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Environmental factors and the infectious disease caused by the protozoan parasite, Perkinsus marinus, in eastern oysters (Crassostrea virginica)

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ENVIRONMENTAL FACTORS AND THE INFECTIOUS DISEASE CAUSED BY THE PROTOZOAN PARASITE, *PERKINSUS MARINUS*, IN EASTERN OYSTERS (*CRASSOSTREA VIRGINICA*)

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Abstract

Temperature and salinity are two important factors limiting the distribution and abundance of *Perkinsus marinus*, a protozoan parasite of Eastern oysters *(Crassostrea virginica)*. Results of laboratory studies are consistent with field observations and clearly demonstrate that *P. marinus* susceptibility and disease advancement are positively correlated with temperature, salinity and *in situ* number of infective cells. Laboratory findings also suggest that environmental degradation may enhance the epizootic, although disease caused by *P. marinus* in oysters is known to be predominantly exacerbated by elevated temperature and salinity. Oysters' cellular defence mechanisms appear ineffective in defence against *P. marinus*. Also, pollutant exposure caused no significant effects on defence-related activities measured in oysters.

Introduction

Infectious diseases have devastated the natural and cultivated populations of several commercially important bivalve species. The most noticeable pathogens are protozoans from the Apicomplexa and Acetospora phyla. The protistan, *Perkinsus marinus* (Dermo), which parasitises American (eastern) oysters (*Crassostrea virginica*), is an apicomplexan in the class Perkinsasida (Levine 1988). It was originally described by Mackin, Owen, and Collier as *Dermocystidium marinum* (Mackin *et al.* 1950). Four life stages, meront (trophozoite), prezoosporangia, zoosporangia, and biflagellate zoospores have been identified and described (Perkins 1966, Perkins 1988). Immature meronts (merozoites, 2-4 µm and coccoid) are usually found in the phagosomes of oyster haemocytes. Meronts (mature merozoites,10-20 µm) have centric vacuoles and often contain a refringent vacuoplast. The mature meront (schizont, 20 to 40 µm) contains 8 to 32 cells. Prezoosporangia (hypnospores)are sometimes observed in moribund and dead oyster tissues, and can enlarge to 150µm. When tissue-associated merozoites/meronts are placed in fluid thioglycollate medium (FTM) for 4 to 5 days, they develop into prezoosporangia (hypnospores). Prezoosporangia are characterised by having a large vacuole and an eccentric nucleus adjacent to the cell wall. Zoosporulation (production of biflagellate zoospores) usually occurs after incubating thioglycollate-cultured prezoosporangia in estuarine water (20 - 22 ppt) for 4-5 days.

The distribution of *P. marinus* is widespread along the East Coast of the United States and into the Gulf of Mexico. This parasite has caused severe oyster mortality from the mid-Atlantic to the Gulf since the 1950s. Presently, *P. marinus* is the most prevalent parasite of the eastern oyster in mid-Atlantic. In this presentation, recent studies on the relationship of environmental factors and the disease caused by this parasite are discussed.

Effects of temperature and salinity on P. marinus infection

Extensive field and laboratory studies have been conducted to examine the disease processes and transmission dynamic of *P. marinus* (see reviews by Andrews 1988, Andrews and Ray 1988, Burreson and Ragone Calvo 1996, Chu 1996, Soniat 1996). Transmission of infections occurs from oyster to oyster. The three life stages, meront, prezoosporangia and biflagellate zoospore are infective (Chu 1996). The merozoite/meront stage is believed to be the primary agent for disease transmission (Perkins 1988, Chu 1996). Field observations have pointed to temperature and salinity as two important environmental factors regulating the infection and progression of *P. marinus* in oysters. In the Chesapeake Bay, Dermo disease prevalence and intensity are maximal during the summer, and increase with rising salinity (Burreson and Ragone Calvo 1996). Dermo-associated mortality usually begins in early summer (June) when water temperature rises to 20°C and peaks between August and September, when the ambient temperature reaches up to 25-28°C. Enhanced mortality also occurs during drought periods.

