was significantly greater (38.74 mm, S.E. = 1.31, range = 2.5 – 83.7 mm) than the second assessment (25.42 mm, S.E. = 1.44, range = 3.7 – 69.3 mm) (Table 3). Mean weight of exposed cultch was 7.22 kg/m^2 (S.E. = 0.75) on the first assessment, not significantly more than the second assessment (5.62 kg/m^2, S.E. = 0.72) (Table 3). Mean density of oyster drills was 0.73/m^2 (S.E. = 0.14) on the first assessment, similar to 0.83/m^2 (S.E. = 0.17) found on the second assessment (Table 3). Mean density of hooked mussels was 870.42/m^2 (S.E. = 137.66) on the first assessment, significantly more than the second assessment, 481.25/m^2 (S.E. = 87.29) (Table 3).

The first assessment in 2000 showed that the unrestored oyster resource remained consistent with historical records with regard to mean density of oysters and mean weight of exposed cultch as recorded in 1969; and mean density and mean height of oysters, and mean weight of exposed cultch per unit area as recorded in 1998 (Table 1). However, a

significant difference was found in the density of oyster drills between 1998 and 2000 (unrestored area).

Results of this study demonstrated that cultch planting was effective in increasing oyster density. Mean oyster density was more than eight times greater between the restored area (49.93/m^2) and unrestored area (5.87/m^2) nine months after shell planting. Alabama oyster harvesters tend to tong on reefs with a marketable oyster density of at least 9,880 harvestable oysters/ha (approximately 1 harvestable oyster/m^2) (May 1971). Therefore, Whitehouse Reef has the potential to be productive if adverse factors affecting oyster survival are alleviated. However, due to the relatively short time frame (nine months) between the 1999 cultch planting effort and the 2000 assessment, and evidence of oyster die-offs at Whitehouse Reef, it is difficult to judge the potential for production of market-sized oysters there.

Review of size-class data shows that the 25.5 – 50.8 mm size class was well represented on the reef for all but the second assessment of 2000 (Fig. 2 – 4). Smaller spat and larger oysters were lacking (Fig. 2 – 4). Studies have shown variable results for spat set in the Whitehouse Reef area of Mobile Bay. Lee (1979) found a range of 0.0 – 0.4 spat/m^2/day at a location adjacent to Whitehouse Reef. Hoese *et al* (1972), although they reported no discrete numbers for the sampling station adjacent to Whitehouse Reef, found setting rates ranging from 0 to approximately 6 spat/m^2/day with an approximate setting season average (May – November) of 1 spat/m^2/day. Saoud (2000) found much higher rates of spat set at Whitehouse Reef (up to 1,730 spat/m^2/day). Eckmayer (1979) reported that spat set at Whitehouse Reef was good, but the reef was prone to effects of spring floods, and suggested Whitehouse Reef would be a good source of seed oysters.

Results of the first and second assessments of 2000 indicated a significant decrease in both mean density of oysters and mean density of hooked mussels, suggesting periodic adverse environmental conditions, such as low D.O. may be causing oyster die-offs on Whitehouse Reef. Mean density of oyster drills increased between the two assessments but this increase was not significant. Through personal observation of dead oysters on the second assessment, I saw only one sign each of oyster drill and crab predation.

Comparisons of historical mean oyster density revealed that the unrestored oyster population has remained relatively stable on Whitehouse Reef since 1969 (Table 1). Mean oyster density was $5.19/m^2$, $7.21/m^2$ and $5.87/m^2$ in 1969, 1998, and 2000, respectively. These data further suggest that something is limiting oyster production on Whitehouse Reef from achieving levels similar to that of Alabama's most productive reef, Cedar Point Reef, where mean density of oysters was $56.5/m^2$ in 1969 and $151.8/m^2$ in 1998 (May 1971; Smith 1999). Mean weight (kg/m^2) of exposed cultch on Cedar Point Reef was 1.10 kg/m^2 in 1998 (Smith 1999). In 1998, exposed cultch may have been limited on Whitehouse Reef (0.20 kg/m^2) compared to Cedar Point Reef. However, present levels of exposed cultch (0.93 kg/m^2 on the unrestored area, and 5.62 to 7.22 kg/m^2 on the restored area) show that cultch is not limited on Whitehouse Reef. Low spat fall and periodic adverse environmental parameters, such as low D.O., are potential causes of lack of production on Whitehouse Reef.

Klondike Reef

Coordinates of the boundary of Klondike Reef are reported in Table 2. The western boundary of the reef was easily distinguishable, as the gradient from shell bottom to mud bottom was distinct. However, the eastern boundary of the reef was more difficult to determine as the reefs proximity to shore borders it with hard, sandy bottom, typical of the perimeter of Mobile and Bon Secour Bays (May 1971).

During summer, 2000, Klondike Reef was assessed in order to quantify the condition of the oyster resource before undertaking an oyster shell cultch planting effort. The restoration effort was completed during fall, 2000 (personal communication, M. Van Hoose, AL MRD). This was the first shell planting effort on Klondike Reef since 1944.

Eleven transects were completed, taking 165 samples, of which less than 25% (40 samples) yielded live oysters and/or exposed cultch material. The remaining 125 samples were on mud bottom overlying the reef. The assessment revealed that the oyster resource on Klondike Reef was scarce. Mean density of oysters was $0.16/m^2$ (S.E. = 0.05, range = 0 – 7) (Table 5). Mean height of the 26 oysters found was 18.70 mm (S.E. = 2.87), with a range of 4.6 – 55.2 mm (Table 5). Mean weight of exposed cultch was 0.03 kg/m^2 (S.E. = 0.005) (Table 5). There were neither oyster drills nor hooked mussels found on Klondike Reef. A review of oyster size-classes (Fig. 5) showed that more small spat ($\leq$ 25.4 mm) were present than large spat and oysters ($\geq$ 25.5 mm), suggesting that a limiting factor is preventing oyster survival. However, spat set, to the extent that cultch is available, may be adequate in replenishing the population.

Historically, Bon Secour Bay's oyster reefs (including Klondike Reef) were favored as producing Alabama's better quality oysters (Engle 1945; May 1971).

However, due to pollution and dredging these reefs are now unproductive (Engle 1945). Through an assessment completed in 1943, Engle (1945) found the oyster population on Klondike Reef to be "scattered and meager". However, although Engle (1945) reported no specific data, he mentioned the presence of market-sized oysters of good quality, and differentiated the quality of market-sized oysters between eastern and western Klondike Reef, suggesting there were sufficient numbers of these oysters with which to make a judgment. During the study, Engle (1945) found no oyster drills, and few mussels attached to oysters. In 1947, two months after a hurricane, Engle (1948) completed another assessment of Alabama's oyster producing grounds. Although this study determined the hurricane caused no detrimental effect to Mobile Bay or Bon Secour Bay reefs, some useful information was collected. Engle (1948) stated most of the dredged bottom material from Klondike Reef consisted of live small oysters, no market-sized oysters, and evidence that the 1947 spat set was abundant. Engle (1948) also observed many drills, signs of mortality caused by drills, and abundant mussels. As of 1969, per May's (1971) assessment of Klondike Reef, mean density of oysters was $0.85/m^2$ compared to $0.16/m^2$ and mean weight of exposed cultch was more than ten times greater in 1969 than in 2000 ($0.39\ kg/m^2$ vs. $0.03\ kg/m^2$) (Table 5).

From the current study it appears the oyster resources on Klondike Reef are at least as limited as two other unproductive reefs in Bon Secour Bay, Fish River and Shellbank Reefs, as reported by Smith (1999). Mean density of oysters was $0.16/m^2$ on Klondike Reef compared to $1.6/m^2$ and $2.7/m^2$ on Fish River and Shellbank Reefs, respectively. Mean height of oysters on Klondike Reef was 18.7 mm, compared to 30.6 mm and 33.2 mm on Fish River and Shellbank Reefs, respectively. Cultch was more

limited at Klondike Reef, which had 0.03 kg/m^2 mean weight of exposed cultch in 2000, compared to 1.00 kg/m^2 and 0.74 kg/m^2 on Fish River and Shellbank Reefs in 1998, respectively.

Previous setting studies adjacent to Klondike Reef reveal that oyster spat set is limited. Hoese *et al* (1972) completed a study in Alabama and, although they reported no data for the sampling station adjacent to Klondike Reef, they generalized that the area received negligible spat fall, less than stations adjacent to Fish River Reef (no data) and Shellbank Reef (0 – 10 spat/m^2/day). Lee (1979) also found spat set at a station adjacent to Klondike Reef limited (from 0.0 to 1.0 spat/m^2/day) as compared to a recent study conducted by Saoud (2000) during peak setting periods at Fish River Reef (170 to 280 spat/m^2/day) and Shellbank Reef (660 to 1,450 spat/m^2/day). Although spat set is considered negligible and periodic anoxic events are likely to occur (Saoud 2000), oyster production will not increase on Klondike Reef without the addition of cultch material. Further research on spat set as well as water quality on Klondike Reef would be advisable before considering a cultch planting effort there.

