Year 2000 Maryland Oyster Disease Status Report

Technical Report: FS–SCOL–01-1

MARYLAND DEPARTMENT OF NATURAL RESOURCES
FISHERIES SERVICE
SARBANES COOPERATIVE OXFORD LABORATORY
OYSTER DISEASE RESEARCH PROJECT
OXFORD, MARYLAND

May, 2001

Year 2000 Maryland Oyster Disease Status Report

Technical Report: FS-SCOL-01-1

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ABSTRACT

As a component of the annual Modified Fall Survey (MFS) of Maryland Chesapeake Bay eastern oyster (*Crassostrea virginica*) populations, a 43-sample subset was established in 1990 to assess oyster parasite distributions, prevalences and infection intensities. Due to their virulence, the protozoan parasites *Haplosporidium nelsoni* (MSX disease) and *Perkinsus marinus* (dermo disease) are primary pathogens of concern. To correlate environmental influences on these oyster disease pathogens, annual disease variables are compared to physical variables, freshwater inflow and annual average winter water temperature. Percent total oyster mortality is also compared to annual disease variables to estimate population influences of these pathogens on eastern oysters.

In 2000, *H. nelsoni* was found on 64% of sampled oyster bars, compared to 67% in 1999. *Perkinsus marinus* was found on all sampled bars in 2000, which was similar to 1999 although the proportion of relatively severe mean intensities declined. The slight decrease in *H. nelsoni* and *P. marinus* infection measures in 2000 coincided with an increase in freshwater inflow and decrease in average winter water temperature. Freshwater inflow was significantly associated with both pathogens while average winter water temperature was not. Percent mortality increased, suggesting that the slight decreases in oyster disease pathogen activity were insignificant.

For comparison to the MFS data, disease data for the State seed areas are also reported. Conservative management activities are suggested due to the sustained high prevalences of *H. nelsoni* and *P. marinus* in these areas.

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INTRODUCTION

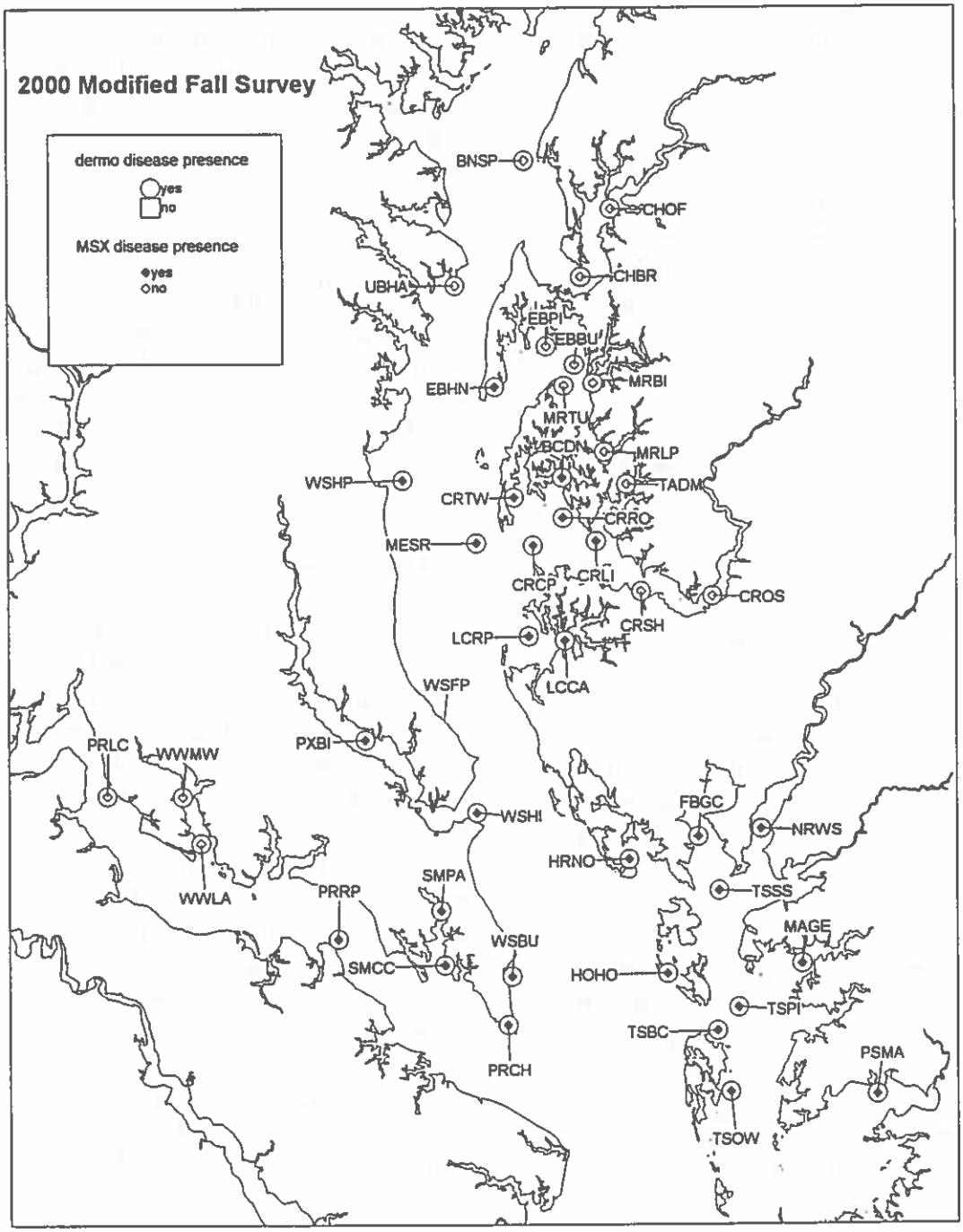
As a component of the annual Maryland Modified Fall Survey (MFS) of its Chesapeake Bay eastern oyster population (*Crassostrea virginica*), a 43-sample subset was established in 1990 to assess oyster parasite distributions, prevalences and infection intensities (Map 1) (Smith and Jordan 1993). Due to their virulence, the protozoan parasites *Haplosporidium nelsoni* (MSX disease) and *Perkinsus marinus* (dermo disease) are primary pathogens of concern. For *H. nelsoni*, infection prevalence at each site and annual percent frequency of MFS-sampled populations infected by *H. nelsoni* are reported. Sample infection intensity is not reported because no validated clinical scale exists. For *P. marinus*, both infection prevalence and mean infection intensity are reported, as well as adjusted sample prevalence, which combines sample prevalence and mean intensity in a single, unitless measure.

Annual *H. nelsoni* and *P. marinus* infection statistics are compared to the physical variables, average winter water temperature and freshwater inflow. Comparison with these physical variables is used to correlate environmental influences on pathogen virulence and distribution. Freshwater inflow rates, measured at major estuary tributaries, reflect and predict long-term salinity effects on host/pathogen interactions, as well as the prominence of freshet effects. The average winter water temperature was used to test whether low winter temperatures can cause measurable pathogen decline. Abnormally cold winter temperatures have been hypothesized to inhibit *H. nelsoni* and *P. marinus* pathology and transmission (Ford and Haskin 1982; Burreson and Ragone Calvo 1996). Comparison of the disease parameters with total oyster mortality was used to correlate population influences of pathogens on eastern oysters. Also reported, to further elucidate pathogen influences on oyster population dynamics, is an apparent variability in susceptibility to diseases by oysters of different age/size classes.

Disease data from State oyster seed areas are reported to compare with the MFS disease subset. Due to the dynamic nature of the replenishment program, in which annual disease samples correspond with oyster seed plantings, very few seed areas are repeatedly sampled annually. Therefore, to help infer any influence of oyster disease on seed oyster health, comparison with the MFS oyster disease subset may be necessary.

The Cooperative Oxford Laboratory Oyster Disease Research Project generates all disease data annually for approximately 2,000 adult and juvenile (seed) oysters from the Maryland portion of Chesapeake Bay, and has done so since 1989. This report provides an annual comparison of disease distributions, prevalences, and intensities to previous years' data; and compares both physical and population parameters to detect correlations with disease variables. This report is designed to be published annually, so that the current year can be compared to previous ones. For some analyses, data from the previous ten years are pooled to look for long-term trends. Ten-year ranges are re-calculated every year to include the new year and subtract the oldest one.

Map 1. Dermo and MSX disease presence from the Maryland Modified Fall Survey, 2000. Refer to Table 1 for the tributary and the bar name that corresponds to each bar code.



MATERIALS AND METHODS

Samples

Samples of 30 eastern oysters (*Crassostrea virginica*) were collected from 43 fixed oyster bars in Chesapeake Bay and sent to the Cooperative Oxford Laboratory (COL) for gross, microbiological and histological examination. For each sample, the collection date, salinity and temperature were recorded and an accession code specifying sampling date and location was assigned. Upon arrival at COL, the samples were held in flow-through tanks constantly supplied with water from the Tred Avon River until the samples were processed. Samples were necropsied daily as they arrived, to minimize holding time.

For every sample, the shell height of each oyster from hinge to bill was measured; and the two outer valves (or shells) of the oyster were pried open by inserting an oyster knife through the hinge ligament. The condition of the oyster meat (glycogen and gamete content) was recorded, using a qualitative 9-point scale, and the intensity of shell ectoparasites estimated, using a 7-point scale. Other gross abnormalities of sample oysters were also noted.

