

## QUARANTINE OF OYSTER SHELL CULTCH REDUCES THE ABUNDANCE OF *PERKINSUS MARINUS*

DAVID BUSHEK,<sup>1,\*</sup> DONNIA RICHARDSON,<sup>2</sup> M. YVONNE BOBO<sup>2</sup> AND LOREN D. COEN<sup>2</sup>

<sup>1</sup>USC-Baruch Marine Field Laboratory, Georgetown, South Carolina 29442; and <sup>2</sup>South Carolina Department of Natural Resources, Marine Resources Research Institute, Charleston, South Carolina 29412

**ABSTRACT** Oyster shell is the preferred substrate for replanting oyster beds and restoring oyster reefs. If pathogens remain viable in tissues attached to shell, then planting shell may inadvertently serve as a vector for pathogen transmission. Limited local shell sources may exacerbate the problem by increasing the risk of spreading novel strains into new areas if shell is derived from other regions. In South Carolina, the primary source of oyster shell is currently the Gulf of Mexico, where the protozoan oyster pathogen *Perkinsus marinus* (Mackin et al. 1978) has been problematic. Although *P. marinus* is present in South Carolina waters, different strains exist in the two regions. Given the detrimental effects of *P. marinus* on oysters, protocols to minimize its spread via planting of shell are needed. We conducted a short-term, replicated experiment to follow changes in *P. marinus* abundance in oyster tissues by placing whole, intact oysters or shucked oysters in shell piles. The amount of oyster tissue present and parasite abundance declined precipitously after one month and was virtually eliminated by three months. Parasite persistence was dramatically longer in whole, unshucked oyster tissues as compared with those associated with shucked oysters. Viability of parasites after one month was, however, unclear. The results support the recommendation that the quarantine of shell for one month or more can dramatically reduce the potential risk of spreading *P. marinus* when planting oyster shell (=cultch) from other geographic areas. This recommendation is applicable to virtually any region, but several parameters such as effects of climatic conditions and shell pile configuration warrant further investigation as does the persistence of other pathogens on shell piles.

**KEY WORDS:** Dermo, disease transfer, oyster, *Perkinsus*, quarantine, restoration, SC, shell planting

### INTRODUCTION

It is now widely recognized that oyster reefs are valuable habitat for a wide variety of organisms (Coen et al. 1999, Luckenbach et al. 1999) and that oyster resources can be enhanced through directed oyster reef restoration programs (Coen & Luckenbach 2000). As a result, oyster reef restoration programs have expanded rapidly throughout the United States in recent years. Unfortunately, oyster shell needed to rebuild reefs is generally scarce in those areas where restoration is needed most. Alternatives to shucked oyster shell cultch include clam shell, whelk shell, coal ash by-products, fossil shell, old porcelain material, marl (sedimentary CaCO<sub>3</sub>), and similar materials (Luckenbach et al. 1999), but the reef-building material of choice is oyster cultch (Coen & Luckenbach 2000, and references therein). The most economical source of plentiful shell for restoration programs along the Atlantic coast of the United States is from the Gulf of Mexico. A major question related to the use of out-of-state shell is its potential to function as a vector of disease and non-native species, especially at a time when invasive species and new disease strains are being introduced around the globe (e.g., see Mann 1983, Naylor et al. 2001, Chapman et al. 2003, NAS 2003).

One potential risk of using oyster cultch from the Gulf of Mexico for replanting and restoring beds and reefs on the Atlantic Coast is the protozoan oyster pathogen *Perkinsus marinus*, which causes dermo disease in the Eastern oyster *Crassostrea virginica* (Gmelin, 1791). This parasite is abundant throughout the Gulf of Mexico (Soniati 1996) and along the Atlantic Coast (Burreson & Ragone Calvo 1996, Ford 1996, Bobo et al. 1997), but different strains inhabit or are predominant in different regions (Bushek & Allen 1996, Reece et al. 1997, Reece et al. 2001). The introduction of additional, possibly more virulent, parasites is a real threat to

any state's oyster populations. Therefore, the development and use of protocols that minimize the survival or persistence of *P. marinus* is warranted.

