Perkinsus marinus tissue distribution and seasonal variation in oysters Crassostrea virginica from Florida, Virginia and New York

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ABSTRACT: Perkinsus marinus infection intensity was measured in eastern oysters Crassostrea virginica collected in October and December 1993, and March, May, and July 1994 from 3 U.S. sites: Apalachicola Bay (FL), Chesapeake Bay (VA), and Oyster Bay (NY). Gill, mantle, digestive gland, adductor muscle, hemolymph, and remaining tissue (including gonadal material and rectum) were dissected from 20 oysters from each site at each collection time. Samples were separately diagnosed for P. marinus infections by incubation in Ray's Fluid Thioglycollate Medium (RFTM) and subsequent microscopic quantification of purified enlarged hypnospores. At all sampling times and sites, average P. marinus infection intensity (g wet wt tissue⁻¹ or ml hemolymph⁻¹) was lowest in hemolymph samples, and generally highest in the digestive gland. Perkinsus marinus prevalence was 100% at both FL and NY sites for each of the 5 collection times, and, for the VA site, was less than 100% in only 1 month (May 1994). Seasonal intensity patterns and mean total body burdens differed among the sites. Average body burden was highest in VA during October and progressively declined to a minimum in May. This decline was probably due to mortality of heavily infected oysters and diminution of parasite activity associated with colder temperatures and reduced salinities. Intensities varied little during the months of October and December at both the FL and NY sites. Minimum average intensities were observed in March in FL oysters and May in NY oysters. Relatively high P. marinus infection levels that persisted throughout the winter in NY oysters compared with VA oysters could reflect constant high salinity in Long Island Sound which favors parasite activity, and also rapid decline in temperature in the fall that may have prevented epizootic oyster mortalities.

KEY WORDS: Perkinsus marinus - Crassostrea virginica - Tissue distribution - Body burdens - Seasonal variation - Geographic variation

INTRODUCTION

The parasite Perkinsus marinus, previously classified as Dermocystidium marinus, is responsible for widespread mortalities in oysters Crassostrea virginica from the Gulf of Mexico to Delaware Bay (Mackin 1953, Andrews 1988, Andrews 1996, Burreson & Ragone 1996, Soniat 1996). Recent documentation of P. marinus in Long Island Sound (Brousseau et al. 1994) and Maine (Kleinschuster & Parent 1995) indicates extension of its range to include most of the U.S. Atlantic coast and the Gulf of Mexico south to Laguna Madre in Texas (Craig et al. 1989), the Florida Keys (Quick & Mackin 1971), and Mexico (Burreson et al. 1994). Oyster mortalities from P. marinus usually peak in late summer-autumn when combined high tempera-

The tendency for low temperature and low salinity to limit the severity of *Perkinsus marinus* infections was recognized by Mackin et al. (1950). In moderate to high salinities, temperatures ≥23°C are usually associated with heavy *P. marinus* infection intensities while temperatures <10°C limit growth of the pathogen (Andrews & Hewatt 1957, Andrews 1965, Perkins 1966). *P. marinus* requires salinities of at least 12% for infections to intensify, and increasing salinity has been positively associated with infection intensity in laboratory and field studies (Andrews 1988, Chu et al. 1993, Ragone Calvo & Burreson 1993).

Despite the strong influence of season and salinity on pathogenicity of *Perkinsus marinus*, these factors do not entirely explain the variability in infection intensity and oyster mortalities. For example, *P. marinus* prevalence and intensity in South Carolina oysters from a relatively low salinity intertidal site were higher than those of oysters from an oceanic site (Burrell et al. 1984). Weighted incidences (WI) of oysters from West Galveston Bay (Texas) measured from May 1979 to September 1981 showed no obvious seasonal pattern, but showed a strong correlation with temperature-salinity interaction (T × S) (Somat 1985). *P. marinus* infection intensity and related oyster mortalities can also be exacerbated by poor nutritional status (Fisher et al. 1992), by high oyster density (Andrews 1996), and by exposure to certain xenobiotic chemicals (Scott et al. 1985, Winstead & Couch 1988, Chu & Hale 1994, Anderson et al. 1996, Fisher et al. 1995). *P. marinus* infection progression and pathogenicity are influenced by numerous fluctuating abiotic and biotic factors.

