



FEATURE ARTICLE:

Declining impact of an introduced pathogen: *Haplosporidium nelsoni* in the oyster *Crassostrea virginica* in Chesapeake Bay

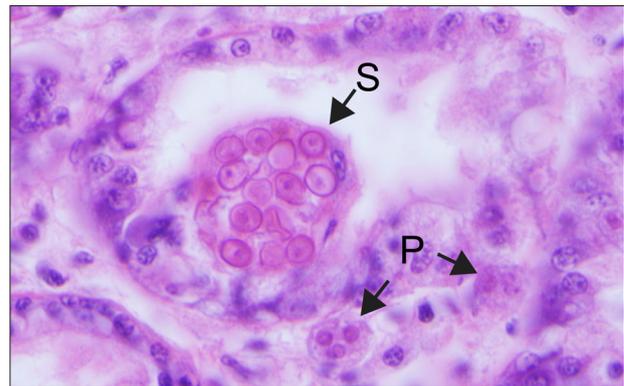
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ABSTRACT: Disease caused by the parasite *Haplosporidium nelsoni* has devastated *Crassostrea virginica* in Chesapeake Bay, exacerbating effects of overharvesting and adversely impacting the ecology of the bay. *H. nelsoni* is thought to persist as an impediment to oyster restoration because strong reproductive contributions from oysters in low-salinity refugia from parasitism have prevented development of disease resistance. On the contrary, long-term data indicate that while infection pressure on naïve sentinels has grown, *H. nelsoni* levels in wild oysters have fallen, with prevalence typically below 20% and advanced infections uncommon. A transplant experiment comparing naïve sentinels with oysters from disease-enzootic populations indicated that these observations represent true disease resistance, and its geographical distribution was revealed by annual fall surveys, and by intensive sampling in 2007 and 2008. Resistance is best developed in the small, polyhaline Lynnhaven River, where salinities are never low enough to suppress *H. nelsoni*. Resistance gradually decreases up the large tributaries and the bay, as revealed by infections reaching higher levels further upstream when drought allows *H. nelsoni* to colonize unselected oyster populations. Elevated disease in small oysters from the Rappahannock River suggests that a non-resistant component of the population persists due to reproductive contributions from unselected oysters in upriver sanctuaries from disease selection, and from susceptible but pre-disease oysters in the lower river. Contributions of these unselected oysters notwithstanding, our results point to substantial reproduction by resistant oysters in disease-enzootic waters, and suggest that reefs in mesohaline-polyhaline salinities, and not only those in disease-free lower mesohaline areas, should be the focus of conservation and restoration efforts.

KEY WORDS: *Haplosporidium nelsoni* · Multinucleated sphere X disease · MSX · *Crassostrea virginica* · Disease resistance · Oyster restoration

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Haplosporidium nelsoni spores (S) and plasmodia (P) in a rare heavy infection of an oyster, *Crassostrea virginica*, from lower Chesapeake Bay

Image: Ryan Carnegie

INTRODUCTION

The protistan parasite *Haplosporidium nelsoni*, the causative agent of MSX (multinucleated sphere X) disease, is notorious for its devastating impacts on populations of the oyster *Crassostrea virginica* along the mid-Atlantic coast of the USA. The parasite was introduced from Asia (Bureson et al. 2000) sometime prior to 1957, when it emerged in Delaware Bay; by 1959, oysters were dying of *H. nelsoni* parasitism also in Chesapeake Bay (Andrews 1962, Haskin et al. 1966). Oyster mortality due to this parasite exceeded 90% on reefs in lower Delaware and Chesapeake Bays during the early years of the epizootic (Ford & Haskin 1982, Haskin & Andrews 1988), an acute impact that presumably reflected a new encounter between an introduced parasite and a naïve host (Bureson et al. 2000). *H. nel-*

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soni supplanted *Perkinsus marinus* as the dominant oyster pathogen in Chesapeake Bay and remained so until the 1980s, when *P. marinus* intensified in activity and colonized previously disease-free oyster populations in waters of salinity <15 ppt (Burreson & Ragone Calvo 1996). The haplosporidian was the only significant pathogen of oysters in Delaware Bay until *P. marinus* colonized this system during its range expansion northward in the 1990s (Ford 1996). *H. nelsoni* persists in both estuaries, and has caused epizootic disease as far north as Maine (Barber et al. 1997) and Nova Scotia (Burreson 2006). In Chesapeake Bay, the ecological effects of *H. nelsoni* and later *P. marinus* parasitism compounded those of overharvesting, and have been profound: loss of a key suspension feeder and habitat provider, shifting the balance from benthic to planktonic production and increasing the transport of planktonic organic matter to deep channels, exacerbating seasonal hypoxia (Kemp et al. 2005).

The impact of *Haplosporidium nelsoni* in Delaware Bay has waned in recent years. The parasite is often undetected in samples from annual autumn surveys conducted by Rutgers University scientists (S. E. Ford & D. Bushek unpubl. data, cited in Ford et al. 2009). This is not due to a change in the parasite's virulence or its disappearance from Delaware Bay, since transplanted oysters from populations unexposed to *H. nelsoni* continue to develop high levels of *H. nelsoni* parasitism, and since an abundance of the parasite, or its DNA, can be detected by PCR in association with oysters without histologically observable infections (Ford et al. 2009). The diminished impact of *H. nelsoni* on native Delaware Bay oysters relates instead to the apparent effects of selection for disease resistance among Delaware Bay oyster stocks. As early as the 1970s, it was clear that naturally selected oysters from *H. nelsoni*-enzootic waters displayed survival superior to oysters from unselected populations (Haskin & Ford 1979, Ford & Haskin 1982). Until the late 1980s, however, disease and mortality caused by *H. nelsoni* were still significant in Delaware Bay, probably because large numbers of unselected, susceptible oysters still inhabited low-salinity refugia from parasitism in the upper bay, and exported larvae to *H. nelsoni*-enzootic waters of the more saline lower bay. A major *H. nelsoni* epizootic in the mid to late 1980s swept even the upper Delaware Bay beds, purging the most susceptible oysters from the population and leaving it generally resistant to serious *H. nelsoni* parasitism (Ford et al. 2009).

Oyster populations in Chesapeake Bay, unlike those in Delaware Bay, are thought to display little resistance to *Haplosporidium nelsoni*. In Virginia, oysters are most abundant in the middle to upper reaches of the large rivers of the lower Chesapeake's western shore, 'where summer-fall salinities are below 20 ppt [and]

where MSX seldom penetrates' (Andrews 1983, p. 268), a function not only of reduced *H. nelsoni* activity there but also of limited predation (Haven et al. 1978) and limited *Perkinsus marinus* parasitism (Burreson & Ragone Calvo 1996). Oysters in these low-salinity sanctuaries from *H. nelsoni* parasitism are still relatively unselected for *H. nelsoni* resistance, and reproductive contributions from this large pool of unselected oysters are believed to have retarded the development of MSX disease resistance in higher salinity waters (Mann et al. 1991). Physical modeling indicating that oyster populations in upstream sanctuaries from disease may supply larvae to disease-intense downstream waters (North et al. 2008) has reinforced the perception that reproductive and larval contributions from upstream oysters are significant.