Little information exists concerning the survival of pathogens on cultch (shell) or the treatment of cultch prior to replanting to prevent the transmission of diseases. Preliminary work revealed that desiccated tissues (adductor muscle) found attached to oyster shell sampled from a large shell pile in Louisiana contained *P. marinus* parasites—putatively identified via body burden assay (Bushek et al. 1994) with Ray's fluid thioglycollate medium (RFTM) (Ray 1966). Subsequently, a sample of fresh shell (aged less than one month) on a shell pile from South Carolina contained whole intact oysters (i.e., not shucked) and dehydrated tissues as above on single valves. Large oysters (>50 mm) contained 12.7 parasites per gram, whereas smaller oysters, which were more commonly found whole and intact, contained 44 parasites per gram wet tissue (Bushek 1997, Bushek 1998). Though these parasite numbers are not particularly high, Mackin (1962) reported that 100–500 cells could initiate an infection, whereas Valiulis (1973) reported the initiation of infections with as few as ten cells. It is entirely possible that the number of parasites had already declined substantially in the oysters examined by Bushek (1997, 1998) while on the shell piles before collection, but the initial infection intensities of the oysters examined were unknown. Furthermore, there was no way to ascertain the viability of the parasites. If those parasites detected are not viable, then they pose no threat to populations where the shells are planted. On the other hand, viable parasites moved with shells planted to restore or enhance oyster populations could actually exacerbate oyster production problems simply by increasing the number of parasites available to infect oysters.

In light of these observations, we undertook an experimental study with the following four objectives: (1) to monitor the persistence or elimination of *Perkinsus marinus* surviving in tissues that remain attached to oyster shell in shell piles; (2) to compare *P.*

\*Corresponding author. E-mail: bushek@hsrl.rutgers.edu

*marinus* persistence in shucked oysters versus whole (initially live, unshucked) oysters (the worst-case scenario); (3) to provide better guidance on how to treat imported shell to protect local oyster resources; and (4) to make management recommendations as appropriate.

#### MATERIALS AND METHODS

This study applied an experimental approach using small (approximately 100 U.S. bushel) shell piles constructed by South Carolina Department of Natural Resources (SCDNR) Marine Resources Division and seeded with *Perkinsus marinus* infected oysters from the Gulf of Mexico. Ninety *P. marinus*-infected oysters (mean shell height  $\pm$  1 SD = 74.8  $\pm$  11.1 mm) were collected from Confederate Reef, West Galveston Bay, Texas, on March 19, 2002, and shipped overnight to SCDNR's Marine Resources Research Institute in Charleston, South Carolina. At the time of collection, salinity was 26 psu and surface water temperature was 25°C. Shell height and whole weight were recorded for each oyster. On March 22, 2002, 45 oysters were shucked, wet meat weight determined, and the meats divided into two equal samples. Care was taken to divide tissues equally during all dissections. One half of the soft tissues were removed for time zero sampling and the other half was returned to the valves which were then loosely held together with rubber bands to form a container for placement in experimental shell piles as described below.

Time zero samples (March 22, 2002) were further divided into three equal samples, each comprising about one-sixth of the entire oyster. One portion was processed for *P. marinus* body burden analysis using standard protocols (Bushek et al. 1994, Fisher & Oliver 1996). The second portion was processed identically, except without the RFTM incubation step for reasons outlined below. The third portion was used to determine tissue dry weight.