Ray's (1952) original technique to diagnose *Perkinsus marinus* infection severity in oysters involves dissection of a select tissue such as rectum or mantle, incubation of the tissue in Ray's Fluid Thioglycollate Medium (RFTM) to enlarge parasites, staining with iodine, and microscopic examination to enumerate parasites. Mackin (1951) and Ray (1952) recognized that variation in parasite density (no. of parasites g⁻¹) within and among different oyster tissues could bias diagnostic methods. New diagnostic methods to quantify *P. marinus* confirmed that parasite distribution varies among oyster tissues. Lewis et al. (1988) first developed a method to quantify parasites, and others (Choi et al. 1989, Fisher et al. 1992) demonstrated that digestive gland tissue had higher parasite densities than other tissues. Gauthier & Fisher (1990) first used the quantitative method to determine the entire oyster's parasite burden, and showed that hemolymph *P. marinus* levels (ml⁻¹) were lower than the total body burdens (g⁻¹). The quantitative total body burden assay is more sensitive than the tissue squash technique (Bushek et al. 1994). However, comprehensive analysis of parasite compartmentalization in all oyster tissues has not yet been performed.

The objectives of this study were to concurrently monitor *Perkinsus marinus* infection patterns in 3 geographically distinct oyster populations, to compare and contrast differences in disease prevalence and intensity at those sites over a 1 yr period, and to study tissue distribution of the parasite by performing quantitative diagnoses on separate oyster tissues. Since individual tissues are often examined to estimate body burden, this approach allowed comparison of the accuracy of using various tissues to indicate total body levels of *P. marinus*. The 3 laboratories participating in the collaborative study employed a standard technique to enumerate parasites in 6 tissues, and calculated total oyster burden by summation.

**MATERIALS AND METHODS**

**Oyster collection.** At each collection time (October and December 1993, March, May, and July 1994), approximately 25 oysters were gathered from 3 sites (see below) using hand tongs in FL and a dredge in VA and NY, placed on ice and transported to the Environmental Protection Agency's Gulf Ecology Division (GED, FL oysters), Virginia Institute of Marine Science (VIMS, VA oysters), or Haskin Shellfish Research Laboratory (HSRL, NY oysters). Florida (FL) oysters were collected from Cat Point Bar in Apalachicola Bay (Northern Gulf Coast of Florida, Fig. 1a), an actively fished oyster bar that experiences diurnal tides and frequent salinity fluctuations. Cat Point Bar salinity typically ranges from 8 to 33°C. Virginia (VA) oysters were collected from Wreck Shoal in the upper James River, an oyster seed bed in a low to moderate salinity area (lower Chesapeake Bay, Fig. 1b). Wreck Shoal has a mean annual salinity of about 14% with minimum salinities typically in March resulting from high runoff, and maximums in October. Annual temperatures usually range from 2 to 28°C. New York (NY) oysters originated from the planting grounds of the Frank M. Flower & Sons Oyster Company¹ in Oyster Bay (Long Island Sound, Fig. 1c). Oyster Bay is well mixed with

¹Mention of commercial names does not constitute endorsement by the U.S. Environmental Protection Agency
Tissue-driven water from Long Island Sound but receives little freshwater input. Salinity is relatively stable, from 25 to 30%. Temperature ranges annually from -1 to 27°C. Ambient salinity and temperature were recorded at each collection time (Table 1).