Disease Assays

After the gross examination, a sample of rectal tissue was excised and incubated in Ray's fluid thioglycollate medium (Ray 1952) at 28°C for 7 days. After incubation, tissues were teased into small pieces and stained with diluted (1:3) Lugol's iodine, for detection of *P. marinus*. *Perkinsus marinus* infection intensity was recorded for each oyster, using a modified Mackin scale (Ray 1966). Infection intensity was rated on a 0 (uninfected) to 7 (heavily infected) relative categorical scale.

After rectal excision, a transverse section of the oyster was dissected for histological examination (Howard and Smith 1983). The oyster tissues were fixed in Davidson's fixative (Shaw and Battle 1957) for 48 hours and processed by routine procedures for paraffin histology. Sections were stained with hemotoxylin to reveal nuclear morphology, and counterstained with eosin Y-phloxine B. Each slide was then microscopically examined to detect *H. nelsoni* (MSX) and other pathological conditions.

Disease Analysis Variables

For each oyster sample analyzed and annual MFS conducted, values for the following disease variables were calculated and analyzed. Example calculations are shown in Appendix II.

Sample % prevalences were calculated for *H. nelsoni* and *P. marinus* infections as the percent proportion of assayed sample oysters that were infected:

(sample infected n / sample n) (100).

Sample mean intensity is a categorical mean calculated for *P. marinus* infections designed to reflect the severity of disease for each infected sample. This measure was calculated from the equation

$$\sum_{i=0}^{7} n_i\,(i) \,/\, \text{sample } n,$$

where

n_i = number of individual oysters in each infection intensity category

i = infection intensity.

This variable is synonymous with **sample weighted prevalence** (Mackin 1955).

Sample adjusted prevalence, is a unitless index with range of 0–7, which predicts *P. marinus* infections impacts by combining the proportion of infected oysters in a sample with the sample mean intensity, and was calculated as the product of proportional sample prevalence and sample mean infection intensity:

(sample prevalence)(sample mean intensity).

Survey mean adjusted prevalence, for *P. marinus* infections, is a mean of all sample adjusted prevalences during an annual MFS (n bars):

$$\left(\sum_{i=1}^{n\text{bars}} \text{sample adjusted prevalence}\right) / n \text{ bars.}$$

Annual percent frequency, reflects the extent, usually geographic range, of *H. nelsoni* infections, as the percent of MFS sampling sites where this pathogen was detected in a given survey year. It is calculated as:

(n bars infected / n total bars) (100).

Physical Environmental Variables

Annual percent frequency of *H. nelsoni* infections and annual survey mean adjusted prevalence of *P. marinus* infections among the MFS bars were compared to average annual freshwater inflows (cubic feet per second) into Chesapeake Bay, and average annual winter water temperatures. Inflow data, expressed as discharge, were obtained from the U.S. Geological Survey data of monthly cumulative inflows to Chesapeake Bay from above the mouth of the Potomac River.

(http://md.usgs.gov/monthly/bay1.html)

Water temperature data were obtained from the Chesapeake Bay Program Data hub.

(http://www.chesapeakebay.net/data/index.htm)

The purpose of these comparisons is to infer the influence of climate on oyster pathogens and diseases. The mean annual inflow was calculated from November of the preceding year through October of the sampling year to correlate the yearly discharge during the 12 months preceding sample collection, since most of the samples were collected during the month of October. The average annual winter water temperature was calculated from four sites along the main channel of Chesapeake Bay that range from upper to lower portions of the Bay, to give a representative value. Monthly averages from December of the previous year through March of the year of interest were pooled to calculate the annual winter average.

Oyster Mortality Estimates

The annual precent frequency of *H. nelsoni* infections and mean adjusted prevalence of *P. marinus* infections among the MFS bars was compared to total estimated mortality of small and market-size oysters. Total mortality was calculated as the total number of dead oysters divided by the combined total number of living and dead oysters. The proportion was multiplied by 100 to yield percent mortality. Dead oysters (boxes) include moribund oysters unable to close their valves (gapers) and empty valves still articulated by their hinge ligament. The integrity of the hinge ligament indicates a relatively recent death (Christmas et al. 1997).

RESULTS

Disease Comparisons, MFS Sites

Haplosporidium nelsoni infections were found in 27/42 (64%) MFS bar samples examined in 2000, compared to 28/42 (67%) MFS bar samples examined in 1999 (Table 1). Within the past ten years, the percent frequency of *H. nelsoni* infections in 2000 ranks third, only exceeded in 1992 and 1999 (Fig. 1). This indicates that, among the past ten years, conditions were well suited for *H. nelsoni* pathogenicity in 2000.

Perkinsus marinus was found on all 42 bars in 2000. A similar widespread distribution for *P. marinus* was observed in 1999, with only one upstream Potomac River bar (Lower Cedar Point) free of this pathogen. The annual survey mean adjusted prevalence of *P. marinus* infections in 2000 ranks fourth during the past ten years exceeded in 1991, 1992, and 1999 (Fig. 2), which suggests mortality due to *P. marinus* infection was relatively high among the past ten years. In fact, estimated mortality in 2000 ranked second among the past ten years, exceed only in 1992 (MDDNR 2001). Disease data from the last ten years for MFS samples are tabulated in Appendix I.

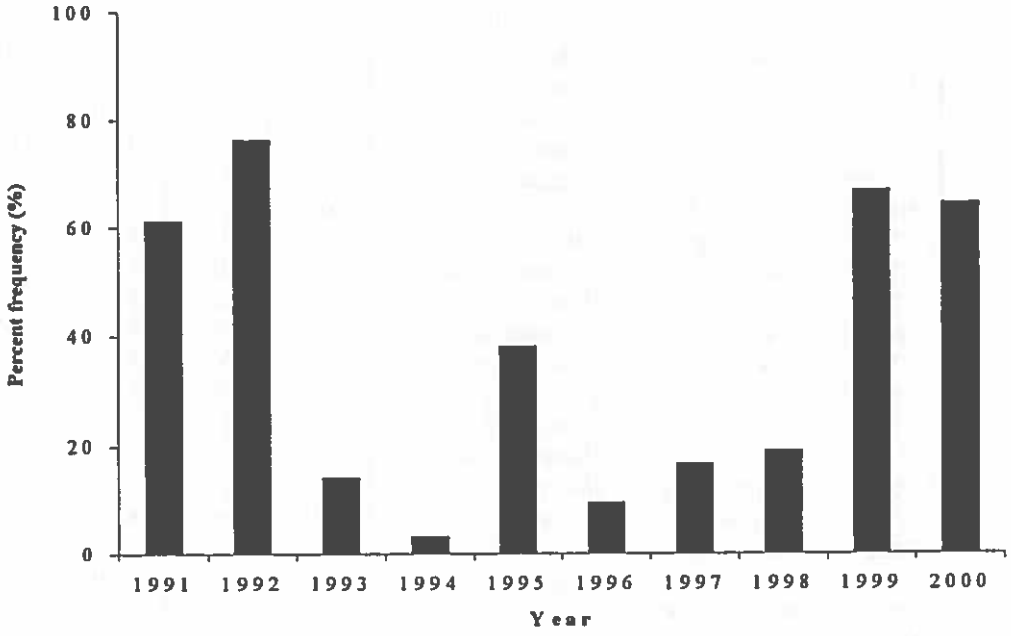


Figure 1. Annual *H. nelsoni* percent frequency, 1991-2000. In 2000, *H. nelsoni* infection frequency ranks third highest among the past ten years.

Table 1. 1999 and 2000 *H. nelsoni* percent prevalences and *P. marinus* sample adjusted prevalences for MFS disease bars.

Code	Region	Bar	H. nelsoni		P. marinus	
			1999	2000	1999	2000
BNSP	Upper Bay	Swan Point	0	0	3.30	1.44
UBHA		Hacketts	0	0	3.20	3.56
WSHP	Mid Bay	Holland Point	0	3	2.60	2.96
MESR		Stone Rock	30	47	4.00	3.32
WSFP		Flag Pond				
WSHI		Hog Island	60	27	5.12	3.26
WSBU	Lower Bay	Butlers	47	17	2.98	2.27
CHBR	Chester River	Buoy Rock	0	0	2.79	3.42
CHOF		Oldfield	0	0	2.91	2.86
EBBU	Eastern Bay	Bugby	0	0	3.90	4.03
EBHN		Hollicutt's Noose	7	10	2.70	4.10
EBPI		Parsons Island	0	0	4.70	3.53
MRBI	Wye River	Bruffs Island	0	0	3.70	3.13
MRLP	Miles River	Long Point	0	0	3.60	3.17
MRTU		Turtleback	0	0	4.30	2.98
CRCP	Choptank River	Cooks Point	13	33	3.16	0.48
CRLI		Lighthouse	13	7	2.00	3.43
CROS		Oyster Shell Point	0	0	1.90	1.63
CRRO		Royston	3	7	3.40	4.56
CRSH		Sandy Hill	0	0	3.30	3.16
CRTW	Harris Creek	Tilghman Wharf	3	27	2.18	3.16
BCDN	Broad Creek	Deep Neck	3	7	4.37	4.00
TADM	Tred Avon River	Double Mills	3	0	4.80	4.73
LCCA	L. Choptank River	Cason	7	27	3.69	3.60
LCRP		Ragged Point	20	47	4.00	3.56
HRNO	Honga River	Normans Addition	63	37	3.26	1.90
FBGC	Fishing Bay	Goose Creek	47	17	5.40	3.04
NRWS	Nanticoke River	Wilson Shoals	4	10	4.30	1.49
MAGE	Manokin River	Georges	40	20	3.26	1.86
HOHO	Holland Straits	Holland Straits	73	40	2.00	0.27
TSBC	Tangier Sound	Back Cove	33	37	5.50	0.49
TSOW		Old Women's Leg	53	30	3.39	1.21
TSPI		Piney Island East	43	53	1.51	1.95
TSSS		Sharkfin Shoal	53	37	4.30	1.82
PSMA	Pocomoke Sound	Marumsco	37	30	3.06	2.51
PXBI	Patuxent River	Broomes Island	3	10	4.60	3.75
SMCC	St. Marys River	Chicken Cock	77	7	5.00	1.12
SMPA		Pagan	3	13	3.30	1.11
WWLA	Wicomico River	Lancaster	0	0	2.08	2.43
WWMW		Mills West	3	0	2.88	3.52
PRCH	Potomac River	Cornfield Harbor	53	17	3.78	1.70
PRLC		Low Cedar Point	0	0	0.00	0.08
PRRP		Ragged Point	13	10	0.09	0.09