Annual spring imports by our laboratory of MSX-naïve, susceptible *Crassostrea virginica* from the disease-free upper Rappahannock River, Virginia, to the disease-intense lower York River have shown *Haplosporidium nelsoni* activity to continue unabated in lower Chesapeake Bay (e.g. Ragone Calvo et al. 2003). Nevertheless, we have begun to note reduced *H. nelsoni* infection, relative to such naïve imports, in wild oysters native to disease-intense waters. At the same time, significant accumulations of large, old oysters in the lower part of the Rappahannock River, an area until recently closed to harvesting and long characterized by high MSX disease pressure, have caught the attention of the harvest community as well as the general public. These observations raised the prospect of resistance to *H. nelsoni*, and prompted a re-evaluation of *H. nelsoni* parasitism in contemporary oyster populations. We describe the results of this investigation in this paper.

MATERIALS AND METHODS

Spring imports. Since 1960, naïve, susceptible *Crassostrea virginica* from lower salinity waters have been deployed each spring to the Virginia Institute of Marine Science (VIMS) beach on the *Haplosporidium nelsoni*-enzootic York River to assess the relative timing and intensity of the annual MSX epizootic. In the early years, these oysters were collected from the James River seed area, typically from Horsehead Rock, which was free of both *H. nelsoni* and *Perkinsus marinus* because of generally low salinities. After invasion of the James River seed area by both parasites in the 1980s, Ross Rock in the upper Rappahannock River was used as the spring import oyster source (Burreson & Ragone Calvo 1996, Ragone Calvo et al. 2003). In late April of each year, ~700 market-sized oysters (>3 inches or 76.2 mm) were collected by dredge,

scrubbed free of fouling organisms, and deployed to cages in the lower intertidal York River at VIMS (Table 1, Fig. 1). Around the first of each month from June through October or November, monthly mortality was determined and samples ($n = 25$) were collected for pathological evaluations (see 'Pathological analyses' below).

Fall survey and James River survey. Since 1986, samples of market-sized oysters ($n = 25$ oysters per sample) have been collected by dredge each October from natural reefs in the Virginia portion of Chesapeake Bay to monitor the distribution and intensity of oyster diseases. Since the late 1990s, the number of sites monitored in each fall survey has approached 30 (Table 1). Four reefs in the James River (Wreck Shoal, Point of Shoal, Horsehead Rock, and Deepwater Shoal)

have been sampled on a more intensive monthly to quarterly basis to generate more detailed data on seasonal oyster disease cycles. Processing for pathological analysis is described in 'Pathological analyses' below.

York River beach trial. In late April 2006, an experiment was initiated to evaluate the relative susceptibility to *Haplosporidium nelsoni* of oysters from 3 Virginia populations. About 500 small oysters (<60 mm) were collected from each of 3 wild oyster reefs — Ross Rock in the Rappahannock River, Aberdeen Rock in the York River, and Wreck Shoal in the James River — and an additional 500 domesticated DEBY-strain oysters (Ragone Calvo et al. 2003) were obtained from a population held by a commercial firm on Mobjack Bay (Table 1, Fig. 1). Ross Rock is free of *H. nelsoni*, and oysters there are highly susceptible to MSX disease, as noted earlier. Oysters from

Table 1. Study and sample locations for the York River beach trial (YRB), the size-specific parasitism study (SSP), and the long-term fall survey (FS) and spring import (SI) programs. One additional site, Shell Bar reef, is listed as temperature (T) and salinity (S) data are presented for this site in Fig. 7

System	Site	Latitude / Longitude	Fall salinity (ppt)	Project
Great Wicomico River	Haynie Bar	37° 49' 47" N / 76° 18' 33" W	10–21	FS
	Shell Bar reef	37° 49' 43" N / 76° 19' 05" W	–	T, S data
	Sandy Point	37° 49' 14" N / 76° 18' 45" W	–	SSP
	Whaley's East	37° 48' 31" N / 76° 18' 00" W	10–21	FS
	Fleet Point	37° 48' 35" N / 76° 17' 19" W	10–21	FS
Rappahannock River	Ross Rock	37° 54' 04" N / 76° 47' 21" W	5–13	FS, SI, YRB
	Bowler's Rock	37° 49' 36" N / 76° 44' 07" W	7–15	FS
	Long Rock	37° 48' 59" N / 76° 42' 50" W	7–15	FS
	Morattico Bar	37° 46' 55" N / 76° 39' 33" W	9–17	FS
	Smokey Point	37° 43' 09" N / 76° 34' 56" W	10–18	FS
	Hog House Rock	37° 38' 30" N / 76° 33' 04" W	10–19	FS
	Middle Ground	37° 41' 00" N / 76° 28' 24" W	10–19	FS
	Drumming Ground	37° 38' 38" N / 76° 27' 59" W	10–20	FS
	Parrot Rock	37° 36' 21" N / 76° 25' 20" W	10–20	FS
Broad Creek	37° 34' 37" N / 76° 18' 03" W	11–20	FS, SSP	
Piankatank River	Ginney Point	37° 32' 00" N / 76° 24' 12" W	11–20	FS
	Palace Bar	37° 31' 36" N / 76° 22' 12" W	12–20	FS
	Burton Point	37° 30' 54" N / 76° 19' 42" W	12–20	FS
Mobjack Bay	Ware River	37° 23' 04" N / 76° 27' 27" W	–	YRB
	Tow Stake	37° 20' 20" N / 76° 23' 10" W	16–24	FS
	Pultz Bar	37° 21' 11" N / 76° 21' 10" W	17–21	FS
York River	Bell Rock	37° 29' 03" N / 76° 44' 59" W	6–25	FS
	Aberdeen Rock	37° 20' 07" N / 76° 36' 02" W	10–23	FS, YRB
	VIMS Beach	37° 14' 52" N / 76° 30' 00" W	–	SI, YRB
James River	Deepwater Shoal	37° 08' 56" N / 76° 38' 08" W	2–15	FS
	Mulberry Point	37° 07' 09" N / 76° 37' 55" W	3–17	FS
	Horsehead Rock	37° 06' 24" N / 76° 38' 02" W	3–18	FS, SI
	Point of Shoal	37° 04' 37" N / 76° 38' 36" W	5–18	FS
	Swash	37° 05' 32" N / 76° 36' 44" W	5–18	FS
	Long Shoal	37° 04' 35" N / 76° 37' 01" W	5–17	FS
	Dry Shoal	37° 03' 41" N / 76° 36' 14" W	7–20	FS
	Wreck Shoal	37° 03' 37" N / 76° 34' 20" W	10–21	FS, YRB
	Thomas Rock	37° 01' 32" N / 76° 29' 33" W	12–22	FS
Nansemond Ridge	36° 55' 20" N / 76° 27' 10" W	13–23	FS	
Lynnhaven River		36° 53' 42" N / 76° 04' 55" W	–	SSP
Seaside Eastern Shore	Mockhorn Channel	37° 16' 00" N / 75° 54' 17" W	–	SSP