**Tissue analysis.** Oysters (20 per site) were processed within 1 d of receipt at each participating facility. Shells were scrubbed to remove fouling organisms and mud, then total weights and shell dimensions (height, length, and width) were obtained. Valves were notched, mantle fluid drained, and oysters exsanguinated via the adductor muscle. After obtaining maximal hemolymph from adductor muscle sinuses, oysters were shucked, and shells and meat were weighed separately. The following tissues were dissected, weighed, minced, and placed into 20 ml RFTM (Ray 1966) containing 5 µl ml⁻¹ Chloromycetin (Sigma Chemical Co., 2.5% in deionized water): gill, including palps (G), mantle (M), digestive gland (DG), adductor muscle (AM), and remaining tissue (REM). The REM included rectal tissue and gonadal material when present. Hemolymph was centrifuged (1500 to 3000 × g, 5 min), the supernate removed, and 5 ml RFTM with 5 µl ml⁻¹ Chloromycetin was added. Nystatin (Sigma Chemical Co., 0.1% in deionized water) was layered on top of all cultures without mixing (2.0 ml for tissue
cultures, 0.5 ml for hemolymph cultures). All samples were incubated at room temperature (±25°C) in darkness for 7 d to allow enlargement of *Perkinsus marinus* hypnospores.

After incubation samples were centrifuged (800 to 2000 × g, 10 to 15 min) and overlying RFTM was drawn off. Sodium hydroxide (2 M NaOH, 30 ml for tissues, 5 ml for hemolymph) was added and samples were placed in a 60°C oven for 2 h to digest remaining oyster tissue. After samples had cooled to room temperature, they were centrifuged as described above and the NaOH supernate was removed. Two additional washes/centrifugation steps were performed with deionized water (DI) to remove all NaOH. In some cases, particularly for the Long Island oysters, a viscous layer formed over the pellet during the wash steps following NaOH digestion. This layer of material was retained to avoid potential loss of parasites. Sample volume (stored in 10%o NaCl) was recorded and stock Lugol's stain was added at the rate of approximately 350 μl ml⁻¹. The final volume was recorded, each sample was vortex mixed and filtered as above. Samples were counted using a Zeiss compound microscope at 100×. Samples were diluted if counts exceeded 300 per 100 μl aliquot, and the entire sample was examined if counts were less than 10. Dilutions were made by removing 10 to 500 μl of original sample and diluting to 1 ml.

For all 3 sites, whole body burdens were calculated by summing *Perkinsus marinus* counts for each separate tissue, then dividing by the undissected whole tissue weight plus the volume of hemolymph (ml) removed. Final parasite densities were expressed as log₁₀ no. of hypnospores (g wet wt tissue)⁻¹ or log₁₀ no. of hypnospores (ml hemolymph)⁻¹.

**Statistical analysis.** Although quantitative *Perkinsus marinus* diagnostic data were obtained at each participating laboratory using a common method, differences in technical personnel and equipment (e.g. centrifuges) could impose variability that would invalidate inter-site statistical comparisons. Calibration samples that consisted of finely homogenized oyster tissue were processed by all participating laboratories during the study and some results varied considerably (Fisher & Oliver 1996). Consequently, inter-site comparisons of *P. marinus* prevalence and intensity have been limited to qualitative observations. Data from each site were subject to 2-way interactive analysis of variance (ANOVA) with date and tissue type as main effects. Where significant interaction was found, Tukey's studentized range test was conducted on all date × tissue type means (including total body burdens) within a given site. If no interaction was present, 1-way ANOVAs were conducted to evaluate infection intensity differences among tissue types within date, and to test for infection intensity differences between dates for each tissue type and for total body burden. Within-site correlational analysis (Pearson's procedure) was conducted separately for data from each site to evaluate the relative accuracy of individual tissue analyses in reflecting whole body results. All data were log₁₀ transformed to satisfy an ANOVA requirement for normality of error terms. For 6 oysters from FL and 2 from NY, 1 or more individual tissue *P. marinus* counts were missing due to technical errors during oyster processing, and whole body burdens were not calculated for these oysters. Differences were considered significant at p < 0.05.