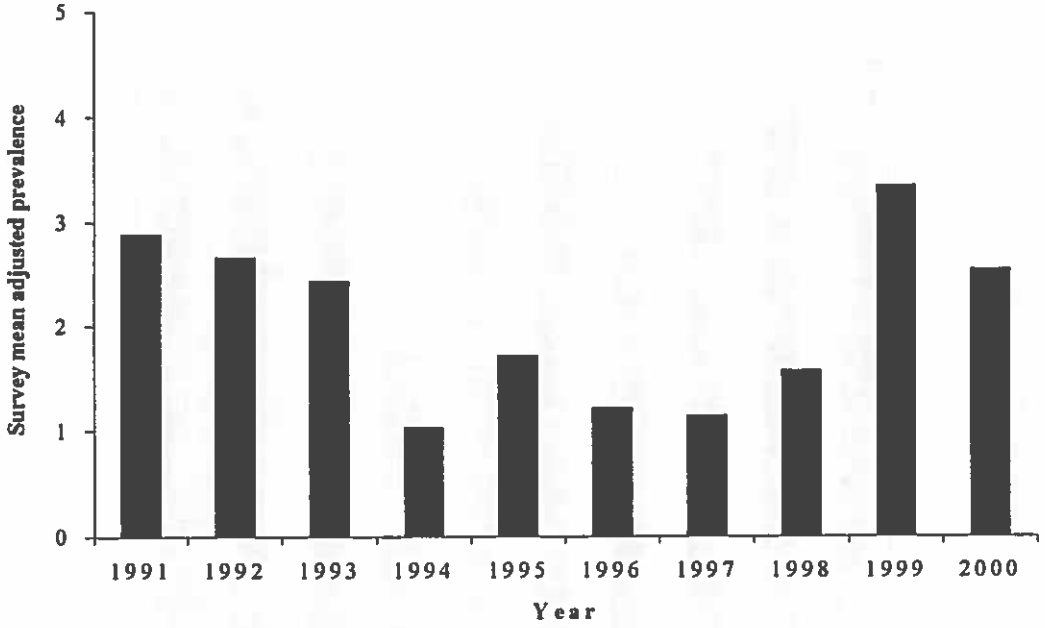


Figure 2. Annual *P. marinus* survey mean adjusted prevalence, 1991-2000. 2000 *P. marinus* survey mean adjusted prevalence ranks as fourth highest over the past ten years.

P. marinus prevalences ≥60 % were recorded among 83% of samples in 2000, which was comparable to 90% of samples with high prevalences observed in 1999 (Fig. 3). *P. marinus* mean infection intensities ≥3.0 on a modified Mackin scale dominated during 1999 (78% of infected oysters), while in 2000, intensities ≥3.0 declined to 54% of infected oysters. Concomitantly, mean intensities 1.0 to 2.9 increased from 24% of samples in 1999 to 38% in 2000 (Figure 4).

Environmental Comparisons

In 2000, *H. nelsoni* annual percent frequency and *P. marinus* survey mean adjusted prevalence decreased, concurrent with an increase in freshwater inflow, compared to 1999 (Fig. 5, A and B). Linear regression of annual percent frequency of *H. nelsoni* infections on freshwater inflow (1991-2000) yielded a regression of:

$$freq = -0.011(inflow)+1.104,$$

where

"freq" is the predicted percent frequency (r^2=0.68, p=0.003).

H. nelsoni percent frequency appears to correlate moderately well with inflow, as indicated by the relatively high r^2 value. Mean adjusted prevalence of *P. marinus* infection on inflow (1991-2000) yielded a linear regression of:

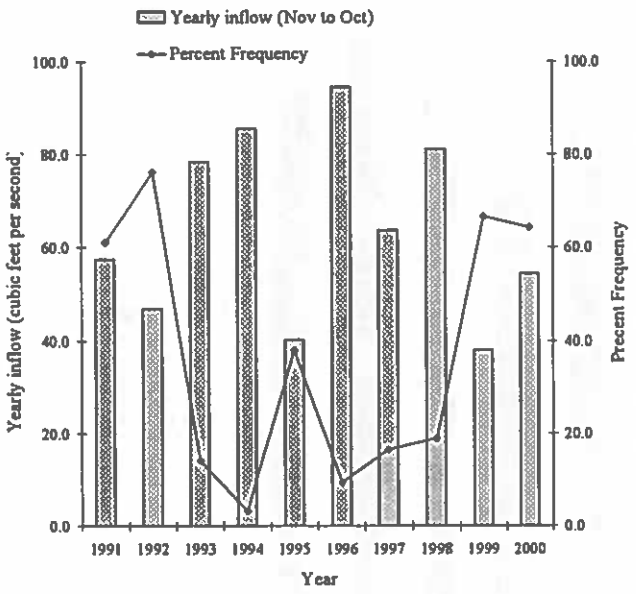
$$prev = -0.027 (inflow)+3.78,$$

where

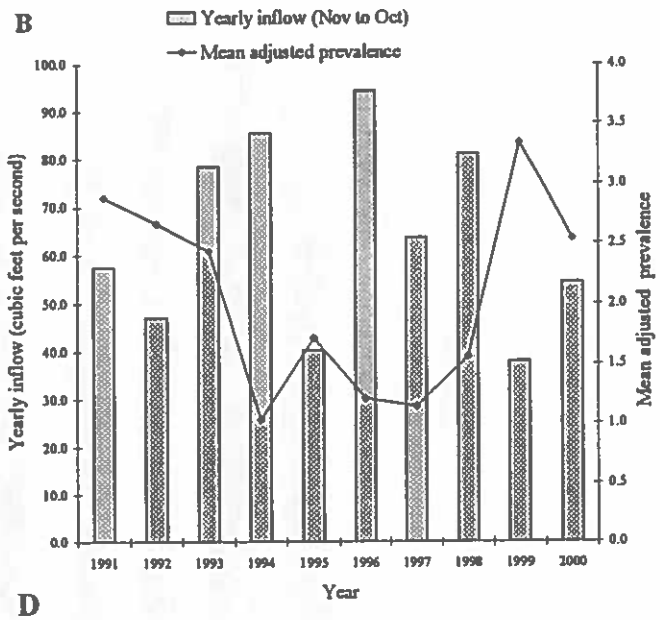
"prev" is the predicted mean adjusted prevalence (r^2=0.43, p=0.040).

Both analyses are consistent with the broad salinity tolerance of *P. marinus* relative to that of the halophilic *H. nelsoni*.

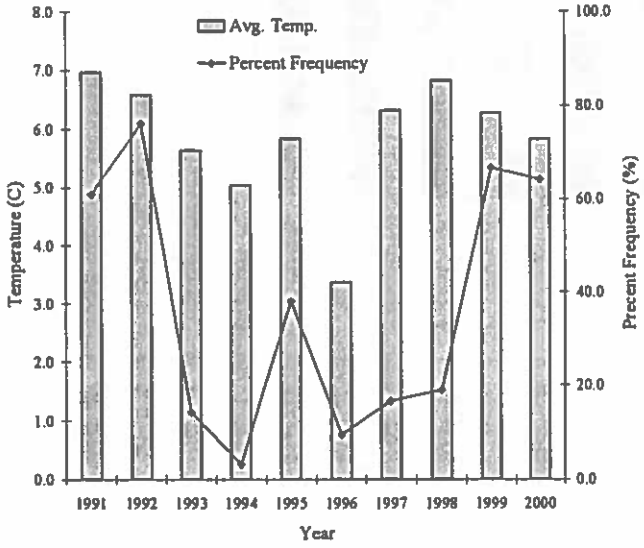
A


Yearly inflow (Nov to Oct)
Percent Frequency
Yearly inflow (cubic feet per second)
Precent Frequency
Year