Table 1. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Whitehouse Reef in 1969[1], 1998[2], and 2000 (unrestored area). Different superscripts in the same row indicate a significant difference, $p \leq 0.05$. Statistical tests were conducted on 1998 and 2000 data only.

	Assessment		
	1969[1]	1998[2]	2000 (unrestored area)
number of samples	152	90	30
mean density (#/m^2) of oysters	5.19	7.21	5.87
mean height (mm) of oysters	n/a	35.57	41.74
mean weight (kg/m^2) of exposed cultch	1.28	0.20	0.93
mean density (#/m^2) of oyster drills	0.00	0.00[a]	0.33[b]
mean density (#/m^2) of hooked mussels	n/a	n/a	145.83

[1] May 1971

[2] Smith 1999

Table 2. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Whitehouse Reef in 2000, unrestored area and restored area. Different superscripts in the same row indicate a significant difference, $p \leq 0.05$.

	Assessment	
	2000 (unrestored area)	2000 (restored area)
number of samples	30	60
mean density (#/m^2) of oysters	5.87^a	49.93^b
mean height (mm) of oysters	41.74	38.74
mean weight (kg/m^2) of exposed cultch	0.93^a	7.22^b
mean density (#/m^2) of oyster drills	0.33^a	0.73^b
mean density (#/m^2) of hooked mussels	145.83^a	870.42^b

Table 3. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Whitehouse Reef in 2000, first and second assessments of restored area. Different superscripts in the same row indicate a significant difference, $p \leq 0.05$.

	Assessment	
	2000 (first assessment)	2000 (second assessment)
number of samples	60	60
mean density (#/m^2) of oysters	49.93^a	8.40^b
mean height (mm) of oysters	38.74^a	25.42^b
mean weight (kg/m^2) of exposed cultch	7.22	5.62
mean density (#/m^2) of oyster drills	0.73	0.83
mean density (#/m^2) of hooked mussels	870.42^a	481.25^b

Table 4. Klondike reef boundary coordinates from a survey completed during summer, 2000.

Sounding	Latitude	Longitude
KR1	30° 27.69349'	87° 56.12283'
KR2	30° 27.49651'	87° 56.05363'
KR3	30° 27.37259'	87° 55.95063'
KR4	30° 27.24127'	87° 55.58274'
KR5	30° 26.99633'	87° 55.61300'
KR6	30° 26.87466'	87° 55.84088'
KR7	30° 26.74592'	87° 55.83766'
KR8	30° 26.49325'	87° 55.83701'
KR9	30° 26.24735'	87° 55.75590'
KR10	30° 26.10347'	87° 55.76459'
KR11	30° 26.00531'	87° 55.85407'
KR12	30° 26.24767'	87° 55.91555'
KR13	30° 26.49937'	87° 56.03786'
KR14	30° 26.74849'	87° 56.08775'
KR15	30° 27.00051'	87° 56.06876'
KR16	30° 27.25028'	87° 56.09483'
KR17	30° 27.49940'	87° 56.21810'

Table 5. Number of samples, mean density (#/m^2) of oysters, mean height (mm) of oysters, mean weight (kg/m^2) of exposed cultch, mean density (#/m^2) of oyster drills, and mean density (#/m^2) of hooked mussels on Klondike Reef in 1969[1] and 2000. No statistical tests were conducted on these data.

	Assessment	
	1969[1]	2000
number of samples	193	165
mean density (#/m^2) of oysters	0.85	0.16
mean height (mm) of oysters	n/a	18.70
mean weight (kg/m^2) of exposed cultch	0.39	0.03
mean density (#/m^2) of oyster drills	0.006	0.00
mean density (#/m^2) of hooked mussels	n/a	0.00

[1] May 1971

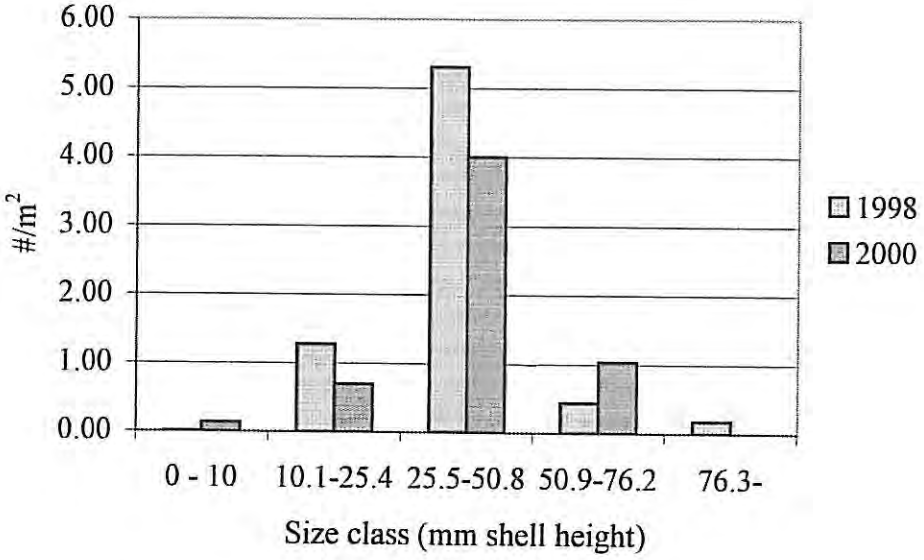

Figure 2. Mean density (#/m^2) of oysters sampled on Whitehouse Reef, per size class, 1998 and 2000 (unrestored area).

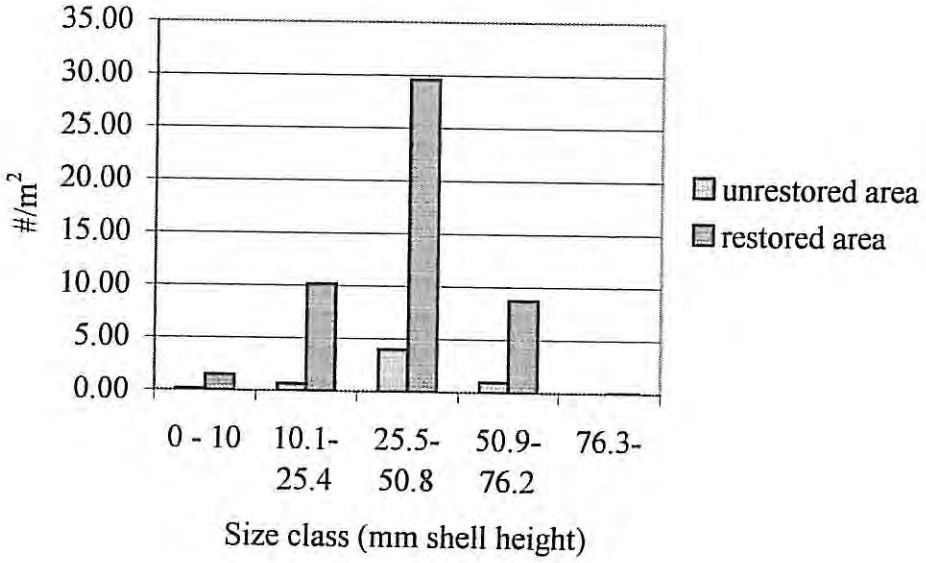

Figure 3. Mean density (#/m^2) of oysters sampled on Whitehouse Reef, per size class, unrestored and restored areas, 2000.

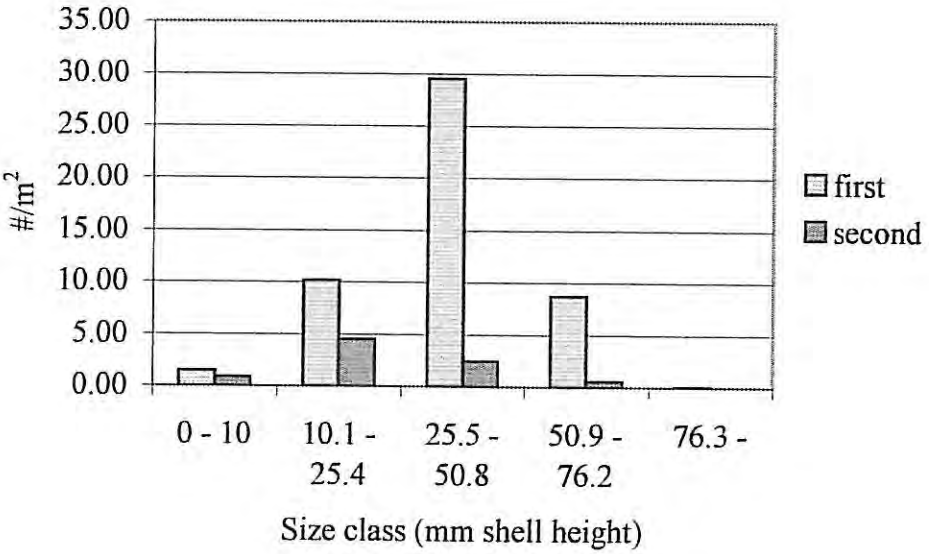

Figure 4. Mean density (#/m^2) of oysters sampled on Whitehouse Reef, per size class, first and second assessments, 2000.