The relationship, documented in field studies, between *P. marinus* infection in oysters and temperature and salinity,

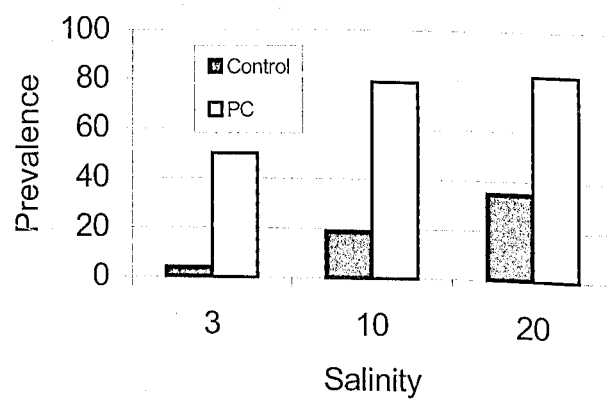

Figure 1: *Perkinsus marinus* prevalence (% of infected oysters) of oysters at 3, 10, and 20 ppt, 35 days after challenge with 10^6 meronts/oyster (N=19-24). Oysters were collected from Deep Water Shoal in the James River, Virginia (a tributary of the southern Chesapeake Bay affected by *P. marinus*). C = Control, PC = *P. marinus* challenged.

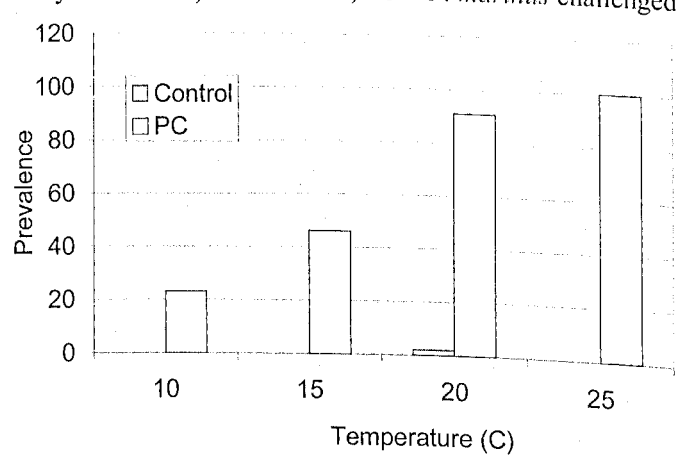

Figure 2: *Perkinsus marinus* prevalence (% of infected oysters) in oysters at 10, 15, 20, and 25°C, 46 days after challenge with 10^6 meronts/oyster (N=40). Oysters were collected from Rappahannock River, Virginia (a tributary of the southern Chesapeake Bay affected by *P. marinus*). C = Control, PC = *P. marinus* challenged.

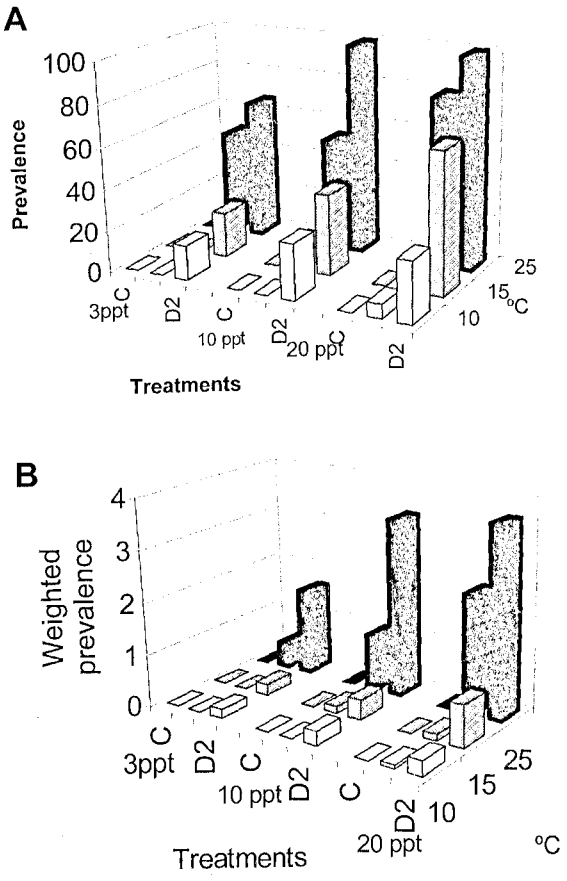

Figure 3. Prevalence (A, % of infected oysters) and intensity (B) of *Perkinsus marinus* in oysters at different temperature and salinity regimes after challenge with two different doses of *P. marinus* infective cells. C = Control, D1= 2.5 x10^3 meronts/oyster; D2 = 2.5 x10^4 meronts/oyster, N=7-15 per group at all temperature-salinity treatments, except the groups at 25C-3ppt treatment (N=3-4 per group). Weight prevalence: sum of disease intensity rank/number of oysters examined (Andrews 1988).