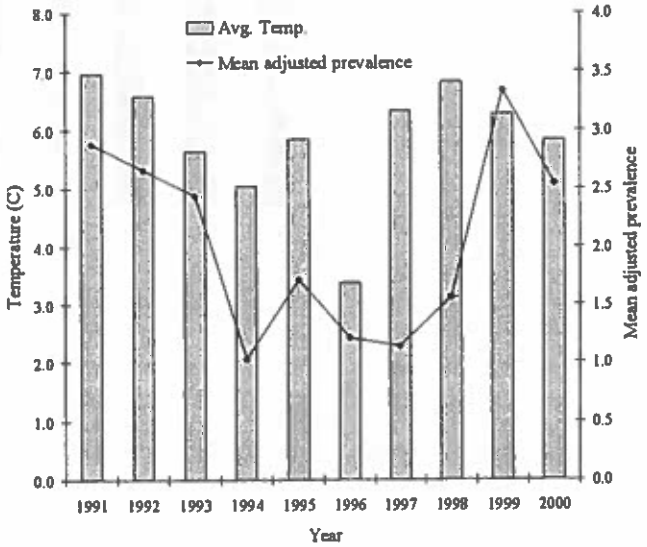
B


Yearly inflow (Nov to Oct)
Mean adjusted prevalence
Yearly inflow (cubic feet per second)
Mean adjusted prevalence
Year

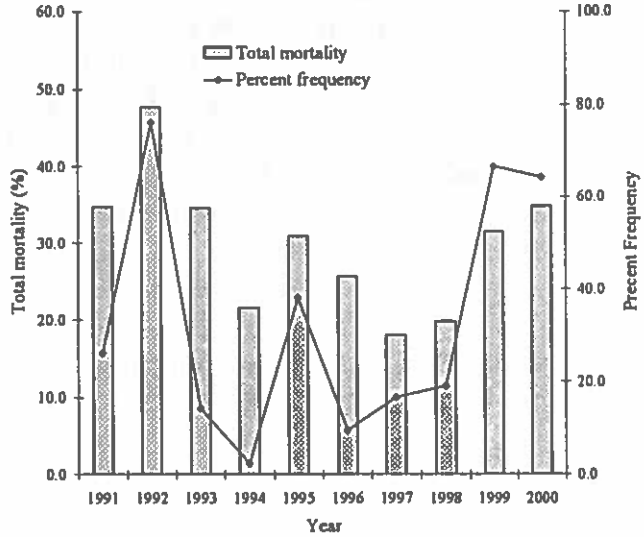
C


Avg. Temp.
Percent Frequency
Temperature (C)
Precent Frequency (%)
Year

D


Avg. Temp.
Mean adjusted prevalence
Temperature (C)
Mean adjusted prevalence
Year

E


Total mortality
Percent frequency
Total mortality (%)
Precent Frequency
Year

F

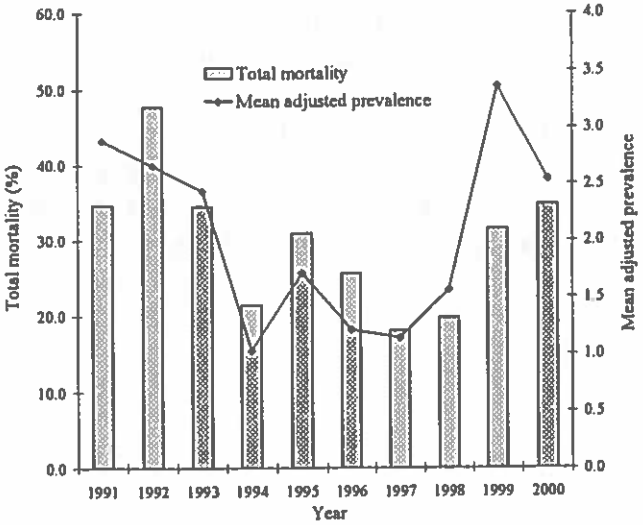
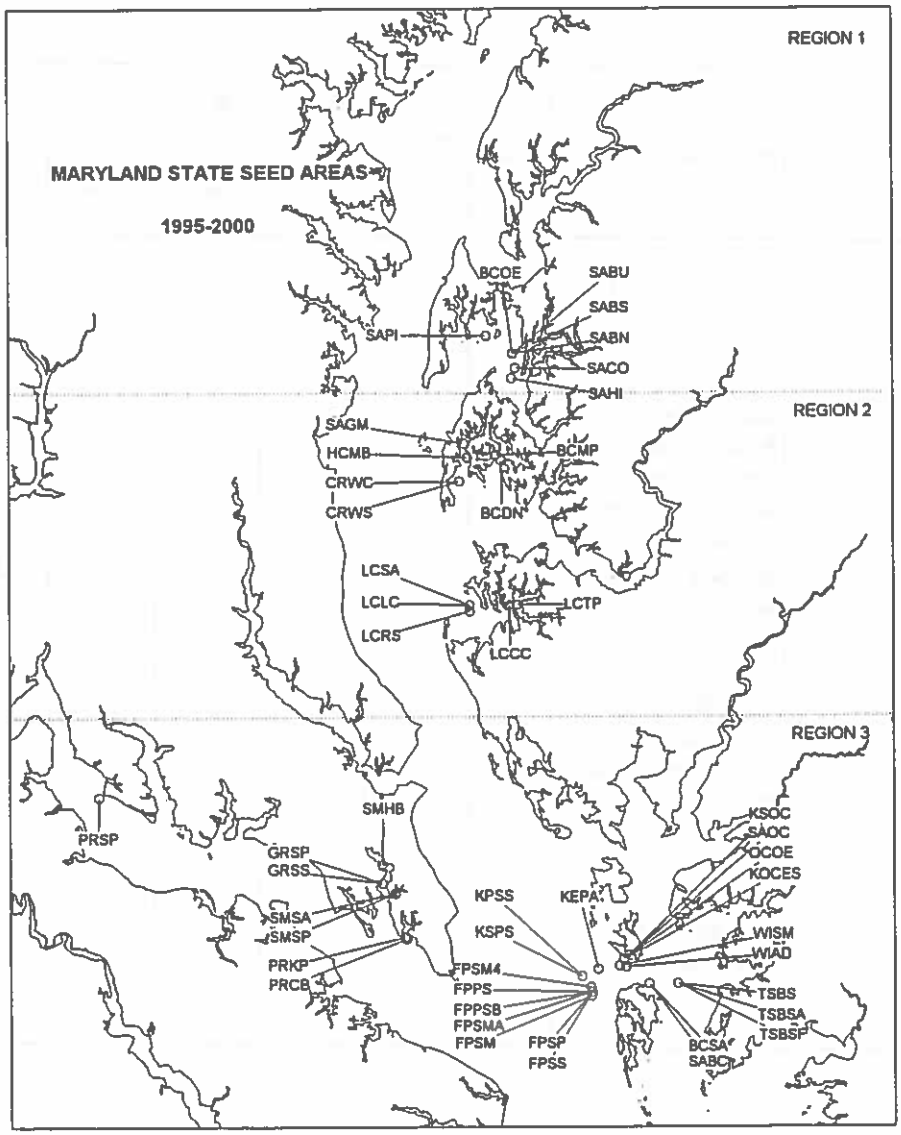

Total mortality
Mean adjusted prevalence
Total mortality (%)
Mean adjusted prevalence
Year

Figure 5. (A through F) **A** and **B**. Annual November to October Chesapeake Bay freshwater inflow vs. *H. nelsoni* percent frequency and *P. marinus* survey mean adjusted prevalence, 1991-2000. In 2000, both *H. nelsoni* and *P. marinus* infection measures declined with an increase in freshwater inflow. **C** and **D**. Annual average winter water temperature vs. *H. nelsoni* percent frequency and *P. marinus* survey mean adjusted prevalence, 1991-2000. In 2000, both *H. nelsoni* and *P. marinus* infection prevalences declined with a decrease in winter water temperature. **E** and **F**. Annual oyster mortality vs. *H. nelsoni* percent frequency and *P. marinus* survey mean adjusted prevalence, 1991-2000. In 2000, oyster mortality increased although there was a marginal decrease in both infection measures.

Table 2. *P. marinus* prevalence and intensity, and *H. nelsoni* prevalence by region on State seed areas, 1999-2000. (1=upper bay, 2=middle bay, 3=lower bay).

Tributary	Seed Areas	Region	P. marinus				H. nelsoni	
			1999		2000		1999	2000
			Prevalence (%)	Mean Intensity	Prevalence (%)	Mean Intensity	Prevalence (%)	Prevalence (%)
Eastern Bay	Bugby N. COE seed	1	7	0.2	50	1.4	0	0
	Bugby	1	63	1.9			0	3
	Coffee	1	90	2.6			0	
	Parson Island	1	53	1.6	3	0.1	0	0
	Bugby N. Seed	1	23	0.6	47	1.1	7	
	Bugby Seed Spat	1	7	0.2	73	2.0	3	0
Choptank River	Wild Cherry Tree	2	43	1.5	20	0.4	27	7
	Wild Cherry Tree Spat	2	3	0.03			0	
	Great Marsh	2	23	0.5			0	
Little Choptank River	Seed Area	2	77	2.1	50	1.5	20	7
Fog Point	State Seed B	3	97	3.9			20	
Kedge Straits	Oyster Creek	3	23	0.6	77	2.2	43	37
	Oyster Creek COE	3	0	0			72	
	Private Seed	3	43	1.2			70	
	Private Seed Spat	3	0	0			37	
	Oyster Creek COE AM	3	3	0.2			57	
St. Mary's River	State Spat	3			13	0.3		0
	State Seed	3			97	4.4		13
	Seed Area	3	90	2.8			60	
	Horseshoe Bend	3	0	0	15	0.2	0	0
Tangier Sound	Back Cove Seed	3			83	2.0		20
	Back Cove Smalls	3	83	3.5			53	
	Back Cove Seed	3	0	0	20	0.2	57	47

As in 1999, *H. nelsoni* infection distribution among the seed areas for 2000 mirrors the MSX disease distribution among the MFS bars, with a wide dispersion northward into the Choptank River and Eastern Bay. A similar 1999/2000 relationship occurred for *P. marinus* infections, whose distribution in 2000 was Bay-wide at varying intensities. Two juvenile oyster samples (TSBS and SMHB) from seed areas that were negative for *P. marinus* in 1999 had prevalences of 20% and 15%, respectively in 2000. Two new 2000 samples (GRSP and GRSS) showed *P. marinus* prevalences of 13% and 97%, respectively. Two other juvenile oyster samples (KOCES and KPSS), that were uninfected in 1999, were not sampled in 2000. A seed area sample (SABC) of relatively large oysters, collected proximal to KOCES and KPSS, was heavily infected by *P. marinus* (83%).