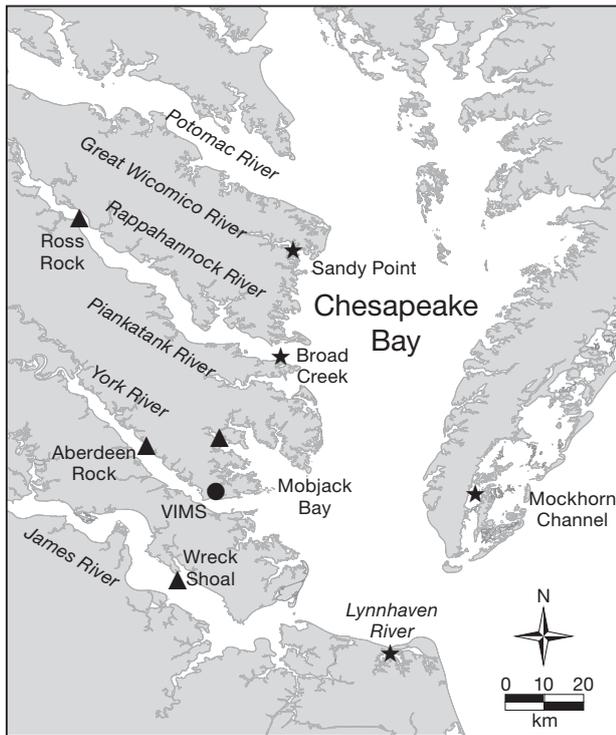


Fig. 1. Study locations in and around Chesapeake Bay, along the mid-Atlantic coast (Virginia/Maryland) of the eastern USA. ▲: source reefs for oysters used in the 2006 beach trial at the Virginia Institute of Marine Science (VIMS, ●) on the York River. Note that Ross Rock on the Rappahannock River is the source of all spring imports to the York River. ★: the 4 populations studied *in situ* in 2007 and 2008

Aberdeen Rock and Wreck Shoal have long been exposed to *H. nelsoni*. The DEBY-strain oysters are named for their Delaware Bay origins and are relatively resistant to both *H. nelsoni* and *Perkinsus marinus* (Ragone Calvo et al. 2003). Oysters from each population were divided among 3 triplicate bags and maintained in a rack-and-bag culture array in the intertidal at the VIMS beach on the York River (Table 1, Fig. 1). Each month from June to November, we determined oyster mortality in each bag and collected 8 oysters from each bag for pathology (see 'Pathological analyses'). Prevalences of *H. nelsoni* infections and the proportions of more intense moderate to heavy infections (both arcsine-transformed) were compared among treatments for each month using analyses of variance (ANOVA, $\alpha = 0.05$) in PASW Statistics 18.0 (SPSS). Multiple comparisons were performed as necessary using a Student-Newman-Keuls test (SNK, Zar 1996).

Size-specific *Haplosporidium nelsoni* parasitism.

To address the question of whether larger oysters were more greatly affected by MSX disease, we evaluated oysters in 4 broad size categories (20.0–49.9, 50.0–76.1, 76.2–99.9, and ≥ 100 mm, ~25 oysters per size category

per sampling date) from each of 4 oyster reefs in 2007 and 2008. Sandy Point reef in the Great Wicomico River was sampled monthly from May to November 2007 and in February and April 2008. Broad Creek reef in the Rappahannock River was sampled from May to November 2007 and from March to October 2008. Reefs in the Lynnhaven River (southern Chesapeake Bay) and at Mockhorn Channel (Virginia's seaside Eastern Shore) were sampled from April to August and in October 2008. Oysters were collected by dredge at Sandy Point and Broad Creek, and by hand from intertidal reefs at the Lynnhaven River and Mockhorn Channel (Table 1, Fig. 1), and oysters were returned to VIMS for pathological analysis (see 'Pathological analyses'). Overall data for monthly prevalence of *H. nelsoni* infections and the proportions of moderate to heavy infections (both arcsine-transformed) were compared by ANOVA ($\alpha = 0.05$) for the York River beach trial as above. Post-hoc chi-squared analyses ($\alpha = 0.05$) were performed to compare observed size-specific proportions of *H. nelsoni* infections reaching moderate or heavy intensity with expected proportions.

Pathological analyses. Collected oysters were measured and shucked, and soft tissues were evaluated for gross signs of pathology. After removal of sections of mantle and rectum for Ray's fluid thioglycollate medium culture for *Perkinsus marinus* diagnosis (results of which are not the focus of this manuscript), the remaining soft tissues were fixed in Davidson's fixative (Shaw & Battle 1957) and processed for paraffin histology using standard methods. Hematoxylin and eosin-stained transverse sections (6 μm) including mantle, gills, gonad, stomach, intestine, and digestive gland were evaluated on an Olympus BX51 light microscope for the multinucleate plasmodia, occasionally accompanied by sporocysts and, more rarely, spores in digestive tubules of advanced cases, that are characteristic of *Haplosporidium nelsoni* infections of oysters. When *H. nelsoni* infections were detected, intensity of infection was rated rare (1 to 10 *H. nelsoni* cells observed), light (>10 cells observed, but focal in distribution or systemic with only 1 to 2 parasites per field at 400 \times magnification), moderate (infection systemic, with 3 to 4 parasites observed per field at 400 \times magnification), or heavy (infection systemic with parasites very abundant in connective tissues of all organs). For each sample, prevalence (expressed as a percentage: $100 \times$ number of infected individual oysters \div number of oysters in the sample; Margolis et al. 1982) was calculated, and the proportion of oysters seriously infected with *H. nelsoni* was determined from intensity data. This proportion was calculated as the number of oysters characterized by infections of moderate or greater intensity \div the number of oysters in the sample, following the procedure of Haskin & Ford (1982).