<table>
<thead>
<tr>
<th>Date</th>
<th>Florida</th>
<th>Virginia</th>
<th>New York</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct 1993</td>
<td>27.0</td>
<td>19.5</td>
<td>25.0</td>
</tr>
<tr>
<td>Dec 1993</td>
<td>32.0</td>
<td>10.5</td>
<td>26.0</td>
</tr>
<tr>
<td>Mar 1994</td>
<td>6.3</td>
<td>5.0</td>
<td>25.0</td>
</tr>
<tr>
<td>May 1994</td>
<td>18.4</td>
<td>8.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Jul 1994</td>
<td>12.0</td>
<td>17.0</td>
<td>26.0</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oct 1993</td>
<td>24.6</td>
<td>14.4</td>
<td>16.7</td>
</tr>
<tr>
<td>Dec 1993</td>
<td>15.8</td>
<td>12.0</td>
<td>8.1</td>
</tr>
<tr>
<td>Mar 1994</td>
<td>17.5</td>
<td>9.0</td>
<td>2.2</td>
</tr>
<tr>
<td>May 1994</td>
<td>24.0</td>
<td>19.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Jul 1994</td>
<td>26.6</td>
<td>27.6</td>
<td>24.2</td>
</tr>
</tbody>
</table>
RESULTS

Florida

Perkinsus marinus prevalence was 100% for oysters collected from FL. Log$_{10}$ total body burdens ranged from 2.15 to 5.52, and the combined mean over all dates was 4.19. Sixty-two percent of whole-body infections ranged from 4.0 to 5.0 (Fig. 2a). There was significant interaction between date and tissue type for the FL oyster data. The highest month's average total body burden of *P. marinus*, measured in July, was significantly higher than the average for March (Table 2a, Fig. 3a), but was not significantly different from any other monthly mean body burdens. Counts from separate tissues reflected the same month-to-month trend, with the March mean always lower than that of other sample dates (Table 2a). Hemolymph data showed a slightly different pattern, with the December sample having the highest counts compared to other months (Table 2a, Fig. 3a). Hemolymph diagnoses were significantly lower than those for all other tissues and body burden in all months except December, when adductor muscle and hemolymph results were statistically indistinguishable (Table 2a). For all months except October, the highest *P. marinus* intensity was found in the digestive gland (Table 2a). In 21 of 100 cases, no parasites were found in hemolymph samples, but body burdens ranged from 2.4 to 4.9 for FL oysters that had negative hemolymph diagnoses. For the other 5 tissues diagnosed, there were 4 instances in which an individual tissue contained no *P. marinus* (3 adductor muscle and 1 gill), although parasites were recorded for other tissues and whole body burdens ranged from 2.4 to 4.3. Using data from all dates combined, each tissue was significantly correlated with body burden, and digestive gland showed the strongest correlation with $r^2 = 0.795$ (Table 3). Although significantly correlated with body burden, the correlations with both hemolymph ($r^2 = 0.241$) and adductor muscle ($r^2 = 0.251$) were weak (Table 3).