Map 2. Maryland Seed Areas, 1995-2000. Refer to Table 1.3 for the tributary and the barname that corresponds to each barcode.

Disease Susceptibility

Disease data by size range were added to a historic database from 1991 to present (n=8,360) to continually monitor disease susceptibility (Fig. 6). The percentage of *H. nelsoni* and/or *P. marinus* infected oysters was reported for each 5mm size class from 21 to 115 mm shell height. Data are reported only if size class n≥100. Apparent *H. nelsoni* infection susceptibility peaks in the 41-45 mm shell height oyster size class (yearlings), whereas apparent *P. marinus* infection susceptibility peaks in the 61-65 mm shell height size class (2-yr). Oysters are maximally susceptible to *H. nelsoni* infections at 1+ yr., and to *P. marinus* infection at 2+ yr.

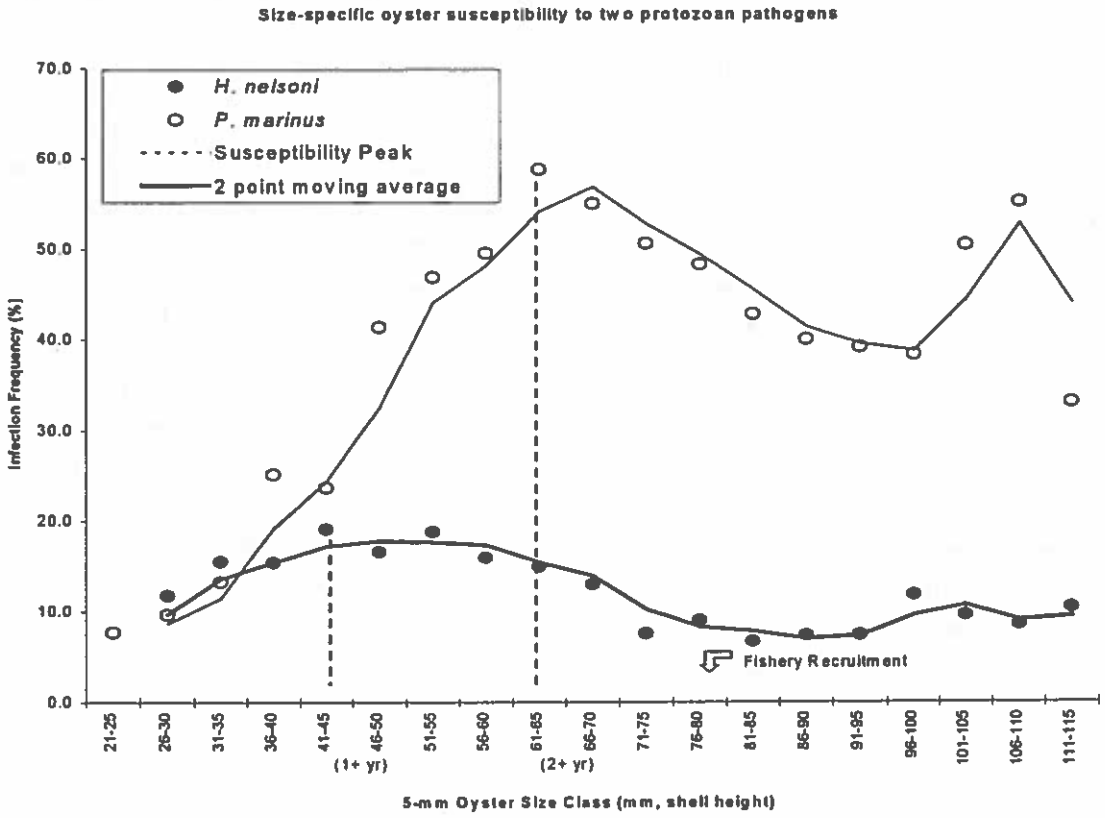


Figure 6. Size-specific susceptibility to the oyster pathogens, *H. nelsoni* and *P. marinus* (n=8,360). Infection frequencies peak in the 41-45 mm and 61-65 mm size classes, respectively, suggesting that apparent size class of maximum susceptibility to both diseases preceeds oyster fishery recruitment. Plotted 2-point moving average is based on the current point and the previous point.

DISCUSSION

For 2000, *H. nelsoni* and *P. marinus* infection prevalences, distributions, and intensities decreased from peak 1999 levels, although the declines were marginal. Among the past ten years, *H. nelsoni* annual percent frequency and *P. marinus* survey mean adjusted prevalence ranked third and fourth respectively, in 2000, compared to second and first, respectively, in 1999. The geographic distribution of both pathogens in 2000 remained similar to 1999, with *P. marinus* enzootic throughout Maryland's Chesapeake Bay, and *H. nelsoni* near the historic northern limit of its distribution.

The decline of *P. marinus* survey mean adjusted prevalence was more pronounced than the slight decline in *H. nelsoni* percent frequency. Since it combines *P. marinus* sample prevalence and sample mean intensity, adjusted prevalence is a more sensitive measure of virulence. An analysis of *P. marinus* prevalences by three categorical ranges (<30%, 30%-59%, and ≥60%) revealed that the proportion of sample prevalences ≥ 60% declined during 2000, with a concurrent increase of sample prevalences in the 30% to 60% range. Sample prevalences of ≥ 60% *P. marinus* infection have been suggested to potentially culminate in an annual mortality that may exceed 50% (Krantz 1995). By three Mackin scale ranges (<1.0, 1.0-2.9, and >3.0), infection intensities in the range ≥ 3.0 declined with a concurrent increase in the 1.0 to 2.9 range. These declines coincide with an increase in Chesapeake Bay freshwater inflow and a decrease in average winter water temperature.

The declines in freshwater inflow and mean winter water temperature in 2000 suggest that both measures could account for changes in oyster disease measures for 2000. In 1999, low freshwater inflow explained most disease measure consequences, as affirmed by linear regression analysis. For 2000, linear regression analysis further indicated that freshwater inflow influences oyster disease more than winter water temperature.

In general, both *H. nelsoni* infection percent frequency and *P. marinus* survey mean adjusted prevalence were inversely correlated with freshwater inflow over the past 10 years, although the effects on MSX disease appear stronger. *P. marinus* mean adjusted prevalence appears less influenced by freshwater inflow, which is consistent with the broad osmotic tolerance of the parasite (Dungan and Hamilton 1995). Prevalence and intensity of *P. marinus* infections fluctuate annually, but the parasite has maintained a chronic presence throughout Chesapeake Bay since 1987 (Burreson and Calvo 1996). The chronic infestations of *P. marinus* have led to lower annual variation, indicated by a comparatively weaker correlation of *P. marinus* mean adjusted prevalence on freshwater inflow, than for that of *H. nelsoni* percent frequency.

Although the mean winter temperature declined in 2000 compared to 1999, it has been thought that mid-Atlantic coast temperatures do not fall low enough to result in any substantial control of *H. nelsoni* infections (Ford and Haskin 1982). Moreover, winter *P. marinus* infection prevalence has been shown not to decline as dramatically as once hypothesized (Burreson and Ragone-Calvo 1996). The absence of a strong statistical correlation between infection rates and winter water temperature

over the last ten years supports the hypothesis that winter temperature does not fall low enough in Chesapeake Bay for oyster disease control.

Freshwater inflow may have accounted for, in most oysters from MFS bars, phagocytosis of *H. nelsoni* by oyster phagocytes which has been suggested to be due to the parasite's physiological inability to tolerate lower salinities (Ford and Haskin 1988). In general, *H. nelsoni* engulfed by phagocytes appeared dense with indistinct nuclei and usually had other non-engulfed, moribund *H. nelsoni* cells proximal to the phagocytized cells. This observation is consistent with histological observations of degenerating *H. nelsoni*, and with observations that oyster hemocytes do not phagocytose live *H. nelsoni* (Ford and Haskin 1982; Ford et al. 1990). Interestingly, oysters from most of the MFS bars, including bars in the lower (high-salinity) bay, showed *H. nelsoni*-infected oysters with phagocytzed parasite cells. Traditionally, the high salinity infection cycle of *H. nelsoni* is relatively insensitive to river flow changes (Paraso et al. 1999). Whether the increased freshwater inflow throughout 2000 challenged the physiological integrity of *H. nelsoni*, or whether Chesapeake Bay oysters have developed some MSX-resistance has not been verified. However, it is encouraging that possible pathogen morbidity and/or oyster resistance has been observed in the lower Chesapeake Bay.