was reaffirmed through detailed and comprehensive laboratory studies (Chu and La Peyre 1993a, Chu and La Peyre 1993b, Chu *et al.* 1993, Ragone and Burreson 1993). The effects of temperature and salinity on *P. marinus* infectivity and pathogenicity in oysters have been examined in two separate studies (Chu and La Peyre 1993a, Chu *et al.* 1993). *P. marinus* prevalence and infection intensity in oysters was found to significantly and positively correlate with experimental temperature and salinity. Five weeks after inoculation into the shell cavity with 10^6 meronts isolated from infected tissues, the *P. marinus* prevalence (% of infected oysters) in oysters were 50, 79, and 82% at 3, 10, and 20 ppt, respectively (Figure 1). Infection intensity also rose with increasing salinity. Heavy infection was found in both 10 and 20 ppt, but not in 3 ppt. Holding infected oysters collected from the field at four different salinities (6, 9, 12 and 20 ppt) for eight weeks inhibited infection progression in oysters at salinity 12 ppt and below (Ragone and Burreson 1993). In oysters initially inoculated with 10^6 freshly isolated meronts and held at 10, 15, 20 or 25°C for 46 days *P. marinus* prevalence was 23, 46, 91, and 100% respectively (Figure 2). Infection intensity increased with temperature, and moderate and heavily infected oysters were found only at 20 and 25°C.

Unlike the other oyster protozoan parasite, *Haplosporidium nelsoni*, *P. marinus* can tolerate low temperature and salinity. Low salinity (3 and 6 ppt) inhibited intensification but did not eliminate infection (Chu *et al.* 1993, Ragone and Burreson 1993). Low winter temperatures (5°C or below) for extended time periods (6-8 weeks) did not warrant a lower summer infection prevalence and/or intensity in subsequent year (Burreson and Ragone 1996). In *in vitro*, prezoosporangia survived up to four days at 9°C (Chu and Greene 1989).

The defence-related haemocyte activities and their expression in relation to *P. marinus* infection at a range of temperature and salinity conditions have also been examined (Chu and La Peyre 1993a, Chu *et al.* 1993). Results suggest that the oysters' cellular mechanisms are ineffective in defence against *P. marinus*. Although oysters at high temperature had higher concentrations of circulating haemocytes, percentage of granulocytes and phagocytic capability, they did not have fewer or less intense *P. marinus* infections. No significant salinity effect was noted on oyster cellular activities. However, plasma lysozyme activity negatively correlated with temperature, salinity, and *P. marinus* infection.

Synergistic effects between temperature, salinity and concentration of P. marinus infective particles

In addition to temperature and salinity, the number of *P. marinus* cells that the oyster is exposed to is also critical for infection transmission. It has long been suggested that dilution of infective particles may be partially responsible for the reduced *P. marinus* prevalence and intensity observed in low salinity areas (Mackin 1962). This is probably true, since in nature, water inputs and/or fresh water runoff not only dilute the salinity, but also reduce the *in situ* concentration of *P. marinus* infective cells in estuaries. Laboratory studies revealed that a dose between 10 and 100 meronts or prezoosporangia per oyster is the minimal number required to initiate *P. marinus* infection (Chu 1996, Chu and Volety 1997). The interactions between temperature, salinity and concentration of *P. marinus* infective cells have recently been investigated by Chu and Volety (1997). They exposed oysters to 3 different doses (0, 2.5 x 10^3 or 2.5 x 10^4 per oyster) of freshly isolated meronts/merozoites at 9 salinity-

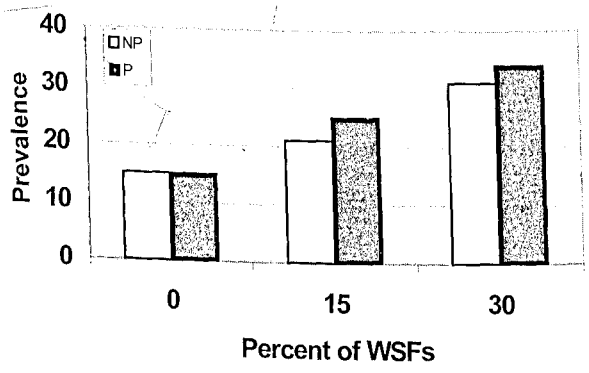

Figure 4: Prevalence (% of infected oysters) in *Perkinsus marinus* challenged (P) and non-challenged (NP) oysters after exposure to 0, 15, and 30% WSFs (N=28-33). Experimental oysters were collected from the Rappahannock River, Virginia (a tributary of the southern Chesapeake Bay affected by *P. marinus*). Oysters were exposed to WSF dilutions for 35 days, and then were challenged with *P. marinus*. WSF exposure was continued for an additional 21 days.