Environmental parameters. Water temperature and salinity were recorded using a hand thermometer and refractometer during visits to sites for field collections. At the Lynnhaven River and Mockhorn Channel, these parameters were recorded for shallow surface water just offshore of sampled intertidal oyster reefs. At Sandy Point and Broad Creek, tested water was collected 1 m above subtidal oyster reefs using a Niskin bottle. For a more detailed perspective on water temperature and salinity in the lower Rappahannock River and the Great Wicomico River, mean monthly data were obtained for Broad Creek in the Rappahannock and for Shell Bar reef, near Sandy Point in the Great Wicomico, from the VIMS Molluscan Ecology Program.

RESULTS

Haplosporidium nelsoni levels in spring import deployments

Haplosporidium nelsoni is typically first detected in July in naïve sentinels deployed to the York River each April (Fig. 2). July levels of *H. nelsoni* vary, but August levels are uniformly high, with parasite prevalence exceeding 70% and with serious infections common. Prevalence and intensities decrease in late summer and fall, as *Perkinsus marinus* levels rise (Burreson & Ragone Calvo 1996). Peak annual prevalence of *H. nel-*

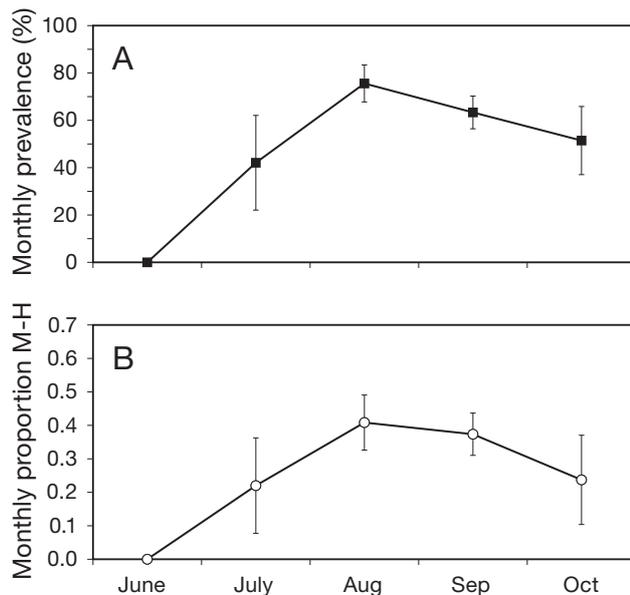


Fig. 2. *Haplosporidium nelsoni* infecting *Crassostrea virginica*. Infection trend in spring imports to the York River, Virginia. (A) Mean monthly prevalence of *H. nelsoni*, 1993–2008. (B) Mean monthly proportion of oysters with infections reaching moderate to heavy (M–H) intensity, 1993–2008. Error bars = 95% confidence intervals

soni infections has averaged $82.1 \pm 4.8\%$ (95% confidence interval [CI]) since 1993 (Fig. 3A). The proportion of oysters with serious infections has been more variable. The annual maximum proportion of infections of moderate or greater intensity has ranged from 0.24 to 0.68, averaging 0.48 ± 0.07 (mean with 95% CI; Fig. 3B).

Long-term comparison with *Haplosporidium nelsoni* levels at Wreck Shoal

Long-term data for spring import deployments reveal *Haplosporidium nelsoni* parasitism to be increasingly intense. While *H. nelsoni* parasitism abated in some years, the maximum annual prevalence of *H. nelsoni* has risen steadily since the parasite's emergence in 1959 (Fig. 4). Mean maximum annual prevalence of *H. nelsoni* infections in spring import deployments increased from 54.4% in the 1960s to 63.5% in the 1990s, and 84.7% from 2000 to 2008. The trend in *H. nelsoni* infection prevalence among wild oysters at Wreck Shoal collected during the fall and James River surveys was less straightforward. Prevalence during the initial epizootic in 1960 and 1961 was 32 to 36%. *H. nelsoni* receded after 1961 and then reemerged in 1987, when, along with *Perkinsus marinus*, it invaded Wreck Shoal and the James River seed area. From 1987 to 1995, the Wreck Shoal trend in *H. nelsoni* infection prevalence was similar to the trend in the spring imports. In 1996, these trends diverged with maximum annual prevalence of *H. nelsoni* infections consistently high in spring imports, but variable and decreasing in

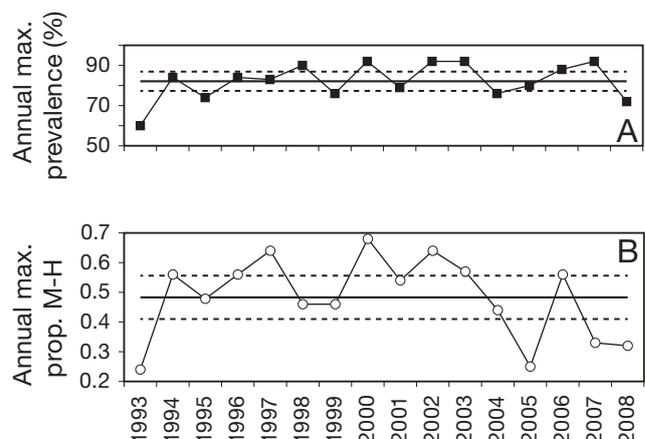


Fig. 3. *Haplosporidium nelsoni* infecting *Crassostrea virginica*. Peak annual infection levels in Spring Imports to the York River, Virginia, 1993–2008. (A) Annual maximum (max.) percent prevalence. (B) Maximum annual proportion (prop.) of oysters with more serious moderate to heavy (M–H) infections. In both panels, solid horizontal lines indicate the mean for the period. Hatched lines indicate 95% confidence intervals for the means

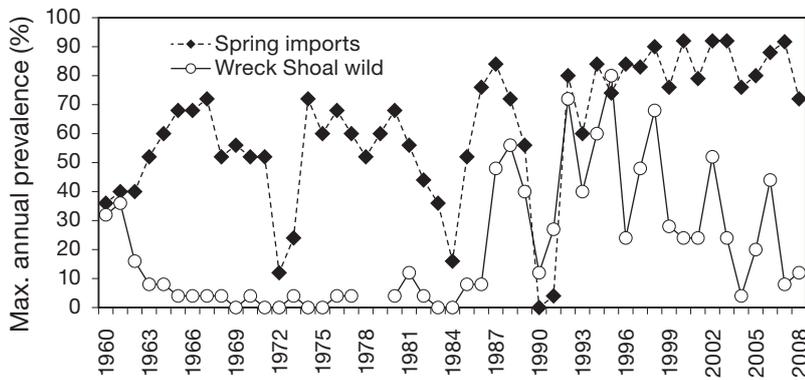


Fig. 4. *Haplosporidium nelsoni* infecting *Crassostrea virginica*. Maximum (Max.) annual prevalence in naïve, susceptible spring imports to the York River and in wild oysters at Wreck Shoal, 1960–2008