For the seed areas sampled in 2000, *H. nelsoni* and *P. marinus* infection prevalences, distributions, and intensities were similar to 1999 sampled seed areas. *Haplosporidium nelsoni* infections were found in 58% of sampled seed areas in 2000 compared to 65% in 1999. *Perkinsus marinus* infections were found in 100% of sampled seed areas in 2000 compared to 80% in 1999. Two samples of juvenile seed oysters from sites negative for *P. marinus* in 1999 were *P. marinus*-infected in 2000.

Generally, oysters of less than about 30 mm shell height are free of *P. marinus* infections (Burreson and Ragone-Calvo 1996) with maximum apparent susceptibility to *P. marinus* infection observed in larger (older) oyster size classes. Juvenile oysters have been suggested to be refractile to infection, but in 2000, continued high infection pressure may have caused some juvenile oysters to succumb to *P. marinus*. From 1995-2000, a relatively constant infestation rate by *H. nelsoni* has been maintained in lower-bay seed oysters, which coincides with the susceptible host size class and the salinity requirements of *H. nelsoni*.

Due to the sustained high prevalences of *H. nelsoni* and *P. marinus* infections in 2000 feral oyster seed stocks, management activities should be conservative. Shellstock transplanting activities have been advised to avoid areas where *P. marinus* infection prevalence exceeds 60%, due to an increased tendency for disease mortality in such oysters (Krantz 1995). Both oyster diseases significantly correlated with oyster mortality. Due to the relatively wide distribution of *H. nelsoni* and the bay-wide distribution of *P. marinus* above 60% prevalences, a regionalized management strategy should be maintained, which protects low-disease areas from introductions of shellstocks with high disease loads, and which maximizes survival to market size of limited 2000 spatsets.

LITERATURE CITED

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

Christmas, J., McGinty, M., Randle, D. and Jordan, S. 1997. Oyster shell disarticulation in three Chesapeake Bay tributaries. *Journal of Shellfish Research* **16**:115-123.

Dungan, C.F. and Hamilton, R.M. 1995. Use of tetrazolium-based cell proliferation assay to measure effects of in vitro conditions on *Perkinsus marinus* (Apicomplex) proliferation. *Journal of Eukaryotic Microbiology* **42**:379-388.

Ford, S.E. and Haskin, H.H. 1982. History and epizootiology of *Haplosporidium nelsoni* (MSX), an oyster pathogen in Delaware Bay, 1957-1980. *Journal of Invertebrate Pathology* **40**:118-141.

Ford, S.E. and Haskin, H.H. 1988. Comparison of in vitro salinity tolerance of the oyster parasite, *Haplosporidium nelsoni* (MSX) and hemocytes from the host, *Crassostrea virginica*. *Journal of Comparative Biochemistry and Physiology* **90A**:183-187.

Ford, S.E., Kanaley, S.A. and Ashton-Alcox, K.A. 1990. In vitro recognition and phagocytosis of the oyster pathogen MSX. *Journal of Shellfish Research* **8**:468.

Howard, D.W. and Smith, C.S. 1983. *Histological techniques for marine bivalve mollusks*. NOAA Technical Memorandum NMFS-F/NEC-25.

Krantz, G.E. 1995. *Maryland Oyster Population Status 1993 and 1994: Biological Seasons*. Chesapeake Bay Research and Monitoring Division CBRM-OX-95-1. pp12.

Mackin, J.G. 1955. *Dermocystidium marinum* and salinity. *Proceedings of the National Shellfish Association* **46**:116-128.

MDDNR 2001. *Maryland Oyster Population Status Report, 1996-2000 Fall Surveys*. Fisheries Service, Cooperative Oxford Laboratory Program and Shellfish Program.

Paraso, M.C., Ford, S.E., Powell, E.N., Hofmann, E.E., and Klinck, J.M. 1999. Modeling the MSX parasite in eastern oyster (*Crassostrea virginica*) populations. II. Salinity Effects. *Journal of Shellfish Research* **18**:501-516.

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

Ray, S.M. 1966. A review of the culture methods for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proceedings of the National Shellfisheries Association* 54:55-59.

Shaw, B.L. and Battle, H. I. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Canadian Journal of Zoology* 35:325-347.

Smith, G. and Jordan, S. 1993. *Monitoring Maryland's Chesapeake Bay Oysters.* Chesapeake Bay Research and Monitoring Division CBRM-OX-93-3.

APPENDIX I

Table I.1. *Haplosporidium nelsoni* % prevalence on MFS bars, 1991-2000.

Region	Bar	Code	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
Upper Bay	Swan Point	BNSP		0	0		0	0	0	0	0	0
	Hacketts	UBHA		3	0	0	0	0	0	0	0	0
Mid Bay	Holland Point	WSHP		13	0	0	0	0	0	0	0	3
	Stone Rock	MESR		43	0	0	3	0		0	30	47
	Flag Pond	WSFP		53	0	0	10	0	0	0		
	Hog Island	WSHI	0	43	0	0	3	0	0	0	60	27
Lower Bay	Butlers	WSBU	0	50	0	0	13	0	7	3	47	17
Chester River	Buoy Rock	CHBR		0	0		0	0	0	0	0	0
	Oldfield	CHOF		0	0		0	0	0	0	0	0
Eastern Bay	Bugby	EBBU	7	3	0	0	0	0	0	0	0	0
	Hollicutt's Noose	EBHN		17	0	0	0	0	0	0	7	10
	Parsons Island	EBPI		7	0	0	0	0	0	0	0	0
Wye River	Bruffs Island	MRBI		0	0	0	0	0	0	0	0	0
Miles River	Long Point	MRLP		0	0	0	0	0	0	0	0	0
	Turtleback	MRTU		0	0	0	13	0	0	0	0	0
Choptank River	Cooks Point	CRCP	7	73	0	0		0	3	0	13	33
	Lighthouse	CRLI		53	0	0	0	0	0	0	13	7
	Oyster Shell Point	CROS		30	0		0	0	0	0	0	0
	Royston	CRRO		33	0	0	0	0	0	0	3	7
	Sandy Hill	CRSH		13	0		0	0	0	0	0	0
Harris Creek	Tilghman Wharf	CRTW	0	40	0	0	0	0	0	0	3	27
Broad Creek	Deep Neck	BCDN		30	0	0	0	0	0	0	3	7
Tred Avon River	Double Mills	TADM		17	0	0	0	0	0	0	3	0
L. Chop. River	Cason	LCCA		43	0	0	0	0	0	0	7	27
	Ragged Point	LCRP	20	57	0	0	0	0	0	0	20	47
	Normans Additior	HRNO	0	53	0	0	20	0	0	3	63	37
	Goose Creek	FBGC	7	27	7	0	7	0	0	0	47	17
	Wilson Shoals	NRWS		57	0		3	0	0	0	4	10
Manokin River	Georges	MAGE	7	23	0	0	20	0	0	0	40	20
Holland Straits	Holland Straits	HOHO	17	13	13	0	10	0	10	0	73	40
Tangier Sound	Back Cove	TSBC	10	27	33	0	0	7	3	10	33	37
	Old Women's Leg	TSOW	13	23	30	10	17	20	4	23	53	30
	Piney Island East	TSPI	17	17	20	0	10	23	13	17	43	53
	Sharkfin Shoal	TSSS	20	40	17	0	23	7	0	20	53	37
Pocomoke Sound	Marumsco	PSMA	13	20	0	0	10	3	11	7	37	30
Patuxent River	Broomes Island	PXBI		20	0	0	0	0	0	0	3	10
St. Marys River	Chicken Cock	SMCC	0	57	0		0	0	0	0	77	7
	Pagan	SMPA		0	0		0	0	0	0	3	13
Wicomico River	Lancaster	WWLA		0	0		0	0	0	0	0	0
	Mills West	WWMW		0	0		0	0	0	0	3	0
Potomac River	Cornfield Harbor	PRCH	0	57	0	0	17	0	0	3	53	17
	Low Cedar Point	PRLC		0	0		0	0	0	0	0	0
	Ragged Point	PRRP	0	0	0		3	0	0	0	13	10

Table I.2. *Perkinsus marinus* % prevalence and mean intensity on MFS bars, 1991-2000.

