CHANGES IN LEVELS OF INFECTION OF OYSTERS BY Perkinsus marinus, WITH SPECIAL REFERENCE TO THE INTERACTION OF TEMPERATURE AND SALINITY UPON PARASITISM

Since the original description as Dermocystidium marinum by Mackin, Owen and Collier (1950), many studies have been conducted on the parasite now called Perkinsus marinus (Levine, 1978). The parasite is a major cause of mortality in its oyster host, Crassostrea virginica (Gmelin) (Ray and Mackin, 1955; Andrews, 1965). Ray (1952, 1966) developed an easy and effective method for its detection that stimulated subsequent work in the field. Numerous studies (Mackin et al., 1950; Mackin 1951, 1956, 1961; Ray 1954a; Hewatt and Andrews 1955; Andrews and Hewatt, 1957; Quick and Mackin, 1971; Ogle and Flurry, 1980) have demonstrated that the parasite is more prevalent at high temperature and high salinity.

This paper documents the seasonal changes in prevalence and intensity of infection of oysters by *P. marinus*, over more than two years from a single reef in Galveston Bay, Texas. Special emphasis is placed on the quantitative relationship between intensity of infection and the interaction of water temperature and salinity.

MATERIALS AND METHODS

Twenty oysters a month were collected for 26 months (between May 1979 and September 1981) from April Fool reef (west central Galveston Bay). The length (umbo-to-bill distance) of the right valve was measured to the nearest millimeter. A small piece of mantle tissue was removed from each oyster for the ex-

amination of parasitism by *P. marinus* using the method of Ray (1966). The level of infection was scored as a disease code number (Quick and Mackin, 1971), which varies as an integer from 0 (uninfected) to 6 (heavily infected). The percent infection (a measure of prevalence) and the weighted incidence (a measure of intensity of infection) of the sample were calculated. Weighted incidence (WI) was determined, after Mackin (1961), as follows:

WI = sum of disease code numbers number of oysters

Temperature (mercury thermometer) and salinity (American Optical refractometer) were recorded at the time of the sampling.

A one-way analysis of variance (ANOVA) was conducted on the WI data. The Duncan Multiple Range Test was used to determine month-to-month differences (Nie et al., 1975). Pearson Correlation Coefficients were calculated between physical parameters and selected oyster and parasite measures. Further details on the sampling site and methods were provided by Soniat (1982).

RESULTS AND DISCUSSION

The WI values varied from 0.10 in August 1979 to 2.35 in September 1981 (Table 1; Figure 1). The ANOVA of the WI data indicated that significant month-to-month differences exist (p < 0.001); results of the Duncan Multiple Range Test are presented in Table 1. The data are variable and do not show obvious seasonal trends. This variability is indicated by the great deal of overlap among the nine groups defined by Duncan's Test. The lack of obvious seasonality is illustrated by the fact that group 1 (lowest WI values) and group 9

Table 1. Three of the nine groups delineated by the Duncan Multiple Range Test. Omitted groups overlap those shown. Months are grouped by weighted incidence (WI) values. There are no significant differences in the WI of the months within a group ($\alpha=0.05$). The WI values are based upon a sample of 20 oysters per month.

Group	Month (and WI)							
1	Aug 79 (0.10)	July 79 (0.20)	Feb 81 (0.20)	July 81 (0.25)	Mar 80 (0.35)	June 79 (0.50)	Mar 81 (0.50)	
	May 79 (0.55)	Feb 80 (0.60)	Nov 79 (0.70)	Sept 79 (0.85)	Jan 80 (0.85)	Apr 80 (1.05)		
5	M ay 79	Feb 80	Nov 79	Sept 79	Jan 80	Apr 80	Oct 79 (1.15)	
	Jan 81 (1.15)	Dec 79	Aug 81	Dec 80	May 80	Oct 80		
9	Dec 79 (1.35)	Aug 81 (1.50)	Dec 80 (1.52)	May 80 (1.55)	Oct 80 (1.55)	Sept 80 (1.60)	Nov 80 (1.80)	
	Apr 81 (1.90)	June 81 (1.90)	May 81 (2.05)	Sept 81 (2.35)				

(highest WI values) contain months representing every season of the year. Percent infection values, which ranged from 5% in August 1979 to 80% in May and June 1981, were closely associated with WI values (Figure 1).

The length of oysters used in this study ranged from 6.5 to 13.9 cm. Oysters

of roughly the same size were selected since it is known that larger (older) oysters tend to have higher levels of parasitism than smaller (younger) ones (Ray, 1954b). The lack of correlation (p > 0.05) between oyster length and WI (Table 2) in this study suggests that this variable was adequately controlled.

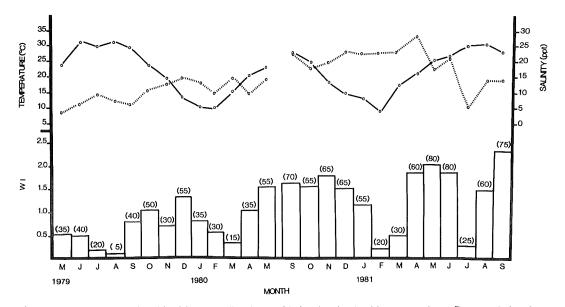


Figure 1. Monthly weighted incidence (WI) values of infection by *Perkinsus marinus*. Percent infection values are shown in parentheses above the WI bars. Each value is based upon a sample of 20 oysters. Corresponding temperature (solid line) and salinity (broken line) values are shown at the top of the graph.