Virginia

Perkinsus marinus prevalence was 98% for oysters collected from Wreck Shoal, VA. Two oysters sampled in May 1994 were negative. Body burdens for individuals ranged from 0 to 7.13 with a grand mean of 3.37. Forty percent of infections were less than 2.0, and 36% were at least 3.0 or greater (Fig. 2b). Two-way interactive ANOVA results showed significant interaction between date and tissue type. The greatest infection intensities were observed in October 1993 for all tissues and body burden (Table 2b, Fig. 3b). Mean total body burden declined between October and May, and average body burdens in March, May, and July were significantly lower than those measured in either October or December (Table 2b). The March and May mean body burdens were the lowest intensities for any site during the study. In all months, parasite densities in hemolymph were lower than in all other tissues. Counts for adductor muscle were next lowest. Digestive gland tissue had the highest counts of all tissues in December 1993, March 1994, and May 1994, whereas REM ranked highest in October 1993 and mantle tissue ranked highest in July 1994 (Table 2b). Hemolymph samples were negative for 22 oysters in which *P. marinus* was detected in other tissues, and body burdens from these oysters ranged from $<1.0$ to 4.0. For the other 5 tissues, negative diagnoses were obtained 52 times: 10 mantle, 6 digestive gland, 11 gill, 18 adductor muscle and 7 REM. Whole body burdens for oysters that yielded some negative and some positive individual soft tissue diagnoses ranged from $<1.0$ to 4.3, and most were below 2.0. All results from separate tissue diagnoses correlated strongly with body burden, and correlation coefficients were much higher than
The density of parasites in hemolymph was high temperatures favor development of heavy infection. Also significantly lower than those for all other months in oyster *Perkinsus marinus* the highest intensity of burden was around 4.0 for all collection times except for May, when it declined significantly to 2.29 (Table 3). Fifty-eight percent of infections were in strong with body burden; the highest r² was 0.910 for the 3.0 to 5.0 range (Fig. 2c). In contrast to data from FL and VA, there was no significant interaction between date and tissue type for the NY data. Mean total body burden was around 4.0 for all collection times except for May, when it declined significantly to 2.29 (Table 2c, Fig. 3c). The May values for individual tissues were also significantly lower than those for all other months (Fig. 3c). The density of parasites in hemolymph was consistently lower than in the other 5 tissues, and digestive gland had the highest density at all times of year (Table 2c). No parasites were found in 10 hemolymph samples from oysters with body burdens that ranged from 0.3 to 4.2. There were negative diagnoses for 5 tissue samples: once each for mantle, digestive gland and adductor muscle, and twice for gill. Whole body burdens of these oysters ranged from 0.3 to 4.2. *P. marinus* intensities in all 6 tissues correlated strongly with body burden; the highest r² was 0.910 for REM (Table 3).

**DISCUSSION**

The highest intensity of *Perkinsus marinus* in oyster tissue is typically found in late summer and fall when high temperatures favor development of heavy infec-

<table>
<thead>
<tr>
<th>Tissue</th>
<th>October</th>
<th>December</th>
<th>March</th>
<th>May</th>
<th>July</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM</td>
<td>3.77 (0.22) def</td>
<td>2.84 (0.29) fg</td>
<td>0.56 (0.12) hj</td>
<td>9.08 (0.04) j</td>
<td>1.47 (0.22) ghij</td>
</tr>
<tr>
<td>M</td>
<td>6.13 (0.18) a</td>
<td>4.69 (0.30) bcde</td>
<td>1.10 (0.25) hj</td>
<td>0.64 (0.19) hj</td>
<td>3.83 (0.34) def</td>
</tr>
<tr>
<td>DG</td>
<td>6.15 (0.15) a</td>
<td>5.27 (0.21) abc</td>
<td>1.84 (0.33) ghij</td>
<td>1.06 (0.19) hj</td>
<td>3.32 (0.46) ef</td>
</tr>
<tr>
<td>G</td>
<td>6.12 (0.17) a</td>
<td>4.64 (0.31) bcde</td>
<td>0.99 (0.22) hij</td>
<td>0.73 (0.21) hij</td>
<td>3.57 (0.36) ef</td>
</tr>
<tr>
<td>AM</td>
<td>5.63 (0.19) abc</td>
<td>4.26 (0.31) cde</td>
<td>0.88 (0.24) hj</td>
<td>0.34 (0.17) ij</td>
<td>2.79 (0.40) fg</td>
</tr>
<tr>
<td>REM</td>
<td>6.33 (0.15) a</td>
<td>5.25 (0.19) abc</td>
<td>1.69 (0.28) ghij</td>
<td>0.86 (0.24) hj</td>
<td>3.30 (0.40) ef</td>
</tr>
<tr>
<td>Total</td>
<td>6.01 (0.14) ab</td>
<td>4.96 (0.18) abcde</td>
<td>1.53 (0.27) ghij</td>
<td>0.91 (0.20) hj</td>
<td>3.41 (0.37) ef</td>
</tr>
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</table>

**Table 2.** *Crassostrea virginica.* Means (and standard errors) of log_{10}-transformed *Perkinsus marinus* counts in different oyster tissues from 3 sites at 5 times of the year. (a, b) Data analyzed by interactive ANOVA with effects = date and tissue type; (c) data analyzed by 1-way ANOVA within date with effect = tissue type. HM: hemolymph; M: mantle; DG: digestive gland; G: gill; AM: adductor muscle; REM: remainder. Total: summed body burden. Means that share a letter are not significantly different.