Region	Bar	Code	1991		1992		1993		1994		1995	
			P	I	P	I	P	I	P	I	P	I
Upper Bay	Swan Point	BNSP	27	0.7	23	0.4	37	0.8	3	0.0	20	0.2
	Hacketts	UBHA	27	0.8	57	1.2	97	3.2	23	0.5	90	2.5
Mid Bay	Holland Point	WSHP	47	1.1	80	2.4	93	3.0	36	1.1	87	2.9
	Stone Rock	MESR	27	0.9	100	4.4	100	3.5	90	2.5	87	2.2
	Flag Pond	WSFP	97	2.6	97	5.7	88	2.7	30	0.8	87	3
	Hog Island	WSHI	97	4.5	100	4.2	93	2.4	37	1.0	93	2.7
Lower Bay	Butlers	WSBU	100	4.0	81	2.4	97	3.3	80	2.1	87	2.5
Chester River	Buoy Rock	CHBR	90	2.5	97	2.8	93	3.3	10	0.3	67	1.7
	Oldfield	CHOF	20	0.5	37	0.9	83	2.4	20	0.6	83	2.3
Eastern Bay	Bugby	EBBU	100	4.0	73	1.8	100	3.0	43	0.8	83	2.6
	Hollicutt's Noose	EBHN	73	2.0	82	2.1	97	2.7	70	1.7	90	2.8
	Parsons Island	EBPI	97	3.6	80	2.1	100	3.3	93	3.1	70	2.1
Wye River	Bruffs Island	MRBI	100	3.3	93	3.0	83	2.6	63	1.3	73	2.1
Miles River	Long Point	MRLP	97	4.3	86	3.0	77	2.6	60	2.0	67	2.2
	Turtleback	MRTU	100	3.3	77	1.6	100	3.3	60	1.2	100	2.8
Choptank River	Cooks Point	CRCP	23	0.3	87	3.7	97	4.2	90	3.0	0	0.0
	Lighthouse	CRLI	100	4.0	100	4.6	93	3.2	47	1.2	90	3.3
	Oyster Shell Point	CROS	60	1.7	100	3.9	93	2.8	10	0.3	68	1.8
	Royston	CRRO	100	4.5	97	4.8	100	3.3	80	2.0	63	2.0
	Sandy Hill	CRSH	100	5.7	100	4.2	100	3.8	83	2.3	89	3.4
Harris Creek	Tilghman Wharf	CRTW	97	3.0	100	3.4	100	3.2	63	1.9	93	2.5
Broad Creek	Deep Neck	BCDN	100	5.6	100	3.7	100	3.8	67	2.3	97	-9.0
Tred Avon River	Double Mills	TADM	100	4.9	100	4.1	100	3.8	90	2.0	75	2.5
L. Chop. River	Cason	LCCA	100	4.4	90	2.6	93	2.8	7	0.2	93	2.3
	Ragged Point	LCRP	100	4.6	100	5.0	100	3.9	87	2.3	93	2.5
	Normans Addition	HRNO	100	3.4	83	2.0	96	3.6	93	3.3	87	2.8
	Goose Creek	FBGC	100	3.1	100	3.6	87	2.1	53	1.1	87	2.5
	Wilson Shoals	NRWS	100	2.8	90	2.5	83	1.6	40	0.9	63	1.1
Manokin River	Georges	MAGE	93	2.9	58	1.4	30	0.7	50	1.2	87	2.8
Holland Straits	Holland Straits	HOHO	100	4.0	100	3.4	76	2.3	57	1.6	93	3.1
Tangier Sound	Back Cove	TSBC	100	4.2	97	3.3	36	1.0	80	2.2	83	3.0
	Old Women's Leg	TSOW	100	4.5	100	4.0	82	2.0	73	2.1	100	4.2
	Piney Island East	TSPI	100	3.9	87	2.7	83	2.2	87	3.1	93	2.5
	Sharkfin Shoal	TSSS	60	1.2	97	2.8	93	2.2	63	1.4	90	3.0
Pocomoke Sound	Marumsco	PSMA	93	3.3	60	1.3	87	2.5	72	1.6	100	4.2
Patuxent River	Broomes Island	PXBI	100	2.8	63	1.5	87	3.0	40	0.6	43	1.0
St. Marys River	Chicken Cock	SMCC	97	3.1	93	3.2	96	2.6	40	1.0	83	1.9
	Pagan	SMPA	97	2.3	100	3.0	93	2.1	10	0.3	93	2.2
Wicomico River	Lancaster	WWLA	97	2.8	67	1.4	67	1.6	20	0.2	27	0.6
	Mills West	WWMW	80	2.0	90	2.9	63	1.8	20	0.2	57	1.4
Potomac River	Cornfield Harbor	PRCH	83	2.3	100	3.8	93	2.9	77	1.9	93	2.5
	Low Cedar Point	PRLC	10	0.3	23	0.6	7	0.1	83	2.2	13	0.2
	Ragged Point	PRRP	90	2.8	40	0.9	50	1.4	10	0.2	33	0.8

Table L2 cont. *Perkinsus marinus* % prevalence and mean intensity on MFS bars, 1991-2000.

Region	Bar	Code	1996		1997		1998		1999		2000	
			P	I	P	I	P	I	P	I	P	I
Upper Bay	Swan Point	BNSP	-9	0.0	3	0.1	43	1.2	97	3.4	80	1.8
	Hacketts	UBHA	30	0.7	43	1.3	43	1.1	97	3.3	97	3.7
Mid Bay	Holland Point	WSHP	47	1.4	37	1.1	37	0.9	93	2.8	87	3.4
	Stone Rock	MESR	93	2.7	90	2.3	100	3.5	100	4.0	93	3.6
	Flag Pond	WSFP	63	2.0	53	1.2	73	2.3	0	0.0	0	0.0
	Hog Island	WSHI	43	1.2	47	1.3	97	3.2	93	5.5	83	3.9
Lower Bay	Butlers	WSBU	60	1.6	57	1.0	97	3.3	93	3.2	83	2.7
Chester River	Buoy Rock	CHBR	13	0.4	7	0.7	33	0.9	93	3.0	97	3.5
	Oldfield	CHOF	0	0.0	10	0.2	33	0.8	97	3.0	93	3.1
Eastern Bay	Bugby	EBBU	80	2.0	70	1.8	60	1.4	100	3.9	100	4.0
	Hollicutt's Noose	EBHN	60	1.4	50	1.0	83	2.5	90	3.0	100	4.1
	Parsons Island	EBPI	73	2.8	63	1.4	80	2.5	100	4.7	100	3.5
Wye River	Bruffs Island	MRBI	67	1.4	17	0.2	57	1.6	100	3.7	97	3.2
Miles River	Long Point	MRLP	20	0.4	23	0.6	100	2.7	100	3.6	97	3.3
	Turtleback	MRTU	83	2.1	83	1.8	50	1.6	100	4.3	97	3.1
Choptank River	Cooks Point	CRCP	60	1.5	70	2.4	87	2.8	93	3.4	40	1.2
	Lighthouse	CRLI	77	1.8	57	1.5	43	1.5	87	2.3	100	3.4
	Oyster Shell Point	CROS	13	0.2	50	0.9	20	0.3	83	2.3	73	2.2
	Royston	CRRO	50	1.1	67	1.5	90	2.5	97	3.5	97	4.7
	Sandy Hill	CRSH	30	0.7	60	1.3	40	1.0	97	3.4	87	3.6
Harris Creek	Tilghman Wharf	CRTW	67	1.3	60	1.0	67	2.0	87	2.5	93	3.4
Broad Creek	Deep Neck	BCDN	83	2.1	100	2.6	97	2.9	97	4.5	100	4.0
Tred Avon River	Double Mills	TADM	70	1.2	82	2.0	100	3.0	100	4.8	100	4.7
L. Chop. River	Cason	LCCA	87	1.9	93	2.4	50	1.4	97	3.8	100	3.6
	Ragged Point	LCRP	97	2.6	97	2.1	87	2.9	100	4.0	97	3.7
	Normans Addition	HRNO	93	2.4	73	1.6	73	2.3	93	3.5	80	2.4
	Goose Creek	FBGC	97	4.0	83	2.0	100	3.0	100	5.4	97	3.1
	Wilson Shoals	NRWS	83	1.8	80	1.9	70	1.6	100	4.3	70	2.1
Manokin River	Georges	MAGE	93	2.0	93	2.2	83	2.4	93	3.5	80	2.3
Holland Straits	Holland Straits	HOHO	83	2.0	67	1.8	57	1.2	80	2.5	30	0.9
Tangier Sound	Back Cove	TSBC	97	3.2	93	2.9	90	2.3	100	5.5	40	1.2
	Old Women's Leg	TSOW	80	2.3	57	1.3	90	3.2	87	3.9	70	1.7
	Piney Island East	TSPI	63	1.7	73	2.2	83	1.9	63	2.4	86	2.3
	Sharkfin Shoal	TSSS	97	2.1	93	2.6	80	2.7	100	4.3	80	2.3
Pocomoke Sound	Marumsco	PSMA	90	2.4	61	2.1	80	2.8	90	3.4	93	2.7
Patuxent River	Broomes Island	PXBI	17	0.4	83	2.1	93	3.0	100	4.6	93	4.0
St. Marys River	Chicken Cock	SMCC	77	1.4	73	1.7	80	1.7	100	5.0	63	1.8
	Pagan	SMPA	82	1.4	86	1.7	73	1.7	97	3.4	68	1.6
Wicomico River	Lancaster	WWLA	56	1.2	80	1.6	37	0.7	83	3	90	3
	Mills West	WWMW	60	1.2	77	1.7	20	0.4	90	3.2	97	3.6
Potomac River	Cornfield Harbor	PRCH	87	2.0	83	1.8	83	2.0	97	3.9	80	2.1
	Low Cedar Point	PRLC	3	0.0	0	0.0	0	0.0	0	0.0	17	0.5
	Ragged Point	PRRP	7	0.2	0	0.0	0	0.0	17	0.5	13	0.7

Table I.3. *Haplosporidium nelsoni* % prevalence on State seed areas, 1995-2000.