It has long been known that salinity and temperature are the primary factors controlling the distribution of P. marinus (Mackin et al., 1950, Ray 1954a). Salinity ranged from 3.0 ppt in May 1979 to 28.5 ppt in April 1981 while temperature varied from 9.3°C in February 1981 to 30.5°C in August 1979 (Figure 1). In the present study a significant relationship (p < 0.01) existed between WI and salinity. Of particular interest is the association between freshets and low WI values. This association could be causal and fresh water may be decreasing the intensity and prevalence of P. marinus; however, low salinities in this study were common in the summer. An alternative explanation of the data is that late summer declines in WI values may have been caused by mortality of heavily infected individuals and the survival of less infected ones (Menzel, personal communication).

The results of this study are exactly opposite to those of Quick and Mackin (1971). They found no relationship between WI and salinity, and a significant relationship between WI and temperature. In this study WI was not significantly correlated with temperature (Table 2); however, it is important to consider the relationship between temperature and salinity, which in this study were inversely correlated (p < 0.05). Environmental conditions during the work of Quick and Mackin (1971) were similar to the present study in that Florida characteristically experiences a summer rainy season. When high

salinities were coupled with high temperatures, highest values of WI occur (Figure 1). A temperature-salinity interaction term (TxS), defined as the product of temperature and salinity, was more closely correlated with WI (p < 0.001) than was temperature or salinity alone.

Although the TxS interaction explains more of the variability in WI than temperature or salinity alone, most of the variability remains unexplained. These oysters were collected from a single reef and were presumably exposed to a similar number of infective elements. Most of the unexplained variability in the data probably resulted from individual differences in resistance to infection. Those differences might be exploited in the development of more resistant stocks of oysters.

ACKNOWLEDGMENTS

This study was made possible by support from the Texas A & M Sea Grant marine fellowship program, Texas A & M University at Galveston, and the Gulf and South Atlantic Fisheries Development Foundation, Inc. Hays Cummins, Jim Nance and J.P. Schmidt assisted in the collection of the oysters. I am especially indebted to Sammy Ray who taught me the Ray technique and helped me in innumerable ways.

LITERATURE CITED

Andrews, J.D. 1965. Infection experiments in nature with *Dermocystidium*

Table 2. Pearson Correlation Coefficients between physical parameters and oyster and parasite data. WI = weighted incidence, L = oyster length, T = temperature, S = salinity, TxS = the product of T = and S = 26.

	WI	L	T	TxS	S
WI T		- 0.38	0.14	0.70***	0.51**
	_		_	_	-0.42*

- marinum in Chesapeake Bay. Chesapeake Sci. 6:60-67.
- Hewatt, W.G., and J.D. Andrews. 1955. Temperature control experiments on the fungus disease, *Dermocystidium marinum*, of oysters. Proc. Natl. Shellfish. Assoc. 46:129-133.
- Levine, N.D. 1978. *Perkinsus* gen. n. and other new taxa in the protozoan phylum Apicomplexa. J. Parasitol. 64:549.
- Mackin, J.G. 1951. Histopathology of infection of *Crassostrea virginica* (Gmelin) by *Dermocystidium marinum*, Mackin, Owen and Collier. Bull. Mar. Sci. Gulf Caribb. 1:72-87.
- ______. 1956. Dermocystidium marinum and salinity. Proc. Natl. Shellfish. Assoc. 46:116-128.
- ______. 1961. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. Publ. Inst. Mar. Sci. Univ. Texas 7:132-299.
- ______, 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.
- Nie, N.H., C.H. Hull, J.G. Jenkins, K. Steinbrenner, and D.H. Bent. 1975. Statistical package for the social sciences. McGraw-Hill, New York. 675 p.
- Ogle, J., and K. Flurry. 1980. Occurrence and seasonality of *Perkinsus marinus* (Protozoa: Apicomplexa) in Mississippi oysters. Gulf Res. Rept. 6:423-426.
- Quick, J.A., Jr., and J.G. Mackin. 1971.

- Oyster parasitism by *Labryinthomyxa* marina in Florida. Fla. Dept. Nat. Resour. Mar. Res. Lab. Prof. Pap. Ser. No. 13:1-55.
- 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.
- ______. 1954a. Biological studies of Dermocystidium marinum a fungus parasite of oysters. Rice Inst. Pamplet Monogr. Biol. Spec. Issue. 114 p.
- rence of *Dermocystidium marinum* in young oysters. Proc. Natl. Shellfish. Assoc. 44:80-92.
- method for detecting *Dermocystidium* marinum with suggested modifications and precautions. Proc. Natl. Shellfish, Assoc. 54:55-80.
- ______, and J.G. Mackin. 1955.
 Studies of pathogenesis of *Dermocystidium marinum*. Proc. Natl. Shellfish. Assoc. 45:164-167.
- Soniat, T.M. 1982. Studies on the nutritional ecology and ecological energetics of oysters from Galveston Bay. Unpublished Ph.D. Dissertation, Texas A & M Univ., College Station, TX 162 p.

Thomas M. Soniat, Department of Biological Sciences, University of New Orleans, Lakefront, New Orleans, LA 70148.