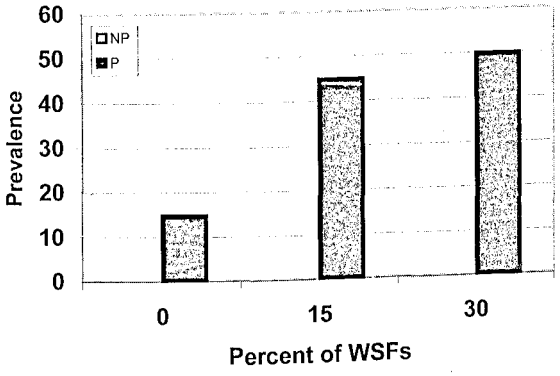

Figure 5: Prevalence (% of infected oysters) in *Perkinsus marinus* challenged (P) and non-challenged (NP) oysters after exposure to 0, 15, and 30% WSFs (N=20). Experimental oysters were obtained from outside the normal geographic range of Dermo (Damariscotta River, Maine). Oysters were exposed to various WSF dilution for 35 days, and thereafter challenged with *P. marinus*. WSF exposure was continued for an additional 35 days. No infection was detected in NP, control oysters.

temperature combinations: 10, 15, 25°C at 3, 10, and 20 ppt for 60 days. Results of statistical analysis showed temperature as the most important factor, followed respectively by the infective cell dose and salinity in determining the susceptibility to *P. marinus* and influencing subsequent disease development in oysters. Increased infection prevalence and intensity occurred at high temperature and salinity, and there was a dose-dependent response to infective particles (Figure 3). Also, temperature in combination with infective particle or with salinity significantly affected disease progression. However, no synergistic effects were noted among temperature, salinity and dosage of infective cells.

Effects of pollutants on P. marinus expression in oysters
Synergetic effects between temperature, salinity, and concentration Pollutant stress has long been suggested to impair immunofunction, leading to outbreak of infectious diseases in aquatic organisms (Sinderman 1983, 1993). Concern over the reduced water quality from pollution has prompted investigations to assess the effect of environmental pollutants on *P. marinus* infection and progression in oysters (Chu and Hale 1994, Anderson *et al.* 1996, Fisher *et al.* 1999, Chu in press). Chu and Hale (1994) investigated the effects of water soluble fractions (WSFs) derived from sediments collected from the Elizabeth River, a heavily polluted subestuary of the Chesapeake Bay. The Elizabeth River sediments were grossly contaminated with polycyclic aromatic hydrocarbons (PAHs), characteristic of creosote. The WSFs generated from the sediments were dominated by lower molecular weight aromatic hydrocarbons and heterocyclic compounds. In two experiments, they exposed oysters to different dilutions (i.e. 0, 15, 30%) of WSFs for 35 days, then challenged them with freshly isolated *P. marinus* meronts and continued the exposure to WSFs for an additional period of 21 or 33 days. Pollutant exposure was found to enhance pre-existing *P. marinus* infections in oysters from an area affected by *P. marinus*, and to increase the susceptibility to experimental infection in oysters from an area outside the normal geographic range of *P. marinus* (Figure 4 and 5). Both occurred in a dose-dependent manner.

To further explore the role of pollution on the onset and progression of infectious disease caused by *P. marinus*, Chu *et al.* (In review) tested the effect of contaminated sediments from the Elizabeth River on *P. marinus* expression in oysters from a *P. marinus* enzootic area (Point of Shoals, James River, VA), with an initial infection prevalence of 39%. The sediments contained predominantly high molecular weight PAHs. PCBs and metals were also present therein. Oysters were daily exposed to 0, 1.0, 1.5, or 2.0 g of suspended contaminated sediments for 30 days. After 30 days, oysters treated with contaminated sediments had increased disease prevalence. Cessation of exposure to contaminated sediments for 3, 7, and 14 days did not moderate *P. marinus* progression. At the end of the experiment, prevalences were 47, 65, 50, and 74% in oysters provided with 0, 1.0, 1.5, and 2.0g of contaminant sediments, respectively (Figure 6). It is believed that this was not an effect of sediment *per se*. A preliminary experiment examining the effects of varying amounts of non-toxic artificial sediments on *P. marinus* progression indicates that sediment amounts from 0-2 g day^{-1} had no impact on this endpoint. A previous study by Winstead and Couch (1988) also noted that *P. marinus* expression appeared at uncharacteristically low temperatures after toxicant (carcinogen n-nitrosodiethylamine) exposure.