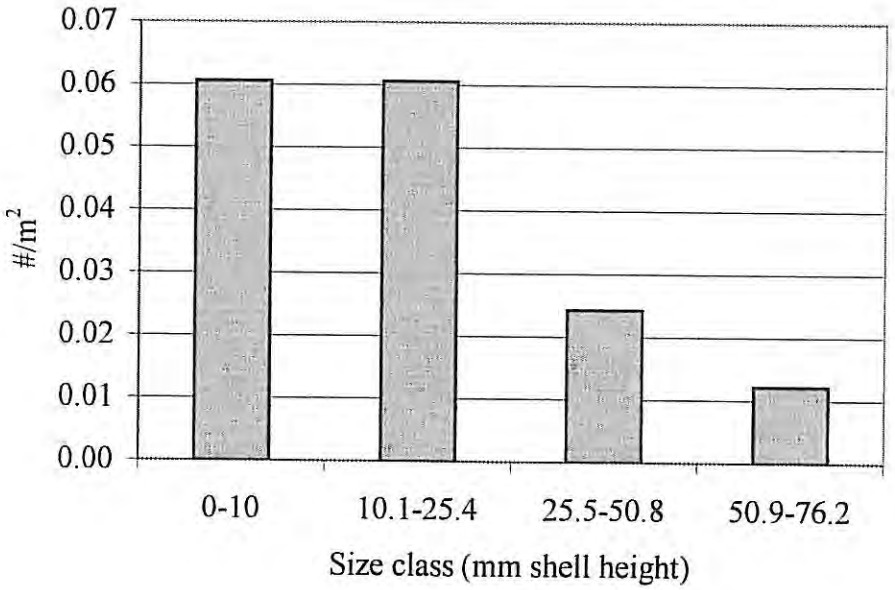

Figure 5. Mean density (#/m^2) of oysters sampled on Klondike Reef, per size class, 2000.

CHAPTER II. PRESENCE OF PERKINSUS MARINUS

ABSTRACT

Perkinsus marinus ("dermo") is responsible for causing substantial oyster mortalities from the Gulf of Mexico to Chesapeake Bay. The prevalence and intensity of "dermo" was assessed, via hemolymph assay, in oysters from Whitehouse Reef and Cedar Point Reef, Alabama, a marginally productive and productive reef, respectively. Monthly oyster samples were obtained from June – September, 2000 in order to coincide with normal seasonal peaks of disease occurrence. Results showed that "dermo" was not a threat to oysters at Whitehouse Reef (mean prevalence: 8.3%, weighted incidence (WI): 0.00). However, the disease was at critical level of intensity at Cedar Point Reef, showing WI for the sampling period of 1.61, and mean prevalence 94.0%. Temperature was conducive to "dermo" proliferation throughout the study period. Further analysis of "dermo" and water quality at these two locations is recommended.

INTRODUCTION

Perkinsus marinus, the etiological agent that causes the disease commonly known as "dermo", is a warm season protozoan parasite (Phylum Apicomplexa) and is responsible for substantial eastern oyster (Crassostrea virginica) mortalities from Chesapeake Bay to the southwest Gulf of Mexico (Andrews and Hewatt 1957; Mackin 1962; Quick and Mackin 1971).

Disease history & taxonomy

Perkinsus marinus was first discovered in Louisiana in the late 1940's and shortly thereafter in Chesapeake Bay (Andrews 1988, 1996; Ray 1996). Its discovery was the result of a research effort to determine the cause of substantial oyster mortalities that were thought to have been the result of oil pollution (Mackin *et al.* 1950; Mackin and Hopkins 1961; Andrews and Ray 1988; Ray 1996; Soniat 1996). Scientists were hired by the oil company to investigate the effect of oil pollution on oysters after substantial lawsuits were filed against the oil company by oystermen. Scientists concluded that oil pollution was not the cause of oyster mortalities, but associated mortalities with certain temporal and environmental parameters (Ray 1996). Mackin, Owen, and Collier discovered the presence of what was considered a fungus and classified it as Dermocystidium marinum (Ford 1996). However, several unique characteristics cast doubt on the classification of D. marinum (Mackin *et al.* 1950). Mackin *et al.* (1950)

noted several differences between D. marinum and the closest congeneric species, Dermocystidium salmonis, such as differences in the vacuoplast and reproduction, and incongruous host and environmental preferences. Mackin (1952) noted that there was no production of a mycelium in D. marinum. Although studied in depth by researchers from Chesapeake Bay to the Gulf of Mexico in both natural and laboratory environments, the organism remained classified as D. marinum until Mackin and Ray (1966) renamed it Labyrinthomyxa marina in 1966. After they observed similar development, cytology, and amoeba-like plasmodia as other Labyrinthomyxa, Mackin and Ray (1966) believed that the parasite should belong in the group of fungi classified as Labyrinthulales. In 1976, Perkins (1976) placed "dermo" in the phylum Apicomplexa due to the presence of an apical complex. L. marina remained classified in the phylum Apicomplexa until 1978 when Levine (1978) showed that zoospores of "dermo" were dissimilar to any other Apicomplexan. Consequently, "dermo" was reclassified as Perkinsus marinus and has remained as such ever since. It's classification as an Apicomplexan is under scrutiny because of several morphological and life cycle characteristics suggesting a relation to flagellates. Molecular sequence data and phylogenetic analysis also suggest it's common ancestry with dinoflagellates (Burreson and Ragone Calvo 1996).

Although the parasite was first identified in the late 1940's, many suspicious oyster die-offs occurred throughout the early 1900's (Hewatt & Andrews 1954). Ray (1996) noted that Owen found "dermo" cells in preserved oyster specimens obtained from Louisiana and displayed at the Chicago World's Fair around 1920. It was difficult to ascertain the cause of early mortalities because of passive grow-out techniques during

which oysters remained on reefs and were not regularly monitored. Tray culture techniques allowed closer observation of oysters and aided in determining timing and subsequent cause of periodic mortalities (Mackin 1951, 1952; Hewatt and Andrews 1954). Before tray culture techniques, mortalities were not observed until harvest in fall, at which point the timing and causes of mortalities could not be accurately determined (Hewatt & Andrews 1954).

Disease distribution

In the Americas, "dermo" is distributed from Tabasco, Mexico to the Damariscotta River, Maine (Burreson *et al.* 1994; Ford 1996). Before it was correctly identified, oyster mortalities in Tabasco were ascribed to oil refinery activities by local oystermen, as they were in Louisiana (Burreson *et al.* 1994). Andrews (1996) noted P. marinus is present in southeast Asia and speculated it was introduced to the Americas by ships during World War II. However, he also noted there were oyster mortalities reported in Virginia prior to 1940. At the time of its discovery in Chesapeake Bay, "dermo" was widespread but absent in the James River, an important oyster culture area for Virginia, in the lower Bay. During the drought of the 1980's the disease spread into other areas including the James River (Andrews 1996). Due to its tolerance of low salinity, it has remained throughout Chesapeake Bay ever since (Burreson and Ragone Calvo 1996). Soniat (1996) speculated P. marinus occurs in all mesohaline estuaries of the Gulf of Mexico, from Mexico to the Everglades of Florida.

Effect on oysters

Oyster infection primarily occurs via the ingestion of waterborne "dermo" cells (Mackin 1952; Ray 1954a). These cells are usually derived from a highly infected "gaper" (moribund oyster that is unable to close it's valves) or a dead oyster that is disintegrating or being scavenged upon (Andrews 1987; Soniat 1996). Consequently, infection begins in the digestive tract and cells may be found throughout the stomach and rectal region (Mackin 1951). Parasitic cells are spread throughout the oyster body via hemolymph sinuses, veins, and arteries (Mackin 1951; Andrews 1987). The parasite lyses oyster cells, and has special affinity for connective tissue, adductor muscle, digestive epithelium, and blood vessels. Eventually, tissues will be composed primarily of "dermo" cells (Mackin 1951; Andrews 1987). The most probable causes of oyster mortality include failure of the circulatory system, blockage of blood sinuses, and lysis of organ tissues (Mackin 1951; Ray 1954a; Andrews and Hewatt 1957; Andrews 1988).