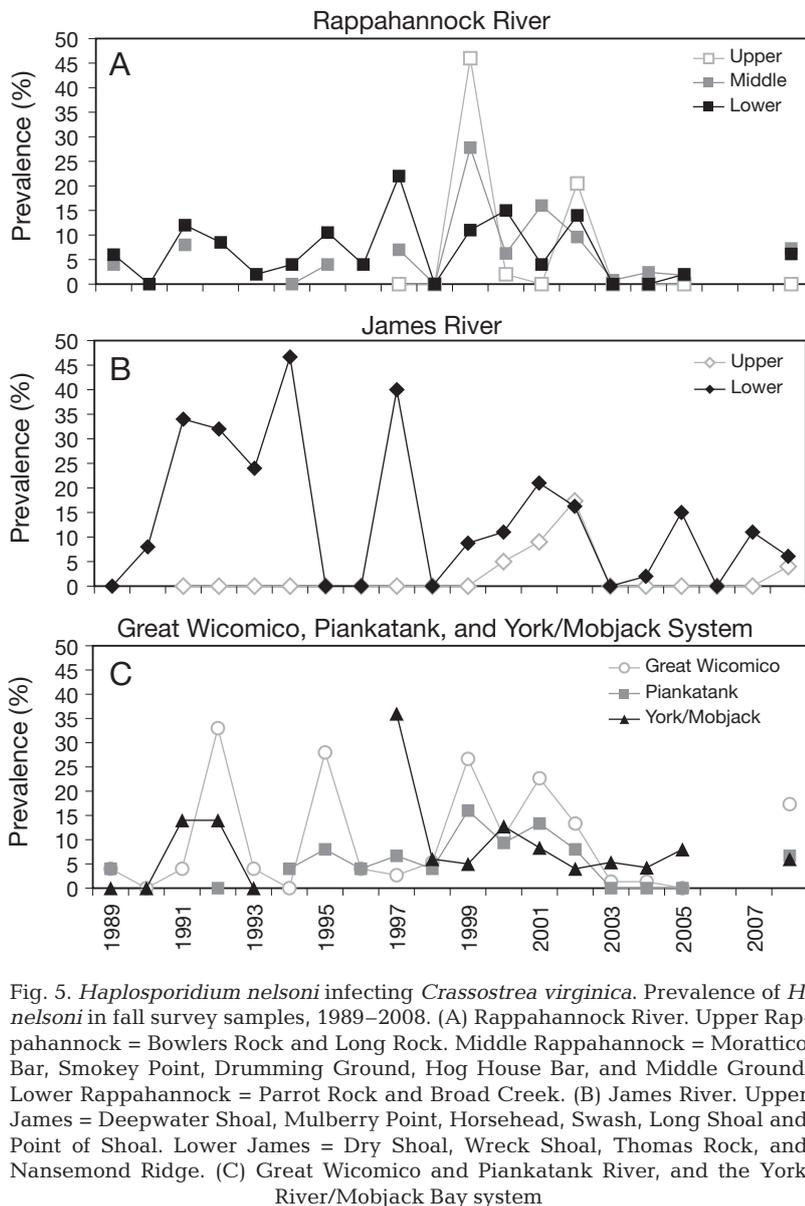


Fig. 5. *Haplosporidium nelsoni* infecting *Crassostrea virginica*. Prevalence of *H. nelsoni* in fall survey samples, 1989–2008. (A) Rappahannock River. Upper Rappahannock = Bowers Rock and Long Rock. Middle Rappahannock = Morattico Bar, Smokey Point, Drumming Ground, Hog House Bar, and Middle Ground. Lower Rappahannock = Parrot Rock and Broad Creek. (B) James River. Upper James = Deepwater Shoal, Mulberry Point, Horsehead, Swash, Long Shoal and Point of Shoal. Lower James = Dry Shoal, Wreck Shoal, Thomas Rock, and Nansemond Ridge. (C) Great Wicomico and Piankatank River, and the York River/Mobjack Bay system

wild oysters at Wreck Shoal. Maximum annual *H. nelsoni* infection prevalence recently has been 2 to 11× higher in spring import deployments to the York River than in wild oysters at Wreck Shoal.

Haplosporidium nelsoni in fall survey samples

Since 1989, fall survey samples have revealed a complex trend in the prevalence of *Haplosporidium nelsoni* infections. In the Rappahannock River (Fig. 5A), *H. nelsoni* was observed in more years and was generally most prevalent at stations in the lower river (Parrot Rock and Broad Creek). The parasite was observed least frequently and at generally lowest prevalence in the upper river (Ross Rock to Long Rock). In the high-disease year of 1999, however, the pattern was reversed, with *H. nelsoni* prevalence increasing sharply in the uppermost Rappahannock. Prevalence of *H. nelsoni* infections was highest in the uppermost Rappahannock in 2002 as well. In the James River (Fig. 5B), as in the Rappahannock, *H. nelsoni* was observed more frequently and at higher prevalence at lower river reefs (Dry Shoal to Nansemond Ridge) than upriver (Deepwater Shoal to Point of Shoal). In the high-disease drought years of 2000 to 2002, however, *H. nelsoni* levels climbed sharply upriver, slightly exceeding downriver levels in 2002. Despite the drought of 2000 to 2002, *H. nelsoni* levels generally decreased in the James River through the period, a trend observed earlier in monthly-quarterly data from Wreck Shoal (Fig. 4). *H. nelsoni* was commonly observed in the 3 smaller systems, the Great Wicomico and Piankatank Rivers, and the York River/Mobjack Bay (Fig. 5C), but in years more favorable for disease it tended to be more prevalent in the Great Wicomico, the northernmost of the 3 systems.

York River beach trial

Haplosporidium nelsoni was already present in the Wreck Shoal, Aberdeen Rock, and DEBY oyster populations at the start of the study, with infection prevalence highest (36%) at Wreck Shoal (Fig. 6). Most infections were light. After some initial mortality that was probably caused by these existing *H. nelsoni* infections, *H. nelsoni* was scarcely detectable by 6 June, when 1 light infection was detected in an oyster from Aberdeen Rock. With *H. nelsoni* levels now minimal, this effectively became the starting point for the experiment. Infections appearing from July on presumably represented acquisition in the York River.