**Florida oysters:** Interaction (date × tissue type) significant, p < 0.0001, all date × tissue type means tested using Tukey's Studentized range test.

**Virginia oysters:** Interaction (date × tissue type) significant, p < 0.0019, all date × tissue type means tested using Tukey's Studentized range test.

**New York**

*Perkinsus marinus* prevalence was 100% for NY oysters throughout the project, and body burdens ranged from 0.23 to 6.41, with an overall mean for the entire study of 3.73. Fifty-eight percent of infections were in the 3.0 to 5.0 range (Fig. 2c). In contrast to data from FL and VA, there was no significant interaction between date and tissue type for the NY data. Mean total body burden was around 4.0 for all collection times except for May, when it declined significantly to 2.29 (Table 2c, Fig. 3c). The May values for individual tissues were also significantly lower than those for all other months (Fig. 3c). The density of parasites in hemolymph was lower than in the other 5 tissues, and digestive gland had the highest density at all times of year (Table 2c). No parasites were found in 10 hemolymph samples from oysters with body burdens that ranged from 0.3 to 4.2. There were negative diagnoses for 5 tissue samples: once each for mantle, digestive gland and adductor muscle, and twice for gill. Whole body burdens of these oysters ranged from 0.3 to 4.2. *P. marinus* intensities in all 6 tissues correlated strongly with body burden; the highest r² was 0.910 for REM (Table 3).
sites illustrate the complex involvement of biological and environmental variables that influence infection intensity and distribution in any given year.

The VA oysters followed the typical seasonal pattern of infection intensity. They displayed very heavy infections \((\log_{10} \approx 6)\) in October, which decreased to light infections by May. Accompanying the decreased intensity were lowered salinities during the winter months, a condition that inhibits parasite activity (Chu & La Peyre 1993, Ragone & Burreson 1993). Six periodic measurements of salinity at Wreck Shoal between February and May 1994 were below 10%. Reduced temperature and salinity probably reduced \(P.\ marinus\) infections, and mortalities of heavily infected oysters may have contributed to the observed decline in average infection intensity over winter.

\(P.\ marinus\) data from the NY site deviate from the sharp seasonal decline in infections usually associated with reduced winter temperatures. Despite the rapid and relatively steep winter decline in temperature, 100% prevalence and average whole-body intensities of approximately 4.0 persisted throughout the winter. The only significant reduction in infection intensity in NY oysters was not seen until May when temperatures were increasing (Fig. 3c). Low autumn temperatures in Oyster Bay could have restricted development of lethal infections, and the constant high salinity (about 25‰ year-round; Table 1) may have allowed moderate to heavy infections to persist through the winter. As suggested by Ragone Calvo & Burreson (1994) the synergistic effect of both low temperature and low salinity is critical in reducing \(P.\ marinus\) infection intensity. Perhaps colder temperatures reduced the destructive activity by \(P.\ marinus\) parasites and associated mortality. Lower reproductive stress, due to a shorter spawning period for NY oysters (Kennedy &

![Graph showing infection intensities for oysters from different locations]

**Table 3. Pearson correlation coefficients \((r^2)\) between individual tissue diagnoses and total body burdens for each site; all significant at \(p < 0.0001\)**

<table>
<thead>
<tr>
<th></th>
<th>Florida</th>
<th>Virginia</th>
<th>New York</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolymph</td>
<td>0.241</td>
<td>0.771</td>
<td>0.738</td>
</tr>
<tr>
<td>Mantle</td>
<td>0.515</td>
<td>0.922</td>
<td>0.881</td>
</tr>
<tr>
<td>Digestive gland</td>
<td>0.795</td>
<td>0.930</td>
<td>0.803</td>
</tr>
<tr>
<td>Gill</td>
<td>0.421</td>
<td>0.922</td>
<td>0.883</td>
</tr>
<tr>
<td>Adductor muscle</td>
<td>0.253</td>
<td>0.902</td>
<td>0.873</td>
</tr>
<tr>
<td>Remainder</td>
<td>0.624</td>
<td>0.974</td>
<td>0.910</td>
</tr>
</tbody>
</table>
Krantz 1982, Brousseau 1995), and more active cellular
defense activities at cool temperatures (Fisher et al.
1989, Fisher et al. 1996) may have protected NY oys-
ters from lethal effects. However there was no decline
in average infection intensity between October and
March; although the link of low temperature to de-
creased P. marinus infection is well documented
(Hewatt & Andrews 1956, Chu & La Peyre 1993,
Ragone Calvo & Burreson 1994).