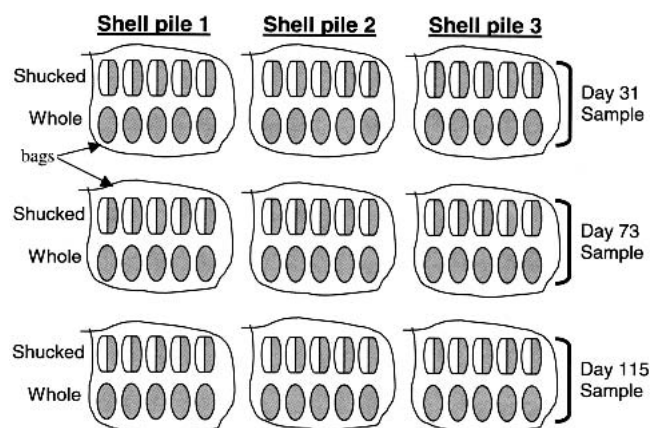
The non-RFTM incubation sample was used as a conservative validation of parasite viability. During RFTM incubation, viable *P. marinus* enlarge forming a prezoosporangium that possesses a NaOH-resistant cell wall (Choi et al. 1989) and stains blue-black with Lugol's iodine (Ray 1952). The total number of *P. marinus* cells in the entire oyster was then estimated from the number of prezoosporangia present in the sample processed for body burden analysis with RFTM incubation. We assumed that enlargement in RFTM indicated cell viability at the time of sampling. Nonviable cells do not enlarge or develop the NaOH-resistant cell wall and are therefore digested during the NaOH incubation step. It is possible, however, that some *P. marinus* parasites form prezoosporangia in moribund and decaying oyster tissues (Ray 1954, Mackin 1962), but this phenomenon is poorly documented, and the viability of such cells is unknown. By processing a portion of each oyster with RFTM and a portion without RFTM incubation, we attempted to determine the proportion of parasites that were responding to the RFTM and therefore viable. The viability of any parasites that enlarged prior to RFTM incubation could not be determined. Hence, our estimate of viability was conservative.

Three experimental piles (each roughly 100 U.S. bushels) were constructed from existing sources of recently shucked Gulf and South Carolina oyster shell. In March 2002, three replicate plastic mesh bags, each containing five whole, unshucked oysters and five of the shucked oysters processed above, were buried approximately 0.5-m deep in each shell pile. Oysters were assigned to treatments (shucked or whole) and to bags based on size to ensure an approximately equal size distribution for subsequent sampling. The total number of parasites added to each pile via shucked or

whole unshucked oysters was estimated from the body burden samples taken above. Because only half of the tissue from shucked oysters was added to shell piles, the total number of parasites added to a pile in shucked oyster tissues was calculated as half the sum of the total body burden estimates. We assumed parasite burdens were similarly distributed among whole unshucked oysters. Because whole unshucked oysters were randomly distributed within size classes, we assumed that each pile received an equivalent number of parasites in total from these oysters. StowAway temperature data loggers from Onset Corporation were deployed within the piles to monitor internal temperature logging every 30 min. A fourth data logger recorded air temperature at the site for a portion of the experiment.

One bag was selected and removed from each pile after 31, 73, and 115 days (Fig. 1) to quantify and process remaining tissues for *P. marinus* body burden as described above with the following differences. On day 31 (April 22, 2002), tissues remaining in shucked oysters were divided into two equal samples and processed for *P. marinus* body burden, one with RFTM incubation and one without as described above. These samples were considered to represent 25% of what would have remained had the entire oyster been placed in the pile. Whole oysters were shucked and tissues divided into three equal portions for RFTM body burden analysis, non-RFTM body burden analysis and dry weight determination. On day 73 (June 3, 2002), samples were processed identically to day 31 except dry weight was not determined; instead, one portion was used in an attempt to isolate and culture *P. marinus in vitro*. Parasites were purified as described by Chu and La Peyre (1993) and cultured as described by Dungan and Hamilton (1995). On day 115 (July 15, 2002), samples were processed similarly except whole oysters were divided into two samples only for RFTM and non-RFTM *P. marinus* body burden estimation. Temperature loggers were downloaded each time that oysters were sampled and then redeployed.