New *Haplosporidium nelsoni* infections were apparent on 5 July, with mean prevalence in the Ross Rocks (mean \pm standard error of the mean [SEM]: $83.3 \pm 11.0\%$) significantly higher than in the other groups (ANOVA: $F_{3,8} = 7.266$, $p = 0.011$; SNK: $p < 0.05$). The proportion of serious, moderate to heavy infections also differed significantly among groups ($F_{3,8} = 4.640$; $p = 0.037$), with the Ross Rocks displaying a marginally higher proportion of serious infections (proportion \pm SEM: 0.54 ± 0.08 ; SNK: $p = 0.051$). Prevalence of *H. nelsoni* infections on 2 August again differed among groups ($F_{3,8} = 11.137$; $p = 0.003$), with prevalence in the

Ross Rocks ($83.3 \pm 4.2\%$) again significantly higher (SNK: $p < 0.05$) than in the other groups. Prevalence decreased relative to July in all but the Ross Rocks group. As in July, the proportions of oysters with serious infections differed among groups ($F_{3,8} = 9.086$; $p = 0.006$), with this proportion significantly higher (SNK: $p < 0.05$) in the Ross Rocks (0.46 ± 0.04) than in the other groups.

By September, only 9 Ross Rock oysters had survived. Mean percent survival through November in the Wreck Shoal, DEBY, and Aberdeen groups, on the other hand, was 25.4, 37.0, and 38.2%, respectively, and *Haplosporidium nelsoni* infection prevalence in each of these groups was $\leq 17\%$.

Size-specific *Haplosporidium nelsoni* parasitism

Results of the 2007 to 2008 study revealed 1 location characterized by enzootic *Haplosporidium nelsoni* activity that intensified in 2008 (Broad Creek), 1 by low *H. nelsoni* activity that became epizootic in 2008 (Sandy Point), and 2 sites characterized by low levels of *H. nelsoni* parasitism in the 2008 evaluation only (Lynnhaven River and Mockhorn Channel). At Broad Creek, *H. nelsoni* was detected in every month in all

size classes (Table 2), with infection prevalence and the proportion of more intense moderate to heavy infections tending to decrease with oyster size, though in neither case significantly (ANOVA: prevalence, $F_{3,56} = 0.627$, $p = 0.601$; serious infections, $F_{3,56} = 2.145$, $p = 0.105$). The monthly time series for the larger oyster size categories (76.2–100.0 and >100 mm) suggested spring to early summer peaks in *H. nelsoni* levels, with 2008 levels markedly higher than those of 2007. In spring 2008, prevalence of *H. nelsoni* among the larger oysters was about twice the maximum observed in 2007 (54.2% in the 76.2–100.0 mm size category, and 48.0% in >100 mm oysters). The proportions of serious infections peaked at 0.20 among the 76.2–100.0 mm oysters and 0.24 among those >100 mm, in both cases in April 2008. Sub-market-sized oysters (50.0–76.1 mm) at Broad Creek displayed a trend in *H. nelsoni* infection prevalence and intensity that was generally similar to the larger oysters, but with prevalences and intensities of parasitism rising to higher peak levels. Prevalence in this size category

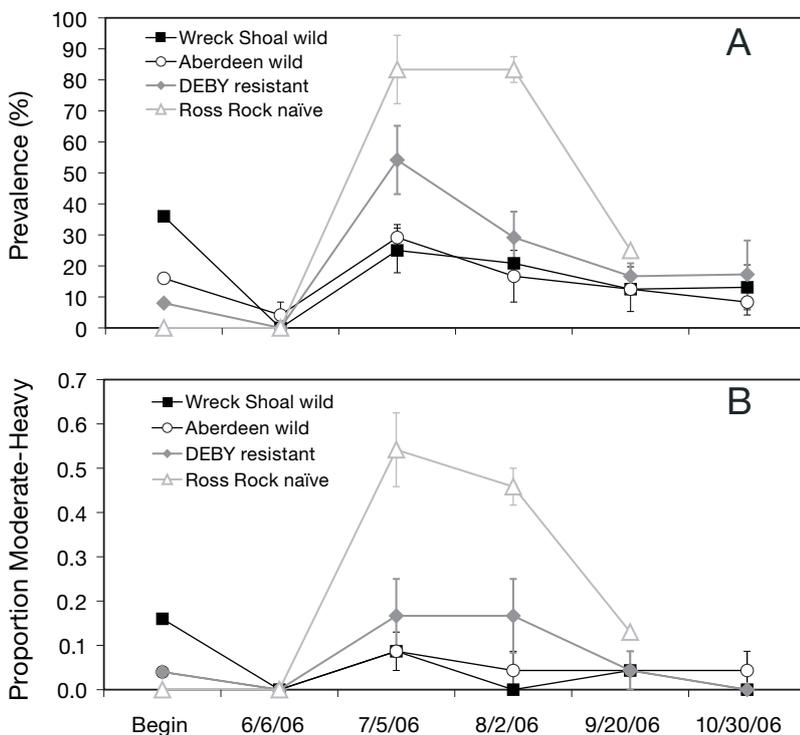


Fig. 6. *Haplosporidium nelsoni* infecting *Crassostrea virginica*. Infection during the beach trial at the York River in 2006. (A) Monthly prevalence of *H. nelsoni*. (B) Monthly proportion of oysters with *H. nelsoni* infections reaching moderate to heavy intensity. Error bars: ± 1 SEM. Dates given as mo/d/yr

Table 2. *Haplosporidium nelsoni* infecting *Crassostrea virginica*. Infections at size-specific parasitism study locations. For each date and oyster size interval, data are provided for sample size (n), percent prevalence (Prev.), and the proportion of oysters with infections of moderate to heavy intensity (Prop. M–H). Dates given as mo/d/yr