During the same time interval, a study of intertidal
oysters at 3 sites on the north shore of Long Island
Sound demonstrated reduced winter Perkinsus mari-
inus infections (Brousseau 1996). At one site (Bridge-
port, CT), prevalence decreased from 100% to 17%
between October 1993 and February 1994. Although
temperatures at Oyster Bay and Bridgeport were simi-
lar, salinity at the Bridgeport site was 15%, which
could have contributed to the apparently lower winter
infection intensities compared with Oyster Bay.
Ragone Calvo & Burreson (1994) found that at high
salinities, winter prevalence did not decline as rapidly,
or to the same degree, at lower salinities at the same
temperature. Light intensity infections in Bridgeport
oysters may have been missed by the mantle/rectal
qualitative tissue squash method which has a detection
limit of about 10^3 [g wet wt tissue^-1] (Bushek et al.
1994).

Despite higher salinities and temperatures in Octo-
ber and December, FL oysters had lower body burdens
of Perkinsus marinus compared with VA oysters. The
autumn intensity peak (and associated P. marinus-
related oyster mortalities) may have occurred earlier
than October in FL, since the average body burden in
July 1994 surpassed that measured in October 1993.
Such a peak during July and August has been previ-
sely reported in Gulf of Mexico oysters (Ray 1954,
Quick & Mackin 1971). Rapid growth of oysters in
Apalachicola Bay can result in market-sized individu-
als in less than 15 mo (Dawson 1955), allowing oysters
to ‘outgrow’ the parasite and reach harvestable size
before P. marinus infections become lethal (Soniat
have used computer simulations to illustrate that oys-
ters inhabiting lower latitudes may sustain a stable
average infection level, although heavy infections and
ensuing mortalities occur, because the rapid growth
rate allows sufficient recruitment to replace dying
adults. In contrast, NY oysters may require 3 yr to
reach market size. Alternatively, a density-dependent
reduction in parasite proliferation at high summer
infection intensities may explain the relatively con-
stant infection levels measured in both FL and NY oys-
ters (Saunders et al. 1993).

Differences in susceptibility among oyster popula-
tions may have contributed to the contrasting patterns
of infection among the 3 sites. Greater resistance to
Perkinsus marinus by South Carolina oysters com-
pared with Virginia oysters was reported by Andrews
(1955). Bushek (1994) showed that oysters from popu-
lations with long exposure to P. marinus developed sig-
ificantly lower infections than oysters with limited
exposure. Oysters inhabiting the New York site have
been exposed to heavy P. marinus parasitism only in the
last few years, in contrast with the Gulf of Mexico
and Chesapeake Bay where P. marinus has been pre-
sent for at least 50 yr (Ray 1954, Ford 1996). In this
study, although moderate-heavy infections persisted in
NY oysters throughout the winter, there was no evi-
dence of infection progression toward levels usually
associated with lethality (10^4 to 10^7 parasites [g wet wt
tissue^-1]). Fisher et al. (1992). Although mortality was
not measured in this study, lethality thresholds for P.
marinus infections may differ among sites.