Data were analyzed by comparing descriptive statistics and plots of relevant parameters over time for shucked and unshucked oysters. A three-way analysis of variance (ANOVA) was used to determine if oysters differed in size among treatments (shucked or unshucked), replicate piles or bags sampled from piles. Following



**Figure 1. Schematic of the experimental design.**  $n = 90$  oysters. Forty-five oysters (partially shaded) were shucked and half of the meat removed for analysis at the start of the study. Completely shaded oysters were placed into shell piles as whole live oysters. Five whole and five shucked oysters were sampled from each pile at each sampling interval.

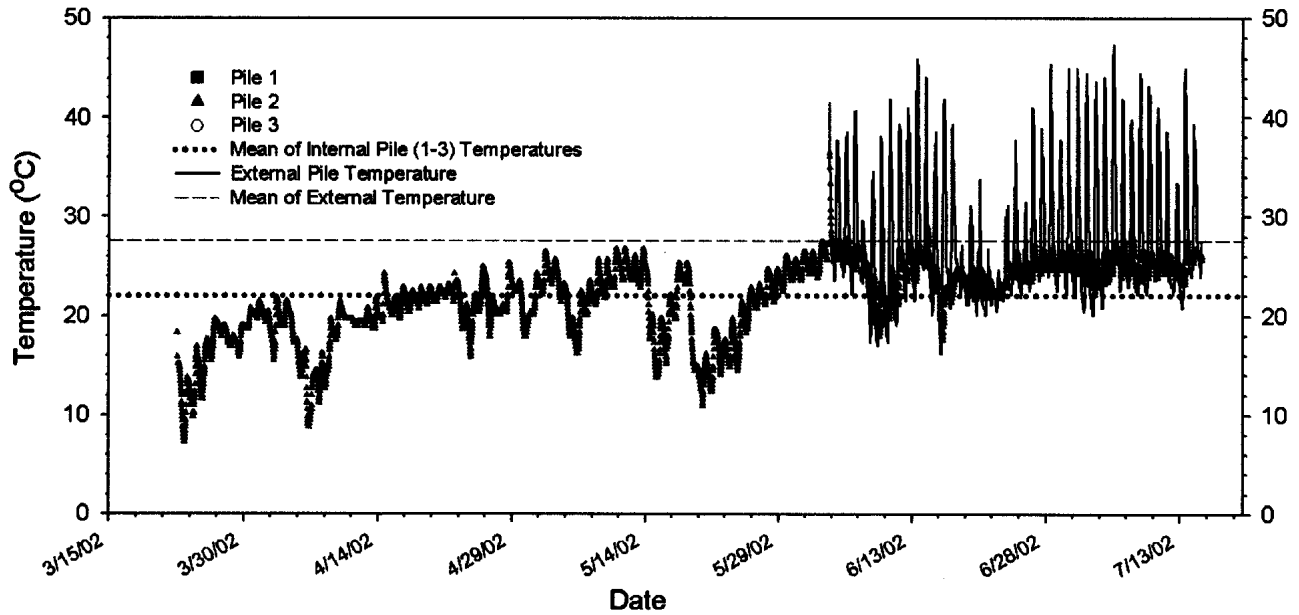


Figure 2. Internal temperatures (symbols) of three shell piles and external temperature (solid line, measured after June 2002) adjacent to the piles measured at 30-min intervals during the study period. Dotted line represents the mean internal temperature of all piles. Dashed line indicates the mean external temperature.

$\log_{10}$  transformation of RFTM counts and assignment of oysters to the various shell piles, mean total body burdens were compared among shell piles. With shell piles as replicates, one-way ANOVAs were also used to compare counts of putative *P. marinus* in RFTM and non-RFTM assays for each treatment on each date. All statistics were carried out using SYSTAT 8.0.