Fisher *et al.* (1999) and Anderson *et al.* (1996) tested independently the effects of tributyltin (TBT) on *P. marinus* progression in eastern oysters. Both research groups observed that TBT exposure significantly intensified *P. marinus* infection and results in greater oyster mortality.

Pollutant exposure may reduce disease resistance by causing physiological stress in the host or suppressing certain host defence mechanisms (Sinderman, 1993). However, such a relationship was not observed in oysters. The mechanisms triggering increased *P. marinus* susceptibility and expression in pollutant exposed oysters are unknown. Although enhanced *P. marinus* expression occurred in contaminated sediment exposed oysters in the above described contaminant sediment exposure experiment, no statistically significant change was noted in any measured humoral or cellular parameters. Similarly, while TBT exposure augmented the progression of Dermo disease, no sig-

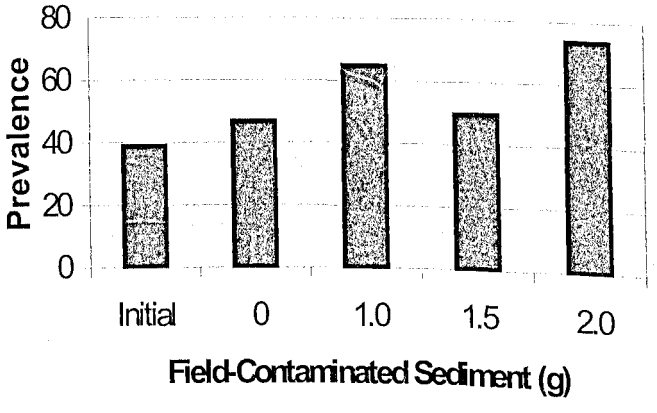

Figure 6: *Perkinsus marinus* prevalence (% of infected oysters) in control and contaminated sediment exposed oysters after 30 days of exposure (N=46-50). Test organisms were collected from Point of Shoals, James River, Virginia (a tributary of the southern Chesapeake Bay affected by *P. marinus*). Control, 0, 1.0, 1.5, and 2.0g contaminated sediments, respectively (corresponding to 0µg, 156µg, 234µg, 312µg PAHs).

nificant effects were observed by Anderson *et al.* (1996) in defence-related activities in oysters.

Field studies also suggest a link between water quality and *P. marinus* infection in oysters. A model developed to investigate the geographical distribution of *P. marinus* on a bay wide scale (Wilson *et al.* 1988) indicates that total PAH concentrations and industrial land use were positively correlated with prevalence.

Other factors

Other environmental factors such as food availability, dissolved oxygen, and turbidity are also believed to play a role in the epizootiology of *P. marinus* in the Chesapeake Bay and Gulf of Mexico. A coupled oyster population- *P. marinus* model suggested that temporal and spatial food supply are important for the host to outgrow of the disease (Hofman *et al.* 1992, Powell *et al.* 1996, Soniat 1996). Reduced ingestion rate due to reduced food supply or increased turbidity can trigger an epizootic (Powell *et al.* 1996, Soniat 1996). Summer hypoxia could be stressful to oysters, thus increasing their susceptibility to disease (Burreson and Ragone 1996).

Summary

Environmental factors play a significant role in regulating the infectious disease caused by *P. marinus* in oysters. Results from both field and laboratory studies reveal the importance of temperature, salinity, and *P. marinus* infective cell dose in controlling *P. marinus* infection in oysters. In addition to high temperature and salinity, environmental pollution could be a potential stressor contributing to magnified *P. marinus* susceptibility and expression in oysters. For better management of oyster resources, further studies are required to further explore the relationship between the host oyster, *P. marinus*, and environmental factors. In particular, studies are necessary to elucidate the cause-effect relationship between pollutants and the actual onset of infectious diseases.

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