Oyster defense against "dermo" involves leukocytosis of parasitic cells and migration of phagocytic cells to the location of "dermo" cells. This results in inflammation of the infected region. Abscesses may occur due to the parasite focusing on one region and reproducing therein. Parasitic cells are transported to areas of elimination, including epithelial layers, by oyster hemocytes (Ragone Calvo and Burreson 1994).

Observable effects of the disease on oysters include reduced growth and fecundity, emaciated and discolored meat, and increased mortality (Mackin 1962; Andrews 1987; Choi *et al.* 1989).

Diagnosis of disease prevalence and intensity

Shortly after the parasite was discovered, Ray (1952) devised a culture technique for the diagnosis of P. marinus. The technique is simple and results may be viewed relatively quickly. Ray's technique utilizes Ray's Fluid Thioglycollate Medium (RFTM), which facilitates visualization of "dermo" cells by causing cells to expand during incubation (Ray 1952; Mackin 1962). Following incubation, cells are stained with Lugol's iodine and counted using a microscope. "Dermo" cells do not reproduce during the RFTM incubation period (Ray 1954a).

Several methods may be utilized to evaluate the level of "dermo" infection in oysters. These include whole body burden, tissue (usually mantle or rectal tissue) assay, and hemolymph assay (Gauthier and Fisher 1990). All techniques but the latter require the oyster be sacrificed, therefore, the hemolymph assay is useful in evaluating disease intensity over time. The body burden assay is the most comprehensive technique, while the hemolymph assay is more sensitive than the tissue assay (Bushek *et al.* 1994). However, due to time and financial constraints the tissue assay technique has become the management standard in evaluating "dermo" disease (Bushek *et al.* 1994).

Mackin (1962) devised a scale for classification of infection intensity, ranging from negative (0) to heavy (5). A light infection usually has little, if any, effect on the host and can be found in the gut epithelium. A moderate infection produces a sub-lethal disease condition in the host (Ray 1954a). A heavy infection involves invasion of all host tissues and some regions incur massive infections, usually resulting in death (Mackin 1951, 1962). Depending upon the technique utilized to evaluate the prevalence and

intensity of infection in oysters, a numerical equation is used to derive a Mackin scale number for each case. A weighted incidence (WI) is then used to evaluate the intensity of infection in an oyster population. The weighted incidence is derived from the sum of Mackin scale intensity ratings divided by the number of oysters evaluated. A WI of 0.5 represents light infections with little or no mortality, a WI of 1.0 represents light infections with some possibly severe cases resulting in mortality, a WI of 1.5 or higher means most oysters are infected, and a WI of 2.0 represents an intense epizootic in which all individuals in the population are likely infected and a majority of the population may be in advanced stages of infection (Mackin 1962). Soniat and Kortright (1998) describe a WI of 1.5 as the critical level of parasitism since a WI of 2.0 represents an intense epizootic. Prevalence of disease is calculated as the ratio of infected oysters to total oysters sampled and is reported as a percentage.

Transmission of disease

The primary mode of transmission is horizontal (oyster to oyster) transmission of infective cells via gapers or decomposing oysters (Ray 1954a, b; Mackin 1962; Andrews 1988). The efficacy of this mode was proven in both feeding and proximity challenges. Feeding challenges involved feeding oysters macerated meats of gapers or highly infected oysters. Proximity experiments, completed in both laboratory and natural environments, involved placing uninfected oysters adjacent to infected oysters either in aquaria or in trays in waterways. Both types of challenge resulted in infection of nearly all naïve oysters, although mortalities occurred more rapidly in feeding experiments, and

proximity of infected to uninfected oysters affected rate of infection (Ray 1954a, b; Hewatt and Andrews 1956; Andrews and Hewatt 1957). Proximity studies showed that isolation of oyster beds is a viable management option in reducing the occurrence of epizootics.

Due to the large number of "dermo" cells required to induce infection, close proximity of infected and uninfected oysters is an important factor in disease transmission (Mackin 1962; Andrews 1988). Mackin (1962) found a dose of 500 prezoosporangia were required to induce infection in oysters whereas Andrews (1988) reported that 10^5 zoospores were required to induce infection. However, continuous small dosages of "dermo" cells may also induce infection over time (Andrews 1988).

Oyster to oyster transmission is further exacerbated by the action of predators and scavengers such as blue crabs (Callinectes sapidus), xanthid crabs (Family Xanthidae), polychaetes (Family Nereidae), and various fishes (Soniat 1996). Live Perkinsus cells have been found in the intestinal tracts of predators and scavengers, including oyster drills (Urosalpinx cinerea) and various fishes; and on the exoskeleton of xanthid crabs (Soniat 1996). White *et al.* (1987) found parasitism by Boonea impressa, an ectoparasitic snail, was capable of transmitting "dermo" between oysters and increasing infection intensity. Eckmayer (1979) reported that leeches (Stylochus sp.) were also a potential disease vector.

Environmental parameters and seasonality

"Dermo" is a warm water disease and is strongly influenced by temperature and salinity. Perkinsus marinus reproduction occurs at temperatures ≥ 20 °C and most rapidly between 25 – 30 °C (Andrews 1988, Brousseau 1996). Oyster infection decreases, or may be expelled completely, at temperatures ≤ 20 °C due to lack of parasite reproduction and expulsion of the parasite via combative action of oyster cells (Andrews 1988; Ragone Calvo and Burreson 1994). Salinity required for replication of "dermo" cells in oysters is 12 – 15‰, development slows in salinities ≤ 10‰, and the parasite can tolerate salinities below 5‰ (Andrews and Hewatt 1957; Andrews 1988; Andrews and Ray 1988). As a result of these environmental requirements, the parasite exhibits distinct seasonality, especially in regions where there are cold winters and relatively short warm seasons, such as Chesapeake Bay and Long Island Sound (Brousseau 1996). Disease prevalence and intensity peak shortly after the warmest periods of summer (Ragone Calvo and Burreson 1994). This peak is followed by an abrupt decline during winter and spring when water temperatures drop below 20 °C. The cycle repeats when spring and summer temperatures exceed 20 °C and mortalities begin to occur 4 – 5 weeks after infection (Andrews 1988, 1996; Ragone Calvo and Burreson 1994).

In the Gulf of Mexico, seasonality of "dermo" is less distinct due to warmer winters and longer, warm summers. In the Gulf, winter temperatures often remain high enough such that the parasite persists but remains relatively dormant, resulting in decreased infection prevalence and intensity (Andrews 1988, 1996). However, the warm season cycle of infection intensity and mortality begins sooner and continues longer,

resulting in greater mortalities in the Gulf (Andrews 1988). The level of over-wintering infections is related to the level of warm season infections and subsequent mortalities (Andrews 1996). Elimination of the disease from mesohaline estuaries will be extremely difficult, if not impossible, given present trends resulting in high warm season mortality and cold season temperatures that allow the disease to persist (Andrews 1988).

Management/control strategies

Early harvest is one of the primary management strategies in reducing mortalities caused by "dermo". Texas reduced the minimum harvest size from 89 mm to 76 mm in an effort to reduce mortalities caused by the disease (Andrews and Hewatt 1988). Gulf oysters have an advantage over "dermo" as their quick growth and high recruitment rates allow them to be harvested before the disease reaches critical levels of intensity (Soniat 1996). Oysters less than two years old showed less susceptibility to disease than did older oysters (Hewatt and Andrews 1954). Ray (1954a) also noted that young oysters were less susceptible than older oysters. Andrews and Hewatt (1957) found that young oysters were easily infected when fed minced oyster tissue derived from heavily infected gapers. This experiment revealed that young oysters were not necessarily more resistant to disease but suggested that perhaps there were mechanical reasons that they were less infected in the wild. Since the amount of water pumped by an oyster is proportional to its size, young oysters may not accumulate enough spore infected water via pumping, thus are unable to contract an infectious dose of spores (Andrews and Hewatt 1957). This

hypothesis assumed that a certain number of spores were required for an oyster to become infected.

Other management strategies include transplanting oysters to low salinity waters, avoiding the use of infected seed, and diverting fresh water to oyster beds. Perkinsus marinus tolerates salinities below 12 – 15‰ and development slows at salinities below 10‰ (Andrews and Ray 1988). Andrews and Ray (1988) suggested an average salinity of 15‰ is sufficient to control parasitism by P. marinus. Fallowing oyster beds during wet or normal years could possibly eliminate "dermo" (Andrews 1988). Due to localization of disease transmission, isolating new oyster beds from infected areas and establishing seed oysters in low salinity areas are also viable management options (Andrews 1988). P. marinus requires dense populations to cause epizootics, therefore, it will thrive in areas where oyster densities are high and recruitment of oysters occurs. The disease is not efficient at spreading between oyster growing areas but the spread of "dermo" is facilitated by transplanting infected oysters (Andrews and Ray 1988).