### RESULTS

A three-way analysis of variance indicated no significant differences among mean sizes of oysters in bags, piles, or treatments ( $P \geq 0.05$  all effects and interactions). Temperature within the piles fluctuated between 6°C and 38°C, with little difference among the piles: means were 21.95°C, 21.83°C, 21.85°C, respectively (Fig. 2). When measured concurrently during the final month of the experiment, temperatures within the three shell piles were cooler on average and less variable than surface temperature (Fig. 2). Temperatures within the piles ranged from 18°C to 37°C with a mean of 25°C, whereas temperatures on the surface ranged from 16°C to 47°C with a mean of 27.5 (Fig. 2). Fluctuations were related to both diurnal (day/night) and climatic changes (e.g., fronts) with external fluctuations always exceeding internal fluctuations. Initial (time zero) RFTM assays indicated 100% prevalence of *P. marinus*. RFTM body burden estimates of *P. marinus* abundance ranged from 180 parasites per oyster to  $27.8 \times 10^6$  parasites per oyster with a median infection intensity of 95,760 parasites per oyster. Summing RFTM estimates of total body burdens from oysters added to specific piles, and assuming that the portion of tissue added to each pile (i.e., not sampled at time zero) contained half of the total body burden, indicated that  $15.8 \times 10^6$  parasites were added to pile one,  $26.5 \times 10^6$  parasites were added to pile two, and  $14.0 \times 10^6$  parasites were added to pile three for a total of  $56.3 \times 10^6$  parasites. A one-way ANOVA on  $\log_{10}$ -transformed estimates from individual oysters indicated that mean body burdens were not significantly different among piles ( $P = 0.513$ ). Assuming a similar distribution of parasite loads in the 45 un-

shucked oysters, a total of  $112.6 \times 10^6$  *P. marinus* parasites were present at the start of the experiment in the whole unshucked oysters and about  $37.5 \times 10^6$  parasites (one-third of the total) were added to each pile in whole unshucked oysters.

Oyster tissues decomposed over time and total parasite abundance declined rapidly (Figs. 3 and 4). After 31 days, only 2 of 15 (13%) shucked oysters contained any tissue (Table 1) and the RFTM assay indicated only 216 parasites remained in tissues from these oysters. No shucked oysters contained any tissue in subsequent samples. In contrast, 12 of 15 (80%) whole oysters contained tissue after 31 days, 10 of 15 (67%) after 73 days, and 2 of 15 (13%) after 115 days (Table 1). The RFTM assay indicated *Perkinsus* abundance dropped 99% by day 31 and declined to only

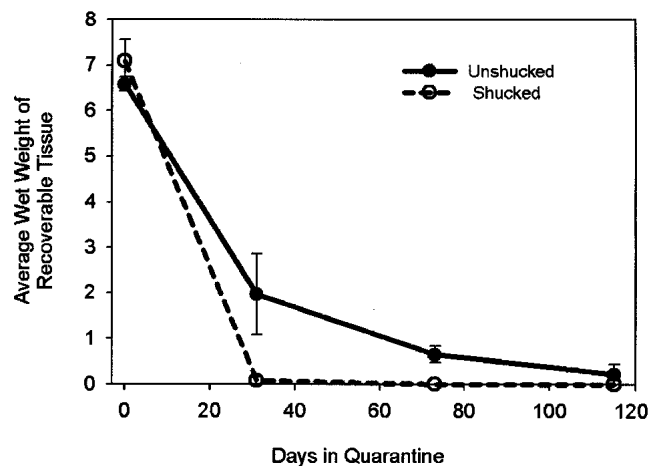


Figure 3. Change in mean wet weight (g) of recoverable tissue in shucked (dashed line) and unshucked (solid line) oysters after 0, 31, 73, and 115 days in shell piles. Error bars represent  $\pm 1$  SEM for the three experimental shell piles.