	— <50.0 mm —			— 50.0–76.1 mm —			— 76.2–100.0 mm —			— >100.0 mm —		
	n	Prev. (%)	Prop. M–H	n	Prev. (%)	Prop. M–H	n	Prev. (%)	Prop. M–H	n	Prev. (%)	Prop. M–H
Broad Creek												
5/1/2007	24	12.5	0.04	26	7.7	0.04	25	28.0	0.00	25	12.0	0.04
6/5/2007	21	14.3	0.05	27	25.9	0.11	27	14.8	0.07	24	16.7	0.08
7/11/2007	25	8.0	0.00	26	7.7	0.04	24	25.0	0.08	25	24.0	0.04
8/7/2007	24	66.7	0.29	25	44.0	0.12	26	15.4	0.00	25	12.0	0.00
9/4/2007	25	28.0	0.08	24	41.7	0.13	28	7.1	0.00	23	13.0	0.04
10/9/2007	25	44.0	0.36	26	23.1	0.04	24	20.8	0.04	25	32.0	0.00
11/13/2007	25	28.0	0.16	23	34.8	0.17	26	11.5	0.00	25	24.0	0.00
3/6/2008	25	48.0	0.12	26	61.5	0.27	24	54.2	0.13	25	48.0	0.08
4/25/2008	24	50.0	0.33	26	69.2	0.19	25	52.0	0.20	25	48.0	0.24
5/29/2008	25	36.0	0.32	25	28.0	0.08	25	28.0	0.12	24	29.2	0.08
6/16/2008	25	28.0	0.24	25	28.0	0.08	25	16.0	0.12	24	16.7	0.08
7/10/2008	25	4.0	0.00	26	19.2	0.04	24	8.3	0.00	7	14.3	0.14
8/5/2008	25	4.0	0.04	24	8.3	0.04	25	8.0	0.04	23	8.7	0.04
9/4/2008	25	8.0	0.00	25	8.0	0.00	25	8.0	0.00	21	4.8	0.00
10/1/2008	26	11.5	0.00	25	16.0	0.04	24	16.7	0.00	12	8.3	0.00
Mean		26.1	0.14		28.2	0.09		20.9	0.05		20.8	0.06
Sandy Point												
5/29/2007	25	0.0	0.00	25	0.0	0.00	21	0.0	0.00	5	0.0	0.00
6/20/2007	23	0.0	0.00	27	7.4	0.00	24	20.8	0.04	2	0.0	0.00
7/19/2007	25	8.0	0.00	25	4.0	0.00	24	4.2	0.00	4	0.0	0.00
8/16/2007	27	3.7	0.00	22	4.6	0.00	20	5.0	0.00	6	0.0	0.00
9/13/2007	25	0.0	0.00	26	0.0	0.00	24	4.2	0.04	2	0.0	0.00
10/15/2007	28	0.0	0.00	22	0.0	0.00	25	0.0	0.00	4	0.0	0.00
11/6/2007	25	4.0	0.04	25	0.0	0.00	22	9.1	0.00	No sample		
2/20/2008	25	12.0	0.04	27	33.3	0.07	23	13.0	0.04	2	0.0	0.00
4/25/2008	21	28.6	0.10	29	55.2	0.24	23	65.2	0.30	1	0.0	0.00
Mean		6.3	0.02		11.6	0.03		13.5	0.04		0.0	0.00
Lynnhaven River												
4/17/2008	25	8.0	0.08	25	8.0	0.08	25	12.0	0.04	25	4.0	0.04
5/16/2008	20	10.0	0.00	29	10.3	0.03	26	3.9	0.04	25	8.0	0.08
6/11/2008	25	0.0	0.00	26	0.0	0.00	24	0.0	0.00	25	4.0	0.00
7/17/2008	25	0.0	0.00	25	4.0	0.00	25	12.0	0.00	25	4.0	0.00
8/27/2008	25	0.0	0.00	25	4.0	0.00	21	0.0	0.00	28	3.6	0.00
10/8/2008	25	0.0	0.00	25	4.0	0.00	25	0.0	0.00	25	0.0	0.00
Mean		3.0	0.01		4.4	0.02		4.7	0.01		3.9	0.02
Mockhorn Channel												
4/17/2008	25	4.0	0.04	26	0.0	0.00	24	8.3	0.00	25	8.0	0.04
5/15/2008	25	0.0	0.00	26	0.0	0.00	24	0.0	0.00	25	0.0	0.00
6/12/2008	25	8.0	0.08	25	0.0	0.00	25	0.0	0.00	25	0.0	0.00
7/16/2008	24	0.0	0.00	27	3.7	0.04	24	0.0	0.00	25	0.0	0.00
8/26/2008	25	4.0	0.00	25	0.0	0.00	25	0.0	0.00	25	12.0	0.04
10/9/2008	24	0.0	0.00	26	0.0	0.00	25	0.0	0.00	1	0.0	0.00
Mean		2.7	0.02		0.6	0.01		1.4	0.00		3.3	0.01

reached 69.2% in April 2008, with the proportion of oysters displaying serious infections reaching 0.27 in March 2008. The smallest oysters (20.0–49.9 mm) were characterized by a more variable prevalence trend than the other size categories, and by infections of greater intensity. *H. nelsoni* infection levels spiked sharply in August 2007, when infection prevalence reached 66.7% and the proportion of oysters with seri-

ous infections was 0.29; in October 2007, when prevalence was 44.0% and the proportion of oysters with serious infections was 0.36; and a third time in April 2008, when prevalence reached 50.0% and a third of small oysters had serious infections.

While the decreasing size category-specific trends in *Haplosporidium nelsoni* infection prevalence and the proportion of oysters with serious infections did not rise

to statistical significance when all oysters in the samples were considered (i.e. both those infected and uninfected), significantly greater proportions of infections reached moderate or greater intensity among smaller than among larger oysters ($\chi^2 = 12.303$; $0.005 < p < 0.01$) when only infected oysters were considered. The proportions of infections reaching moderate or greater intensity were 0.53, 0.31, 0.27, and 0.26 in the 20.0–49.9, 50.0–76.1, 76.2–100.0 and >100 mm size categories, respectively. Considering heavy infections alone, the trend was still significant ($\chi^2 = 9.606$; $0.01 < p < 0.025$). The proportions of infections reaching heavy intensity were 0.33, 0.20, 0.17 and 0.14 in the 20.0–49.9, 50.0–76.1, 76.2–100.0 and >100 mm size categories, respectively. The most intense MSX disease was focused in the smallest segment of the Broad Creek population.

At Sandy Point, *Haplosporidium nelsoni* was detected sporadically before 2008, when prevalence and the proportion of serious infections increased sharply (Table 2). Between May and November 2007, *H. nelsoni* infection prevalence in size classes ≤ 100.0 mm was <10%. (Few larger oysters were found, and *H. nelsoni* infections were not detected.) The June 2007 sample of 76.2–100.0 mm oysters, in which infection prevalence was 20.8%, was the single exception. Infections were generally light, although heavy infections were occasionally observed. By April 2008, prevalence had risen to 28.6, 55.2 and 65.2% in the 20.0–49.9, 50.0–76.1 and 76.2–100.0 mm size classes, respectively. Proportions of seriously infected oysters reached 0.24 and 0.30 in the 50.0–76.1 and 76.2–100.0 mm oysters, respectively, but in the 20.0–49.9 mm oysters, the proportion of serious infections was 0.10. While infection levels peaked in sub-market- and market-sized oysters in April 2008, prevalences and proportions of serious infections did not differ significantly among the 20.0–49.9, 50.0–76.1, and 76.2–100.0 mm size classes (ANOVA: prevalence, $F_{2,24} = 0.499$, $p = 0.613$; serious infections, $F_{2,24} = 0.245$, $p = 0.785$) when the entire time series was considered.