Soniat (personal communication, Nicholls State University) devised a monitoring system to aid oyster resource managers in determining the time to critical level of disease. This system, called Dermowatch, is an internet resource (www.blueblee.com/dermo) that allows managers to input certain environmental parameters, such as temperature and salinity, for their location. With a known weighted incidence of disease at their locale, Soniat can determine how long oysters can remain in such an environment without serious threat of mortality due to P. marinus parasitism. This service can aid in determining which management strategies to utilize (Soniat and

Kortright 1998). Brousseau and Baglivo (2000) developed a temperature-disease course model that allows oyster managers and growers to predict intensity of disease based on temperature patterns. Based on data from Long Island Sound, their model assumes temperature is the main factor regulating "dermo". The model showed a lag time of 53 days illustrated the strongest relationship between temperature and disease intensity. If temperature patterns are known, this model allows managers and growers to predict intensity of disease and plan management strategies accordingly.

MATERIALS AND METHODS

Oyster samples (n = 17 – 30) were collected monthly from June to September, 2000 from Whitehouse Reef in Mobile Bay and Cedar Point Reef in Mississippi Sound, Alabama (Fig. 1, Chapter 1). Oysters were obtained using SCUBA. Only oysters appearing healthy (no gapers) were sampled. Oysters were processed using the hemolymph assay technique (Gauthier and Fisher 1990). Shell height of oysters was measured to the nearest 0.1 mm with a digital caliper, for August and September samples. Water temperature data was obtained from a Hydrolab® located in Bon Secour Bay. Water temperatures in Alabama estuarine waters have been reported to remain relatively consistent throughout, showing a north-south variation of approximately 1 °C and no east-west variation (Lee 1979). Adequate salinity data at the two sites was not available. Unlike temperature, salinity does not remain consistent throughout the Bay (Lee 1979), varying as much as 8.5‰ within a 24-hour period at one site (personal observation). In order to provide evidence that Whitehouse Reef is a potential salinity refuge from Cedar Point Reef, salinity samples were taken once monthly during August and September at the two sites.

Oysters were kept on ice after collection and subsequently allowed to warm to room temperature over several hours before processing. Oysters were notched near the adductor muscle using a power grinder. Hemolymph samples were drawn from the

adductor muscle with a 3-mL syringe equipped with a 20-gauge, 1 – 1½-inch needle. The sample was placed in a 1.5-mL Eppendorf tube and centrifuged at 5000 RPM for 6 minutes to pellet hypnospores. Serum was removed by decanting and 1.0 mL RFTM was added and mixed in the tube, followed by the addition of 50 μL mycostatin to the top of the medium to prevent fungal growth during incubation. The sample was incubated in the dark at room temperature for 5 – 7 days.

Following incubation, samples were centrifuged at 5000 RPM for 6 minutes and the RFTM removed by decanting. One mL NaOH (2M) was added to the tube and allowed to remain for 2 hours. The sample was centrifuged at 5000 RPM for 6 minutes, NaOH was removed by decanting, and the sample washed in distilled water twice. The pellet was resuspended in 1.0 mL of a 1:6 solution Lugol's iodine to stain cells. Each sample was placed in a well in a tissue culture plate, diluted to a countable number and counted using an inverted microscope at 100X magnification. The number of hypnospores/mL of hemolymph was then calculated and translated to the Mackin scale using the equation: cells/mL hemolymph = $42.5(10^{0.68468x})$, where x is the Mackin scale value, ranging from 0 – 5 as discussed above (Gauthier and Fisher 1990).

RESULTS AND DISCUSSION

The equation used to calculate the Mackin scale value resulted in negative values when number of "dermo" cells observed was low. Negative values obtained with the hemolymph assay would have resulted in zero values if the tissue assay were used (personal communication, D. Bushek, University of South Carolina). These negative values were calculated as zeros when computing WI, but all samples with "dermo" cells present were counted as positive when calculating prevalence.

"Dermo" was not a threat to the oyster resource at Whitehouse Reef. Disease prevalence at Whitehouse Reef was 3.3% (n = 30), 6.7% (n = 30), 0.0% (n = 17) and 23.3% (n = 21) for June, July, August, and September sampling periods, respectively (Fig. 1). Intensity of infection at Whitehouse Reef was zero in all but one sample in September, when the sample resulted in an intensity value of 0.29 on the Mackin scale. "Dermo" was prevalent at Cedar Point Reef over the entire study period, with a mean of 94.0% (Fig. 1). Weighted incidence of disease at Cedar Point Reef was 1.52, 1.47, 1.61, and 1.83 for June, July, August and September sampling periods, respectively (Fig. 2). The mean WI for the study at Cedar Point Reef was 1.61. These WI levels indicated a "critical level" of parasitism, according to Soniat and Kortright (1998).

Temperature remained above minimum (20 °C) for P. marinus reproduction throughout the study period (Fig. 3). Salinity data from August showed a 2.2‰

difference between sites with lower salinity at Whitehouse Reef (20.3‰ vs. 22.5‰). However, Whitehouse Reef (25.4‰) had higher salinity than Cedar Point Reef (23.6‰) in September. Salinities were consistently above the lower limit (12‰) for "dermo" replication.

Using size as a surrogate parameter for age (Soniat 1996), it is important to note the oysters sampled at Whitehouse Reef were younger than those at Cedar Point Reef. Shell height of oysters was recorded for August and September samples. Mean height of oysters sampled were 51.2 mm and 48.1 mm at Whitehouse Reef, and 87.5 mm and 85.6 mm at Cedar Point Reef during August and September sampling periods, respectively (Fig. 4). Given historical growth records it can be inferred that oysters obtained from Whitehouse Reef were yearlings while those obtained from Cedar Point Reef were in their second year of growth (Saoud 2000). Lack of prevalence and intensity of "dermo" at Whitehouse Reef may be explained by evidence that oysters generally do not exhibit infection until their second year (Ray 1954a, Andrews 1988), rather than the previous suggestion that Whitehouse Reef is a low salinity refuge from the lower estuary.

Previous studies of "dermo" prevalence and intensity in Alabama yielded varied results. Mackin (1962) noted that Alabama samples showed "very heavy weighted incidences in several reefs in the lower western side of the bay" although no figures were reported. Beckert *et al.* (1972) presented the first documentation of "dermo" in Alabama. They obtained samples of 20 oysters (≥ 63.5 mm shell height) monthly and analyzed tissues for "dermo" using Ray's FTM. Their results showed a WI of 1.89 and prevalence

of infection of 72.7% at Cedar Point Reef, and a WI of 0.31 and prevalence of infection of 28.6% at Whitehouse Reef over a 17-month study period.

During a study of "dermo" prevalence and intensity over 49 sites in the Gulf of Mexico, Craig *et al.* (1989) found that the lowest levels of infection and prevalence occurred in the north central and northeastern Gulf, including Alabama. Their results from Cedar Point Reef showed prevalence to be 83% with a median intensity of 0.33, which was categorized as a "very light" intensity of infection. However, it is important to note that their sampling occurred from January to March, when the mean water temperature was 16 °C and mean salinity was 15‰. As noted above, disease prevalence and intensity declines during cold months when temperature drops below the critical level of 20 °C. Furthermore, Andrews and Hewatt (1988) suggested a mean salinity of 15‰ was sufficient in controlling parasitism by P. marinus. Of the 49 sites studied by Craig *et al.* (1989) in the Gulf, none were free of the parasite. Bruni (1996) conducted a 12-month study of prevalence and intensity of "dermo" infection in cultured oysters in Bon Secour Bay, Alabama. He reported that mean monthly prevalence was consistently above 80% and monthly weighted intensities of infection were consistently below 1.5, with the exception of one monthly sample (May, 1995) which had a WI of 2.4.

The environmental parameters in Alabama in the present study were indicative of drought conditions, which have persisted in the region for the past several years (personal communication, F. Scott Rikard, AUMERC). Such conditions would tend to increase both prevalence and intensity of disease.

Continued year-round studies of prevalence and intensity of "dermo" are recommended to evaluate the effect of the parasite on local populations and over varying environmental and hydrographic conditions. Since oyster height and age differed between sites in the present study, oysters of similar size class should be sampled from reefs. The tissue assay technique should be utilized in order to more accurately compare data to other published reports. Water quality should be constantly monitored at all sites. A comprehensive survey of oystermen of Alabama to learn their observations of the effect of "dermo" on oyster populations may be of interest, especially in lieu of recent drought conditions.