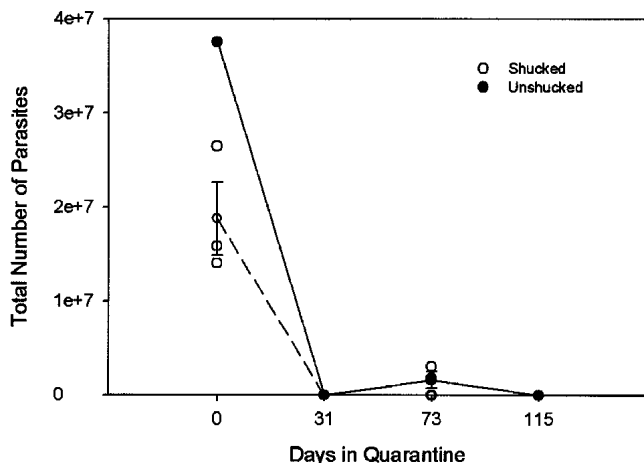


Figure 4. Total estimated number of viable *Perkinsus* cells remaining on shell piles over time in shucked (dashed line) and unshucked (solid line) oysters. Error bars represent  $\pm 1$  SEM for the three experimental shell piles.

0.005% of the original abundance by day 115 (Fig. 4). Attempts to isolate and culture *P. marinus* *in vitro* after 2 mo failed.

At time zero, the number of *P. marinus* detected in the non-RFTM assay (18,000 in all oysters) was significantly less than the number detected in the RFTM assay ( $112.6 \times 10^6$ ,  $P < 0.009$ , one-way ANOVA). In all subsequent samples, there were no significant differences between parasites enumerated in RFTM or non-RFTM assays ( $P > 0.196$  for all dates). Abundances were unexpectedly higher on day 73 compared with day 31 in both RFTM and non-RFTM assays for whole unshucked oysters due to two oysters with high numbers of apparently unenlarged (5–20  $\mu\text{m}$ ) parasites. During RFTM incubation, parasites typically enlarge to 40  $\mu\text{m}$  or more, and observers noted their uncertainty in classifying these unusually small cells as *Perkinsus* while counting them, but small size and microscopic examination was insufficient to disregard them.

## DISCUSSION

Considering that *Perkinsus marinus* continues to plague *Crassostrea virginica* throughout most of the oyster's distribution along the Gulf and Atlantic Coasts of the United States, it is only prudent to minimize the spread and proliferation of *P. marinus* whenever possible. A long-standing recommendation about planting oyster seed where *P. marinus* has been problematic is to avoid the use of *P. marinus* infected seed (Andrews & Ray 1988). Though this may seem to be simple common sense, it is not always followed and, to

TABLE 1.

Number of shucked (S) and unshucked (U) oysters with recoverable tissue remaining in shell piles over time.

	Shucked Oysters			Whole Unshucked Oysters		
	Day 31	Day 73	Day 115	Day 31	Day 73	Day 115
Pile 1	1	0	0	4	3	1
Pile 2	0	0	0	4	5	1
Pile 3	1	0	0	4	2	0
Total	2	0	0	12	10	2

our knowledge, has never been incorporated into the hygiene of handling cultch before it is planted to collect oyster spat. This study demonstrated that *P. marinus* abundances can be high on fresh cultch, but may decline rapidly (within 1–3 mo) in shell piles. Thus, short-term (1–3 mo) shell pile quarantine may reduce or even eliminate the potential reintroduction and subsequent spread of *P. marinus* when shell is planted to replenish, rebuild, or restore oyster reefs and beds. Nonetheless, several parameters need further exploration.

Not surprisingly, tissue decomposition and parasite decline were faster in shucked animals, most likely because the whole unshucked oysters remained alive for some period in the shell piles. Therefore, whole oysters represent the "worst case scenario" and should be used to establish minimum quarantine duration. Interestingly, except for time zero, the non-RFTM assay revealed concentrations of putative *P. marinus* throughout the study similar to those in the RFTM assay. The lack of significant differences between these samples indicates that the parasites counted in the RFTM assay had likely enlarged in the decomposing tissues prior to RFTM incubation. Unfortunately, the viability of these parasites could not be determined. It is generally assumed that only *Perkinsus* species respond to RFTM incubation by enlarging and developing a thick cell wall that stains blue-black with iodine and is resistant to 2 M NaOH. Furthermore, so far as we are aware, no organisms have been reported from oyster tissues that stain blue-black with Lugol's iodine other than *Perkinsus* spp. It is possible, however, that some of the cells detected with the non-RFTM assay were not *Perkinsus*. Regardless, there were several orders of magnitude fewer parasites on each sampling date after the start of the experiment.