Code	Tributary	Seed Areas	R	1995	1996	1997	1998	1999	2000
BCOE	Eastern Bay	Bugby N. COE seed	1					0	0
SABU		Bugby	1			0	0	0	3
SACO		Coffee	1			0		0	
SAPI		Parson Island	1			0		0	0
SABN		Bugby N. Seed	1					7	
SABS		Bugby Seed Spat	1					3	0
SAHI	Miles River	Herring Island SA	1			0			
BCMP	Broad Creek	Mulberry Point	2	0	0				
CRWC	Choptank River	Wild Cherry Tree	2	0	0	0	0	27	7
CRWS		Wild Cherry Tree SP	2					0	
SAGM		Great Marsh	2					0	
LCCC	Little Choptank River	Cedar Cove	2	0					
LCLC		Little Choptank	2	3	0				
LCRS		Ragged Point	2	0					
LCTP		Town Point	2	0	0				
LCSA		Seed Area	2					20	7
HCMB	Harris Creek	Mill Bar	2	0					
FPPS	Fog Point	Private Seed	3	73	0	23			
FPPSB		State Seed B	3					20	
FPSM		State Seed '94	3	40	3	13	7		
FPSM4		Seed/ Sample 2	3						
FPSP		State Spat	3	80	23	23			
FPSS		State Seed '93	3	37					
FPSMA		State Seed '93	3		0				
KEPA	Kedges Strait	EPA Plant	3	23		17			
KSOC		Oyster Creek	3			3	30	43	37
KOCES		Oyster Creek COE	3					72	
KSPS		Private Seed	3			10	7	70	
KPSS		Private Seed Spat	3					37	
OCOE		Oyster Creek COE AM	3					57	
SAOC		Oyster Creek Spat	3				7		
PRCB	Potomac River	Calvert Bay	3	7	0				
PRKP		Kitt's Point	3	67					
GRSP	St. Mary's River	State Spat	3						0
GRSS		State Seed	3						13
SMSA		Seed Area	3	0	0		0	60	
SMSP		Seed Area	3			0			
SMHB		Horseshoe Bend	3					0	0
SABC	Tangier Sound	Back Cove Seed	3			30	30		20
BCSA		Back Cove Smalls	3					53	
TSBS		Back Cove Seed	3		3	23		57	47
TSBS-F		":" Fines	3		0				
TSBSA		Back Cove Seed	3				3		
WIAD	Western Is.	Addition			13				
WISM		State Smalls		39	10	17	7		

Table I.4. Prevalence (P) and intensity (I) of *P. marinus* infections by region on State seed areas, 1995-2000. (R = region, 1 = upper bay, 2 = mid bay, 3 = lower bay)

Code	Tributary	Bar	R	1995		1996		1997		1998		1999		2000	
				P	I	P	I	P	I	P	I	P	I	P	I
BCOE	Eastern Bay	Bugby N. COE seed	1									7	0.2	50	1.4
SABU		Bugby	1					0	0	0	0	63	1.9		
SACO		Coffee	1					0	0	7	0.2	90	2.6		
SAPI		Parson Island	1					7	0.1			53	1.6	3	0.1
SABN		Bugby N. Seed	1									23	0.6	47	1.1
SABS		Bugby Seed Spat	1						.			7	0.2	73	2.0
SAHI	Miles River	Herring Island SA	1					30	0.4						
BCMP	Broad Creek	Mulberry Point	2	100	5.1	57	2.2								
CRWC	Choptank River	Wild Cherry Tree	2	20	0.4	17	0.2	0	0	3	0.03	43	1.5	20	0.4
CRWS		Wild Cherry Tree SP	2									3	0.03		
SAGM		Great Marsh	2									23	0.5		
LCCC	Little Choptank	Cedar Cove	2	100	4.2	97	2.7								
LCLC		Little Choptank	2	90	3.2	53	1								
LCRS		Ragged Point	2	90	3.4										
LCTP		Town Point	2	83	3.5	83	1.6	97	2.4						
LCSA		Seed Area	2									77	2.1	50	1.5
HCMB	Harris Creek	Mill Bar	2												
FPPS	Fog Point	Private Seed	3			33	0.5	100	3.7						
FPPSB		State Seed B	3									97	3.9		
FPSM		State Seed '94	3			90	2.5	100	3.3	100	4.5				
FPSM4		Seed/ Sample 2	3												
FPSP		State Spat	3			50	0.6	100	2.6						
FPSS		State Seed '93	3												
FPSMA		State Seed '93	3			90	3.3								
KEPA	Kedge Straits	EPA Plant	3					63	1.7						
KSOC		Oyster Creek	3					7	0.1	3	0.1	23	0.6	77	2.2
KOCES		Oyster Creek COE	3							0	0	0	0		
KSPS		Private Seed	3					0	0			43	1.2		
KPSS		Private Seed Spat	3									0	0		
OCOE		Oyster Creek COE AM	3									3	0.2		
SAOC		Oyster Creek Spat	3							3	0.03				
PRCB	Potomac River	Calvert Bay	3	97	4	83	2.1	97	3.2						
PRKP		Kitt's Point	3	7	0.07										
PRSP		Swan Point	3	0	0	3	0.03								
GRSP	St. Mary's River	State Spat	3											13	0.3
GRSS		State Seed	3											97	4.4
SMSA		Seed Area	3	97	4.1	80	2.4	80	2.2	63	1.7	90	2.8		
SMSP		Seed Area	3					3	0.03						
SMHB		Horseshoe Bend	3									0	0	15	0.2
SABC	Tangier Snd	Back Cove Seed	3					0	0	7	0.2			83	2.0
BCSA		Back Cove Smalls	3									83	3.5		
TSBS		Back Cove Seed	3			3	0.03	3	0.1			0	0	20	0.2
TSBS-F		":" Fines	3			0	0								
TSBSA		Back Cove Seed	3							85	2.4				
WIAD	Western Is.	Addition	3			0	0								
WISM		State Smalls	3	43	1.5	77	2.2	30	0.6	90	3.3				

APPENDIX II

Example Calculations

Bar = MAGE

Individual oyster #	***P. marinus*** Infection intensity	***H. nelsoni*** Presence (+) or Absence (-)
1	5	-
2	2	-
3	0	-
4	2	+
5	5	-
6	3	-
7	2	-
8	3	+
9	0	-
10	2	-
11	0	-
12	0	-
13	3	-
14	5	-
15	7	-
16	4	-
17	2	-
18	6	-
19	2	-
20	1	-
21	4	+
22	0	-
23	2	-
24	2	-
25	1	+
26	2	-
27	1	-
28	3	+
29	1	+
30	0	-

Sample % prevalence:

(sample infected *n* / sample *n*) (100)

P. marinus
(24/30)(100) = **80.0 %**

H. nelsoni
(6/30)(100) = **20.0 %**

Sample mean intensity: *P. marinus*

$\sum_{i=0}^{7} n_i\,(i)$ / sample *n*

[4(1) + 9(2) + 4(3) + 2(4) + 3(5) + 1(6) + 1(7)]/30 = **2.33**

Sample adjusted prevalence: *P. marinus*

(sample % prevalence/100)(sample mean intensity)

(80.0/100)(2.33) = **1.86**

Bar Code	2000 *P. marinus* sample adjusted prevalence	2000 *H. nelsoni* sample % prevalence
BNSP	1.44	0
UBHA	3.56	0
WSHP	2.96	3
MESR	3.32	47
WSHI	3.26	27
WSBU	2.27	17
CHBR	3.42	0
CHOF	2.86	0
EBBU	4.03	0
EBHN	4.10	10
EBPI	3.53	0
MRBI	3.13	0
MRLP	3.17	0
MRTU	2.98	0
CRCP	0.48	33
CRLI	3.43	7
CROS	1.63	0
CRRO	4.56	7
CRSH	3.16	0
CRTW	3.16	27
BCDN	4.00	7
TADM	4.73	0
LCCA	3.60	27
LCRP	3.56	47
HRNO	1.90	37
FBGC	3.04	17
NRWS	1.49	10
MAGE	1.86	20
HOHO	0.27	40
TSBC	0.49	37
TSOW	1.21	30
TSPI	1.95	53
TSSS	1.82	37
PSMA	2.51	30
PXBI	3.72	10
SMCC	1.12	7
SMPA	1.11	13
WWLA	2.43	0

Survey mean adjusted prevalence: *P. marinus*

$\left(\sum_{i=1}^{n\,\text{bars}} \text{sample adjusted prevalence}\right) / n$ bars

(1.44 + 3.56 + 2.96 + 3.32 + 3.26 + 2.27 + 3.42 + 2.86 + 4.03 + 4.10 + 3.53 + 3.13 + 3.17 + 2.98 + 0.48 + 3.43 + 1.63 + 4.56 + 3.16 + 3.16 + 4.00 + 4.73 + 3.60 + 3.56 + 1.90 + 3.04 + 1.49 + 1.86 + 0.27 + 0.49 + 1.21 + 1.95 + 1.82 + 2.51 + 3.72 + 1.12 + 1.11 2.43 + 3.52 + 1.70 + 0.08 + 0.09) / 42 = **2.54**

Annual percent frequency: *H. nelsoni*

(*n* bars infected / *n* total bars) (100)

(27 infected bars / 42 total bars)(100) = **64.3%**