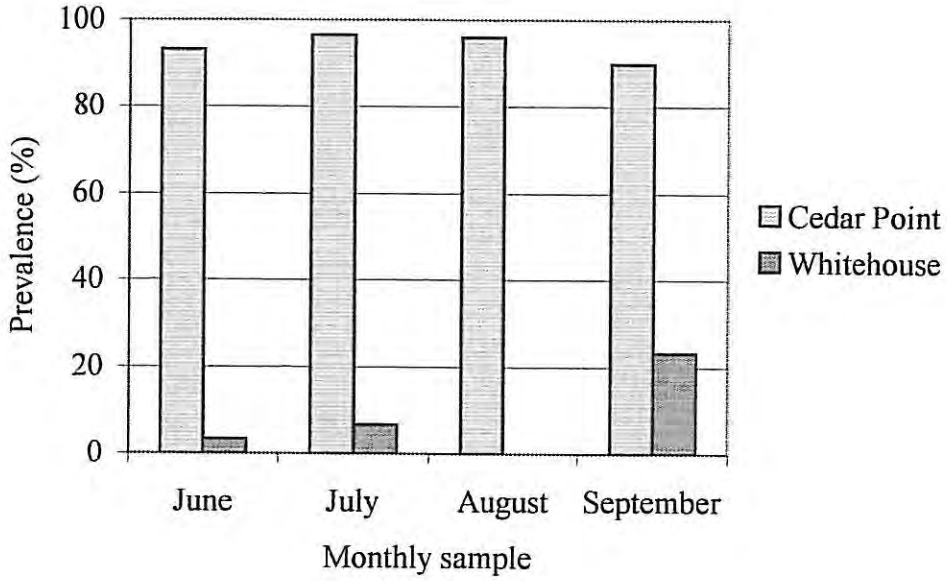

Figure 1. Prevalence (%) of "dermo" infection at Whitehouse Reef and Cedar Point Reef, June through September, 2000 sampling periods.

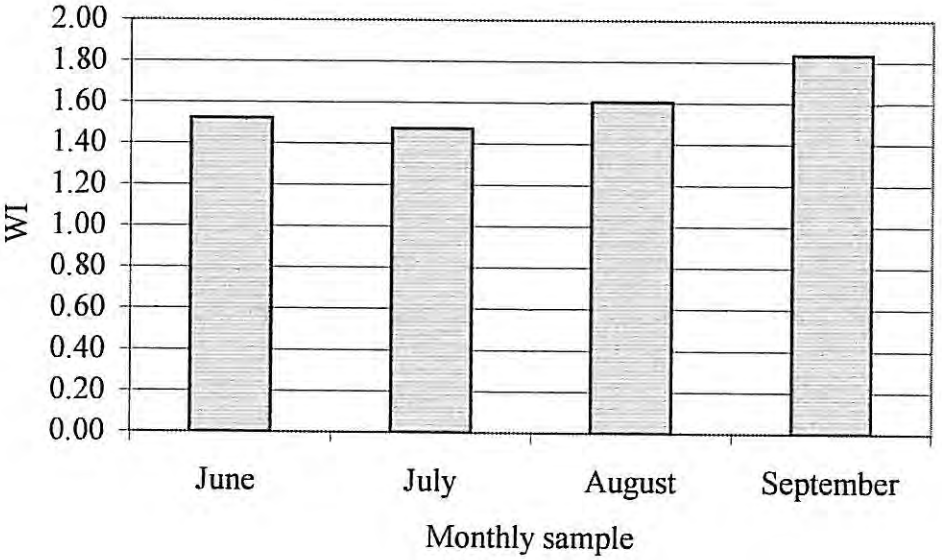

Figure 2. Weighted incidence (WI) of "dermo" infection at Cedar Point Reef, June through September, 2000 sampling periods.

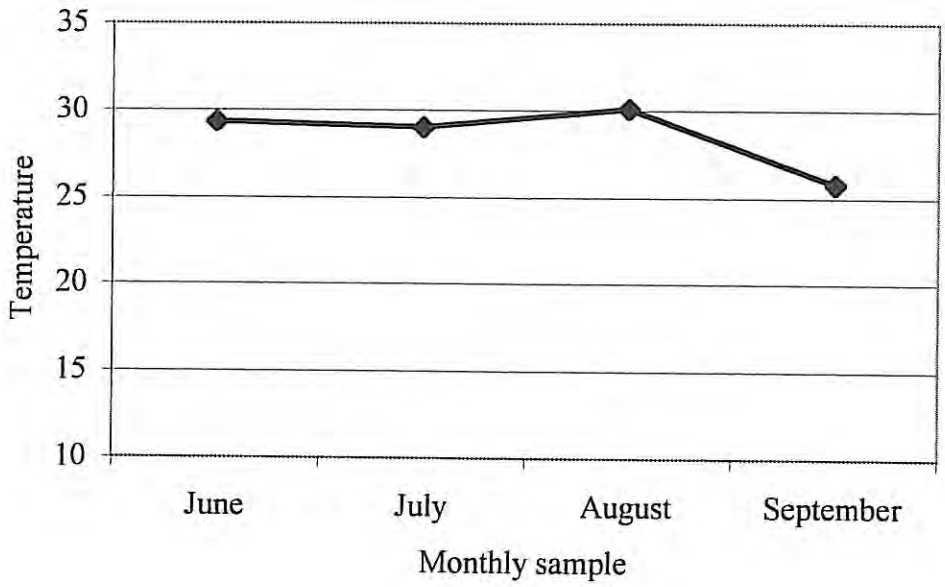

Figure 3. Water temperature (°C) at Bon Secour Bay, Alabama, June through September, 2000 sampling periods.

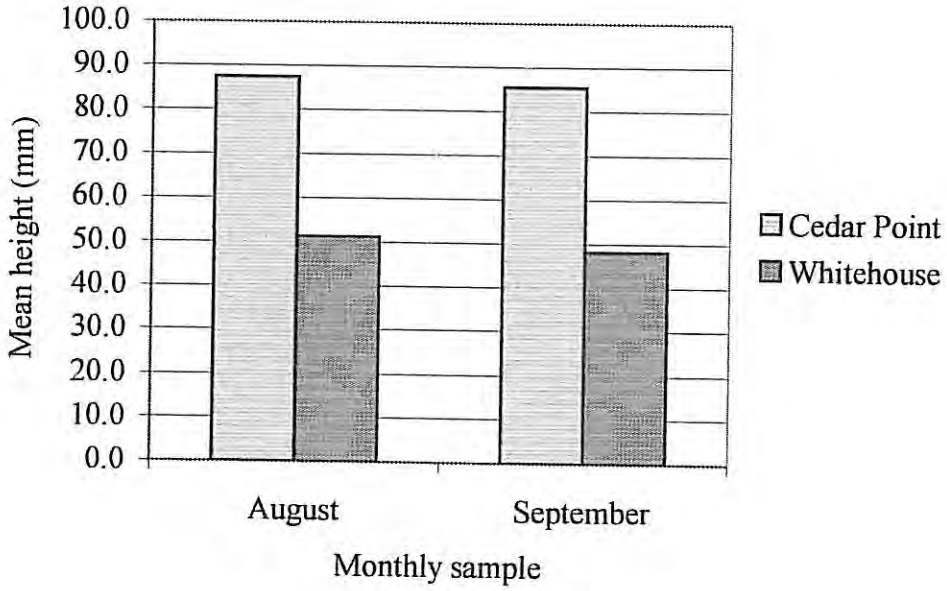

Figure 4. Mean height (mm) of oysters sampled for "dermo" from Whitehouse Reef and Cedar Point Reef, August and September, 2000.

LITERATURE CITED

Alabama Department of Conservation. 1943. Oyster replanting program planned. Alabama Conservation 15(3):5, 15.

Alabama Department of Conservation. 1944. Shells go back to the reefs. Alabama Conservation 15(12):3, 14.

Alabama Department of Conservation. 1945. It's up to the oysters now. Alabama Conservation 16(7):3.

Alabama Department of Conservation. 1947. Rehabilitation of oyster reefs planned. Alabama Conservation 18(10):6.

Alabama Department of Conservation. 1948. 40,000 barrels seed oysters already planted, department's reef building program continues. Alabama Conservation 19(12):7, 10.

Alabama Marine Resources Division. 2000. Alabama commercial/recreational oyster harvest information. State of Alabama Dept. of Conservation and Natural Resources

Andrews, J. D. 1982. Anaerobic mortalities of oysters in Virginia caused by low salinities. Journal of Shellfish Research 2(2):127-132.

Andrews, J. D. 1987. Apicomplexan disease of oysters. Pages 284-290 in C. J. Sinderman and D. V. Lightner, editor(s). Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier Science Publishers, Amsterdam, The Netherlands.