The shell piles used were relatively small (around 100 U.S. bushels) and oysters were buried only 0.5-m deep. This size is reasonable for reef restoration programs in South Carolina (e.g., S.C.O.R.E., [www.csc.noaa.gov/scoysters](http://www.csc.noaa.gov/scoysters)), but is likely much smaller than shell piles produced by commercial shucking operations in other states and used for larger restoration efforts or to replant harvested beds. Decomposition rates are likely to decrease with the size of the shell pile, and we suspect that the abundance of *P. marinus* is closely correlated to decomposition rates of tissue as apparent in this study. It is also likely that decomposition rates are correlated with temperature. Hence, tissue decomposition on shell piles in cooler climates or during cooler portions of the year is apt to be reduced and may increase survival time of *P. marinus* in tissues on the pile.

Thus, though we recommend that shell be quarantined on land for at least a month if not longer, it is clearly apparent that a number of factors should be investigated further. These include temperature and seasonal effects, moisture in the form of humidity or rainfall, and the viability of *P. marinus* in tissues over time. Finally, quarantine impacts for other known or potential oyster pathogens and for human pathogens that are associated with oysters should be investigated similarly.

## ACKNOWLEDGMENTS

The authors thank Dr. Sammy Ray for collecting infected oysters and Jennifer Foster for assistance with RFTM and non-RFTM processing and enumeration of *Perkinsus*. Staff from the SCDNR MRD OFM assisted with shell procurement and shell pile construction. An anonymous reviewer provided comments that

improved the manuscript. Financial support was provided by SCDNR's Marine Resources Division to the Marine Resources Research Institute and by other state funding. This is contract no. 540 of the Marine Resources Research Institute, SCDNR; contri-

bution no. 1382 of the Belle W. Baruch Institute for Marine Sciences and Coastal Research, USC; and contribution no. 2004-3 of the Institute of Marine and Coastal Sciences, Rutgers, The State University of New Jersey.