Parasite virulence may also differ among geo-
graphically separate sites. Bushek & Allen (1996) reported
significantly heavier infections in oysters challenged
with Perkinsus marinus isolates from the U.S. Atlantic
cost compared with those challenged with isolates
from the Gulf of Mexico. Very heavy fall infections in
VA oysters, which probably caused mortalities, com-
pared with more constant, moderate to heavy infec-
tions in FL oysters, may indicate either reduced para-
site virulence or an adaptive response in Gulf of
Mexico host oysters. P. marinus was recently docu-
mented in Maine (Kleinschuster & Parent 1995), sug-
gest the possible emergence of a cold-tolerant
strain that could explain the persistence of winter
infections in NY.

For all sites studied, digestive gland densities of
Perkinsus marinus tended to be the highest of all tis-
sues (Table 2). Similar results were reported by Choi
et al. (1989) and Fisher et al. (1992). Parasite density in
digestive gland tissue was greater than that calculated
for the whole oyster at all sites on all collection dates;
this must be considered if digestive gland intensity is
used as an estimate of parasite density in the total oys-
ter. REM also had relatively high parasite density,
probably due to the inclusion of rectal tissue which is
known to have high levels of P. marinus (Ray 1966).
Correlations between tissue and total body levels of P.
marinus for FL oysters were relatively poor compared
with VA and NY for all individual tissues (Table 3). The
reasons for this are uncertain, but may possibly relate
to technical differences in oyster processing or to the
distribution of P. marinus in FL oyster tissue.

Hemolymph samples contained the lowest parasite
densities of the 6 tissues. This finding, and the rela-
tively high number of missed infections in hemolymph,
was also reported by Bushek et al. (1994) who assayed
0.25 ml hemolymph samples. In our study, an attempt
was made to extract as much hemolymph as possible from the oysters to maximize sensitivity. The average volumes of hemolymph withdrawn at each laboratory were 1.58, 1.42, and 2.28 ml for FL, VA, and NY, respectively; yet infections were still not found in 55 of 303 hemolymph samples. Despite higher intensity infections during certain months in FL oysters relative to VA oysters (Fig. 3a, b), there were about the same number of negative hemolymph diagnoses, suggesting possible differences in the infection process between the sites or technical discrepancies in sample processing. Correlations between total body burdens and hemolymph densities showed a weaker relationship for FL compared with VA and NY data (Table 3). Elimination of *Perkinsus marinus* counts from hemolymph sample volumes less than 1 ml from the correlation analyses did not improve the model fit. Gauthier & Fisher (1990) found a stronger relationship between body burden and hemolymph levels in oysters with relatively high intensity infections, a phenomenon reiterated by Bushek et al. (1994). Although useful for estimating *P. marinus* infections when oysters cannot be sacrificed, the hemolymph assay may underdiagnose moderate to heavy infections. When hemolymph diagnoses are used, investigators should consider that hemolymph infections are consistently lower than whole body burdens. Results from this collaborative study impact past and future research on the oyster parasite *Perkinsus marinus*. Historical prevalence estimates generated from the tissue squash/rating method have probably underestimated true prevalence, as suggested by Bushek et al. (1994). Similarly, the validity of using a single tissue for surveys or monitoring work (Bushek et al. 1994) is also confirmed by our study, since each of the 6 tissues analyzed here reflected the same seasonal trend as did the summed body burdens. The choice of tissue is important depending upon the accuracy required (e.g. hemolymph diagnoses give low estimates of body burden, and digestive gland diagnoses give high estimates). The digestive gland diagnoses were the most sensitive and had the strongest correlation with total body burden. The increased accuracy gained by using the quantitative body burden assay, though more costly and time-consuming than the tissue-rating technique, is desirable for a variety of applications. Computer models suggest that accurate prediction of epizootics requires examination of infection intensity changes in sub-market size classes (Hofmann et al. 1995) which tend to have relatively light infections that could be missed by the single-tissue squash technique (Ray 1954, Soniat 1996). A quantitative, whole-oyster diagnostic method is well suited to study light-intensity, over-wintering infections (Bushek et al. 1994, Ragone Calvo & Burreson 1994, Fisher & Oliver 1996). The quantitative version of Ray's (1952) technique used here allowed detection of distinct seasonal patterns of infection progression at different sites and demonstration of consistent differences in oyster tissue distribution throughout 1 year.

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