Andrews, J. D. 1988. Epizootiology of the disease caused by the oyster pathogen *Perkinsus marinus* and its effects on the oyster industry. American Fisheries Society Special Publication 18:47-63.

Andrews, J. D. 1996. History of *Perkinsus marinus*, a pathogen of oysters in Chesapeake Bay 1950-1984. Journal of Shellfish Research 15(1):13-16.

Andrews, J. D. and W. G. Hewatt. 1957. Oyster mortality studies in Virginia. II. The fungus disease caused by *Dermocystidium marinum* in oysters of Chesapeake Bay. Ecological Monographs 27:1-25.

Andrews, J. D. and S. M. Ray. 1988. Management strategies to control the disease caused by *Perkinsus marinus*. American Fisheries Society Special Publication 18:257-264.

Archer, A. F. 1947. A scientific study of conditions and effects of 1947 hurricane on oyster reefs in Alabama. Alabama Conservation 19(6):7, 12.

Archer, A. F. 1948. Alabama's oyster reefs. Alabama Conservation 19(11):8-9.

Baker, S. M. and R. Mann. 1992. Effects of hypoxia and anoxia on larval settlement,

juvenile growth, and juvenile survival of the oyster *Crassostrea virginica*. Biological Bulletin 182:265-269.

Bault, E. I. 1972. Hydrology of Alabama estuarine areas - cooperative Gulf of Mexico estuarine inventory. Alabama Marine Resources Bulletin 7:1-25.

Beckert, H., D. G. Bland and E. B. May. 1972. The incidence of *Labyrinthomyxa marina* in Alabama. Alabama Marine Resources Bulletin 8:18-24.

Berrigan, M., T. Candies, J. Cirino, R. Dugas, C. Dyer, J. Gray, T. Herrington, W. Keithly, R. Leard, J. R. Nelson and M. Van Hoose. 1991. The oyster fishery of the Gulf of Mexico, United States: A regional management plan. Gulf States Marine Fisheries Commission, Ocean Springs, Mississippi, USA.

Berrigan, M. E. 1990. Biological and economical assessment of an oyster resource development project in Apalachicola Bay, Florida. Journal of Shellfish Research 9(1):149-158.

Brodtmann Jr., N. V. 1991. Engineering and biological studies of reconstructed oyster habitat. Journal of Shellfish Research 10(2):399-403.

Brousseau, D. J. 1996. Epizootiology of the parasite, *Perkinsus marinus* (Dermo) in intertidal oyster populations from Long Island Sound. Journal of Shellfish Research 15(3):583-587.

Brousseau, D. J. and J. A. Baglivo. 2000. Modeling seasonal proliferation of the parasite, *Perkinsus marinus* (Dermo) in field populations of the oyster,

Crassostrea virginica. Journal of Shellfish Research 19(1):133-138.

Bruni, G. W. 1996. Effects of Perkinsus marinus on cultured Mobile Bay oysters (Crassostrea virginica). Master's thesis. Auburn University, Auburn, Alabama, USA.

Burreson, E. M., R. S. Alvarez, V. V. Martinez and L. A. Macedo. 1994. *Perkinsus marinus* (Apicomplexa) as a potential source of oyster *Crassostrea virginica* mortality in coastal lagoons of Tabasco, Mexico. Diseases of Aquatic Organisms 20:77-82.

Burreson, E. M. and L. M. Ragone Calvo. 1996. Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. Journal of Shellfish Research 15(1):17-34.

Bushek, D., S. E. Ford and S. K. Allen Jr. 1994. Evaluation of methods using Ray's Fluid Thioglycollate Medium for diagnosis of *Perkinsus marinus* infection in the Eastern oyster, *Crassostrea virginica*. Annual Review of Fish Diseases 4:201-217.

Butler, P. A. 1949. Gametogenesis in the oyster under conditions of depressed salinity. Biological Bulletin 96:263-269.

Choi, K. S., E. A. Wilson, D. H. Lewis, E. N. Powell and S. M. Ray. 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycollate method. Journal of Shellfish Research 8(1):125-131.

Craig, A., E. N. Powell, R. R. Fay and J. M. Brooks. 1989. Distribution of *Perkinsus marinus* in Gulf Coast oyster populations. Estuaries 12(2):82-91.

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

Dunnington Jr., E. A. 1968. Survival time of oysters after burial at various temperatures. Proceedings of the National Shellfisheries Association 58:101-103.

Eckmayer, W. J. 1979. The oyster fishery in Mobile Bay, Alabama. Symposium on the Natural Resources of the Mobile Estuary, Alabama pp. 189-200.

Engle, J. B. 1945. The condition of the natural oyster reefs and other public oyster bottoms of Alabama in 1943 with suggestions for their improvement. U.S. Department of the Interior Special Scientific Report 29:40 pp.

Engle, J. B. 1948. Investigations of the oyster reefs of Mississippi, Louisiana, and Alabama following the hurricane of September 19, 1947. U.S. Department of the Interior Special Scientific Report 59:72 pp.

Ford, S. E. 1996. Range extension by the oyster parasite *Perkinsus marinus* into the northeastern United States: response to climate change? Journal of Shellfish Research 15(1):45-56.

Galtsoff, P. S. 1964. The American Oyster *Crassostrea virginica* Gmelin. Fishery Bulletin of the Fish and Wildlife Service 64:480 pp.

Gauthier, J. D. and W. S. Fisher. 1990. Hemolymph assay for diagnosis of *Perkinsus*

marinus in oysters *Crassostrea virginica* (Gmelin, 1791). Journal of Shellfish Research 9(2):367-371.

Haywood III, E. L. and T. M. Soniat. 1992. The use of cement-stabilized gypsum as cultch for the Eastern oyster, *Crassostrea virginica* (Gmelin, 1791). Journal of Shellfish Research 11(2):417-419.

Hewatt, W. G. and J. D. Andrews. 1954. Oyster mortality studies in Virginia. I. Mortalities of oysters in trays at Gloucester Point, York River. The Texas Journal of Science 6:121-133.

Hewatt, W. G. and J. D. Andrews. 1956. Temperature control experiments on the fungus disease, *Dermocystidium marinum*, of oysters. Proceedings of the National Shellfisheries Association 466:129-133.

Hoese, H. D., W. R. Nelson and H. Beckert. 1972. Seasonal and spatial setting of fouling organisms in Mobile Bay and eastern Mississippi Sound, Alabama. Alabama Marine Resources Bulletin 8:9-17.

Ingle, R. M. 1951. Spawning and setting of oysters in relation to seasonal environmental changes. Bulletin of Marine Science of the Gulf and Caribbean 1(2):111-135.

Kennedy, V. S. 1996. Biology of larvae and spat. Pages 371-421 in V. S. Kennedy, R. I. E. Newell, and A. F. Eble, editor(s). The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant, College Park, Maryland, USA

Kennedy, V. S. and L. P. Sanford. 1999. Characteristics of relatively unexploited beds

of the Eastern oyster, *Crassostrea virginica*, and early restoration programs. Pages 25-46 in M. W. Luckenbach, R. Mann, and J. A. Wesson, editor(s). Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches. VIMS Press, Virginia Institute of Marine Science, USA.

Krantz, G. E. and J. V. Chamberlin. 1978. Blue crab predation on cultchless oyster spat. Proceedings of the National Shellfisheries Association 68:38-41.

Leard, R. L., R. Dugas, and M. Berrigan. 1999. Resource management programs for the Eastern oyster, *Crassostrea virginica*, in the U.S. Gulf of Mexico...past, present, future. Pages 63-91 in M. W. Luckenbach, R Mann, and J. A Wesson, editor(s). Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches. VIMS Press, Virginia Institute of Marine Science, USA.

Lee, C. K. 1979. Seasonal and spatial study of oyster spat in Mobile Bay and East Mississippi Sound. Master's thesis. University of Alabama, USA.

Levine, N. D. 1978. *Perkinsus* gen. n. and other new taxa in the Protozoan Phylum Apicomplexa. Journal of Parasitology 64(3):549.

Luckenbach, M. W., R. Mann, and J. A. Wesson. 1999. Introduction and overview. Pages 1-2 in M. W. Luckenbach, R. Mann, and J. A. Wesson, editor(s). Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches. VIMS Press, Virginia Institute of Marine Science, USA.

Lund, E. J. 1957. Self-silting, survival of the oyster as a closed system, and reducing

tendencies of the environment of the oyster. Publications of the Institute of Marine Science 4(2):313-319.

MacKenzie Jr., C. L. 1981. Biotic potential and environmental resistance in the American oyster (*Crassostrea virginica*) in Long Island Sound. Aquaculture 22:229-268.

MacKenzie Jr., C. L. 1989. A guide for enhancing estuarine molluscan shellfisheries. Marine Fisheries Review 51(3):1-47.