## LITERATURE CITED

- Andrews, J. D. & S. M. Ray. 1988. Management strategies to control the disease caused by *Perkinsus marinus* in Disease Processes. In: W. S. Fisher (ed.), Marine Bivalve Molluscs. Bethesda, MD: American Fisheries Society Special Publication 18:257-264.
- Bobo, M. Y., D. L. Richardson, L. D. Coen & V. G. Burrell. 1997. A report on the protozoan pathogens *Perkinsus marinus* (Dermo) and *Haplosporidium nelsoni* (MSX) in South Carolina Shellfish populations, with an overview of these shellfish pathogens. SCDNR-MRD-MRRI Technical Report, Charleston, SC, 50 pp.
- Burreson, E. M. & L. M. Ragone Calvo. 1996. Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay, with emphasis on data since 1985. *J. Shellfish Res.* 15(1):109-117.
- Bushek, D. 1997. Letter to SCDNR-OFM outlining results of work on the detection and quantification of the oyster pathogen *Perkinsus marinus* in cultch material used to establish oyster reefs for recreational harvesting of examination of Gulf of Mexico oyster cultch, dated October 30, 1997.
- Bushek, D. 1998. Letter to SCDNR-OFM outlining results of examination of Gulf Oyster Cultch from restaurant pile, dated July 6, 1998.
- Bushek, D., S. E. Ford & S. K. Allen, Jr. 1994. Evaluation of methods using Ray's fluid thioglycollate medium for diagnosis of *Perkinsus marinus* infections in the eastern oyster, *Crassostrea virginica*. *Ann. Rev. Fish Dis.* 4:201-217.
- Bushek, D. & S. K. Allen, Jr. 1996. Host-parasite interactions among broadly distributed populations of the eastern oyster *Crassostrea virginica* and the protozoan *Perkinsus marinus*. *Mar. Ecol. Prog. Ser.* 139:127-141.
- Chapman, J. W., T. W. Miller & E. V. Coan. 2003. Live seafood species as recipes for invasion. *Conservation Biology* 17:1386-1395.
- Choi, K. S., E. A. Wilson, D. H. Lewis, E. N. Powell & S. M. Ray. 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: Quantification of the thioglycollate method. *J. Shellfish Res.* 8(1):125-131.
- Chu, F. L. E. & J. F. La Peyre. 1993. *Perkinsus marinus* susceptibility and defense-related activities in Eastern oysters *Crassostrea virginica* - temperature effects. *Dis. Aquatic Org.* 16(3):223-234.
- Coen, L. D., M. W. Luckenbach & D. L. Breitburg. 1999. The role of oyster reefs as essential fish habitat: a review of current knowledge and some new perspectives. In: L. R. Benaka (ed.), Fish Habitat: Essential Fish Habitat and Rehabilitation. Bethesda: American Fisheries Society, Symposium 22, pp. 438-454.
- Coen, L. D. & M. W. Luckenbach. 2000. Developing success criteria and goals for evaluating oyster reef restoration: ecological function or resource exploitation? *Ecological Engineering* 15:323-343.
- Dungan, C. F. & R. F. Hamilton. 1995. Use of a tetrazolium-based cell proliferation assay to measure effects of in vitro conditions on *Perkinsus marinus* (Apicomplexa) proliferation. *J. Eukaryot. Microbiology* 42(4):379-388.
- Fisher, W. S. & L. M. Oliver. 1996. A whole-oyster procedure for diagnosis of *Perkinsus marinus* disease using Ray's fluid thioglycollate culture medium. *J. Shellfish Res.* 15(1):109-117.
- Ford, S. E. 1996. Range extension by the oyster parasite *Perkinsus marinus* into the northeastern United States: Response to climate change? *J. Shellfish Res.* 15(1):45-56.
- Luckenbach, M. W., R. Mann & J. A. Wesson (eds.) 1999. Oyster Reef Habitat Restoration. A Synopsis and Synthesis of Approaches. Gloucester Point, VA: Virginia Institute of Marine Science Press. 358 pp.
- Mackin, J. G. 1962. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publ. Inst. Mar. Sci.* 7:133-229.
- Mann, R. 1983. The role of introduced bivalve mollusc species in mariculture. *J. World Mariculture Soc.* 14:546-559.
- NAS 2003. Non-native Oysters in the Chesapeake Bay. Washington, DC: National Academies Press. 168 pp.
- Naylor, R. L., S. L. Williams & D. R. Strong. 2001. Aquaculture: a gateway for exotic species. *Science* 294:1655-1656.
- Ray, S.M. 1952. A culture technique for the diagnosis of infections with *Dermocystidium marinum*, Mackin, Owen, Collier, in oysters. *Science* 166:360-361.
- Ray, S. M. 1954. Biological studies of *Dermocystidium marinum*, a fungus parasite of oysters. Rice Institute Pamphlet of Special Issue. 114pp.
- Ray, S. M. 1966. A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proc. Natl. Shellfisheries Assoc.* 54:55-69.
- Reece, K., J. Graves & D. Bushek. 1997. Molecular markers for population genetic analysis of *Perkinsus marinus*. *Mol. Mar. Biol. Biotech.* 6:197-206.
- Reece, K. S., D. Bushek, K. L. Hudson & J. E. Graves. 2001. Geographic distribution of *Perkinsus marinus* genetic strains along the Atlantic and Gulf coasts of the USA. *Marine Biology* 139:1047-1055.
- Soniat, T. 1996. Epizootiology of *Perkinsus marinus* disease of eastern oysters in the Gulf of Mexico. *J. Shellfish Res.* 15:35-44.
- Valiulis, G. A. 1973. Comparison of the resistance to *Labyrinthomyxa marina* with resistance to *Minchinia nelsoni* in *Crassostrea virginica*. Ph.D. Dissertation. Rutgers University, New Brunswick, NJ, USA.