Mackin, J. G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum* Mackin, Owen, and Collier. Bulletin of Marine Science of the Gulf and Caribbean 1:72-87.

Mackin, J. G. 1952. Incidence of infection of oysters by *Dermocystidium marinum* in the Barataria Bay area of Louisiana. Proceedings of the National Shellfisheries Association pp. 22-35.

Mackin, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publications of the Institute of Marine Science 7:132-229.

Mackin, J. G. and S. H. Hopkins. 1961. Studies on oyster mortality in relation to natural environments and to oil fields in Lousiana. Publications of the Institute of Marine Science 7:1-131.

Mackin, J. G., H. M. Owen and A. Collier. 1950. Preliminary note on the occurrence of

a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). Science 111:328-329.

Mackin, J. G. and S. M. Ray. 1966. The taxonomic relationships of *Dermocystidium marinum* Mackin, Owen, and Collier. Journal of Invertebrate Pathology 8:544-545.

Marshall, N. 1954. Factors controlling the distribution of oysters in a neutral estuary. Ecology 35(3):322-327.

May, E. B. 1968. Summer oyster mortalities in Alabama. Progressive Fish-Culturist 30:99.

May, E. B. 1969. Feasibility of off bottom oyster culture in Alabama. Alabama Marine Resources Bulletin 3:1-14.

May, E. B. 1971. A survey of the oyster and oyster shell resources of Alabama. Alabama Marine Resources Bulletin 4:53.

May, E. B. 1972. The effect of floodwater on oysters in Mobile Bay. Proceedings of the National Shellfisheries Association 62:67-71.

May, E. B. 1973. Extensive oxygen depletion in Mobile Bay, Alabama. Limnology and Oceanography 18(3):353-366.

McClellan, H. A. 1965. A survey of the ectoparasites and commensals of the American oyster, Crassostrea virginica, in Mobile Bay, Alabama. Master's thesis. University of Alabama, USA.

Menzel, R. W. and S. H. Hopkins. 1955. Crabs as predators of oysters in Louisiana. Proceedings of the National Shellfisheries Association 46:177-184.

Menzel, R. W., N. C. Hulings and R. R. Hathaway. 1966. Oyster abundance in Apalachicola Bay, Florida in relation to biotic associations influenced by salinity and other factors. Gulf Research Report 2:73-96.

Moore, H. F. 1913. Condition and extent of the natural oyster beds and barren bottoms of Mississippi east of Biloxi. Dept. of Commerce, Bureau of Fisheries 774:41 pp.

Morales-Alamo, R. and R. Mann. 1990. Recruitment and growth of oysters on shell planted at four monthly intervals in the lower Potomac River, Maryland. Journal of Shellfish Research 9(1):165-172.

Ogle, J. T. and K. Beaugez. 1988. Oyster hatcheries on the Gulf Coast: history, current technology and future trends. Journal of Shellfish Research 7(3):505-509.

Osman, R. W., R. B. Whitlatch and R. N. Zajac. 1989. Effects of resident species on recruitment into a community: larval settlement versus post-settlement mortality in the oyster *Crassostrea virginica*. Marine Ecology Progress Series 54:61-73.

Perkins, F. O. 1976. Zoospores of the oyster pathogen, *Dermocystidium marinum*. I. Fine structure of the conoid and other sporozoan-like organelles. The Journal of Parasitology 62(6):959-974.

Pineda, J. and H. Caswell. 1997. Dependence of settlement rate on suitable substrate

area. Marine Biology 129:541-548.

Quayle, D. B. and Newkirk, G. F. 1989. Farming bivalve molluscs: methods for study and development. The World Aquaculture Society and The International Development Research Centre.

Quick, J. A. and J. G. Mackin. 1971. Oyster parasitism by *Labyrinthomyxa marina* in Florida. Florida Department of Natural Resources Marine Research Laboratory, Professional Papers Series 13:1-55.

Ragone Calvo, L. M. and E. M. Burreson. 1994. Characterization of overwintering infections of *Perkinsus marinus* (Apicomplexa) in Chesapeake Bay oysters. Journal of Shellfish Research 13(1):123-130.

Ray, S. M. 1952. A culture technique for the diagnosis of infections with *Dermocystidium marinum* Mackin, Owen, and Collier in oysters . Science 116:360-361.

Ray, S. M. 1954a. Biological studies of *Dermocystitium marinum*. The Rice Institute Pamphlet, Special Issue pp. 1-114.

Ray, S. M. 1954b. Experimental studies on the transmission and pathogenicity of *Dermocystidium marinum*, a fungous parasite of oysters. Journal of Parasitology 40:235.

Ray, S. M. 1996. Historical perspective on *Perkinsus marinus* disease of oysters in the Gulf of Mexico. Journal of Shellfish Research 15(1):9-11.

Saoud, I. G., D. B. Rouse, R. K. Wallace, J. E. Supan and S. Rikard. 2000. An *in situ* study on the survival and growth of *Crassostrea virginica* juveniles in Bon Secour Bay, Alabama. Journal of Shellfish Research 19(2):809-814.

Saoud, I. P. 2000. Environmental parameters affecting the restoration of an oyster reef in Mobile Bay, Alabama. PhD Dissertation. Auburn University, Auburn, Alabama, USA.

Schroeder, W. W., S. P. Dinnel and W. J. Wiseman. 1990. Salinity stratification in a river-dominated estuary. Estuaries 13(2):145-154.

Schroeder, W. W. and W. J. Wiseman. 1986. Low-frequency shelf-estuarine exchange processes in Mobile Bay and other estuarine systems on the northern Gulf of Mexico. Pages 355-367 in D. A. Wolfe, editor(s). Estuarine Variability. Academic Press, New York, USA.

Shumway, S. E. 1996. Natural Environmental Factors. Pages 467-513 in V. S. Kennedy, R. I. E. Newell, and A. F. Eble, editor(s). The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant, College Park, Maryland, USA.

Smith, L. L. 1999. Comparative assessments of three unproductive oyster reefs and a productive reef in Mobile Bay, Alabama. Master's thesis. Auburn University, Auburn, Alabama, USA.

Soniat, T. M. 1996. Epizootiology of *Perkinsus marinus* disease of Eastern oysters in the Gulf of Mexico. Journal of Shellfish Research 15(1):35-43.

Soniat, T. M., R. C. I. Broadhurst and E. L. I. Haywood. 1991. Alternatives to clamshell as cultch for oysters, and the use of gypsum for the production of cultchless oysters. Journal of Shellfish Research 10(2):405-410.

Soniat, T. M. and E. V. Kortright. 1998. Estimating time to critical levels of *Perkinsus marinus* in Eastern oysters, *Crassostrea virginica*. Journal of Shellfish Research 17(4):1071-1080.

Swingle, H. A. 1977. Coastal fishery resources of Alabama. Alabama Marine Resources Bulletin 12:31-58.

Swingle, H. A. and E. A. Hughes. 1976. A review of the oyster fishery of Alabama. Alabama Marine Resources Bulletin 11:58-72.

Tatum, W. M., M. S. Van Hoose, R. W. Havard and M. C. Clark. 1995. The 1995 atlas of major public oyster reefs of Alabama and a review of oyster management efforts 1975-1995. Alabama Marine Resources Bulletin 14:1-7.

Thompson, R. J., R. I. E. Newell, V. S. Kennedy, and R. Mann. 1996. Reproductive processes and early development. Pages 335-370 in V. S. Kennedy, R. I. E. Newell, and A. F. Eble, editor(s). The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant, College Park, Maryland, USA.

Wallace, R. K. 1993. Oysters in Alabama. Alabama Cooperative Extension System ANR-832:2 pp.

Wallace, R. K., K. Heck, and M. Van Hoose. 1999. Oyster restoration in Alabama.

Pages 101-106 in M. W. Luckenbach, R. Mann, and J. A. Wesson, editor(s). Oyster Reef Habitat Restoration: A Synopsis and Synthesis of Approaches. VIMS Press, Virginia Institute of Marine Science, USA.

White, M. E., E. N. Powell, S. M. Ray and E. A. Wilson. 1987. Host-to-host transmission of *Perkinsus marinus* in oyster (*Crassostrea virginica*) populations by the ectoparasitic snail *Boonea impressa* (Pyramidellidae). Journal of Shellfish Research 6(1):1-5.

Widdows, J., R. I. E. Newell and R. Mann. 1989. Effects of hypoxia and anoxia on survival, energy metabolism, and feeding of oyster larvae (*Crassostrea virginica*, Gmelin). Biological Bulletin 177:154-166.

Wiseman, W. J., W. W. Schroeder and S. P. Dinnel. 1988. Shelf-estuarine water exchanges between the Gulf of Mexico and Mobile Bay, Alabama. American Fisheries Society Symposium 3:1-8.