The Lynnhaven River and Mockhorn Channel sites were both sampled 6 times between April and October 2008. Prevalences and intensities of *Haplosporidium nelsoni* infection at these 2 sites were low. The maximum infection prevalence observed in any size category at either site was only 12% (Table 2). The maxi-

imum proportion of serious infections observed at either site was 0.08. No patterns with respect to oyster size could be discerned, as size-specific differences in prevalence and in the proportions of serious infections were not significant at either the Lynnhaven River (ANOVA: prevalence, $F_{3,20} = 0.597$, $p = 0.624$; serious infections, $F_{3,20} = 0.094$, $p = 0.962$) or Mockhorn Channel (ANOVA: prevalence, $F_{3,20} = 0.629$, $p = 0.605$; serious infections, $F_{3,20} = 0.928$, $p = 0.445$).

Measured water temperatures peaked at 28°C in 2007 at Sandy Point; 31°C in 2007 and 28°C in 2008 at Broad Creek; 30°C in 2008 in the Lynnhaven River; and 29°C in 2008 at Mockhorn Channel (Fig. 7A). As a result of the sampling schedule, winter low temperatures at these sites were not defined. Mean monthly data from the VIMS Molluscan Ecology Program revealed generally similar winter temperature profiles for 2 of these sites, Broad Creek and Shell Bar reef, near Sandy Point in the Great Wicomico River. They also provided perspective on inter-annual variation, which was most notable with respect to winter low tem-

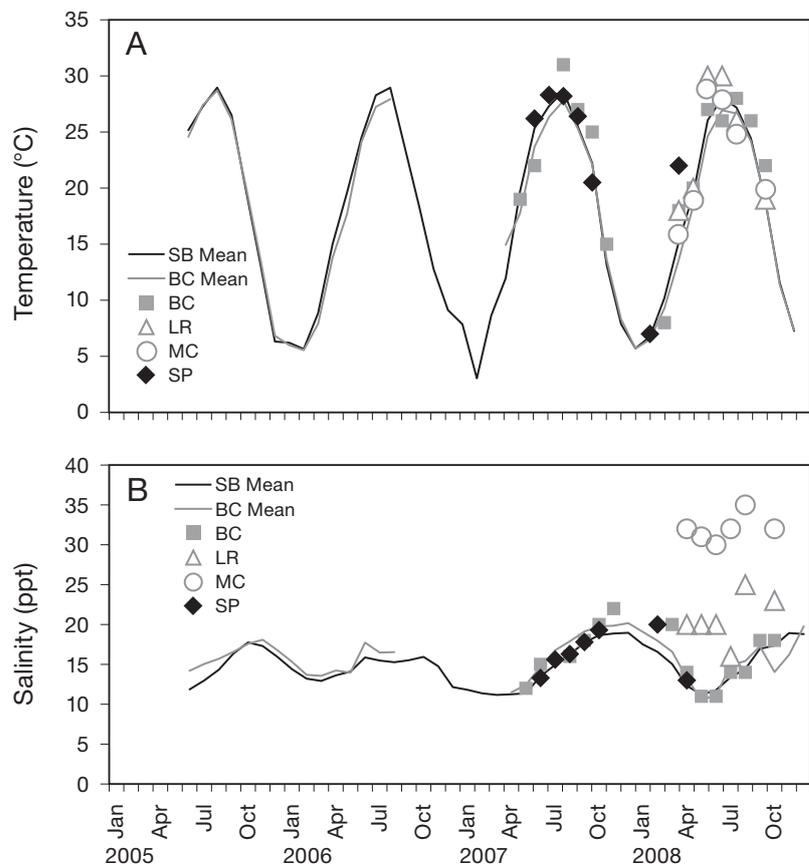


Fig. 7. Temperature and salinity measured during collections at 4 field locations: Broad Creek reef (BC), Lynnhaven River (LR), Mockhorn Channel (MC), and Sandy Point reef (SP). For perspective, monthly mean temperatures and salinities from 2005–2008 are presented for Broad Creek in the Rappahannock River and Shell Bar reef (SB) in the Great Wicomico River, the latter just upstream of Sandy Point reef

peratures. Complete data are only available for Shell Bar reef, but minimum monthly mean winter water temperatures were substantially higher at this location in 2005 to 2006 (5.7°C in February 2006) and 2007 to 2008 (5.7°C in January 2008) than in 2006 to 2007 (3.0°C in February 2007).

Measured salinities ranged from 13 to 20 ppt at Sandy Point, 11 to 22 ppt at Broad Creek, 16 to 25 ppt in the Lynnhaven River, and 30 to 35 ppt at Mockhorn Channel (Fig. 7B). As with the temperature data, mean monthly salinity data from the VIMS Molluscan Ecology Program revealed generally similar profiles for both Broad Creek and Shell Bar reef, with salinities at Broad Creek slightly higher. Salinities at both sites were higher in summer and fall, and lower in winter and spring. They remained higher for longer in 2007 to 2008, however, the product of a period of drought. At Shell Bar reef, mean monthly salinity exceeded 15 ppt for 4 mo from September to December 2005, and for 5 mo from June to October 2006. It exceeded 15 ppt for 8 mo (August to March) in 2007 to 2008. At Broad Creek, mean monthly salinity exceeded 15 ppt for 9 mo from July 2007 to March 2008.

DISCUSSION

Patterns of *Haplosporidium nelsoni* parasitism in Chesapeake Bay are complex with respect to annual and geographic variation, and to which oysters in a population are most affected. The most straightforward observation is the steady increase over time in maximum *H. nelsoni* levels in spring import deployments to the York River. We might ask whether this increase is due to increasing oyster susceptibility or increasing parasite virulence, or to environmental factors increasingly favoring *H. nelsoni*. The *H. nelsoni* susceptibility of oysters in unselected populations would not be expected to increase unless, for example, resistance alleles are linked to others in oysters that are selectively, and increasingly, disadvantageous in low-salinity environments. We do not know this to be the case. An increase in *H. nelsoni* virulence is more plausible, particularly if competition with *Perkinsus marinus* drives directional selection for this. Yet this too is unsupported by empirical evidence. The best explanation for the increase in *H. nelsoni* activity in naïve transplants is that environmental factors increasingly favor the parasite. One contributing factor may be the warming winter water temperatures in the Chesapeake region over recent decades (see Preston 2004). While it is not clear that winter temperatures in Chesapeake Bay are low enough to impact *H. nelsoni* epizootiology, milder winter water temperatures in Delaware Bay have been associated with periods of

more serious *H. nelsoni* activity, with harsher winters, conversely, preceding periods of lower parasite prevalence (Ford & Haskin 1982). Average salinities in the lower York River exceeded 15 ppt in the spring, and 20 ppt in the fall, in the middle part of the last century (Haven et al. 1978). They are similar today, and thus normally in a range that is favorable for *H. nelsoni* (Ford 1985) for the duration of each year's spring import deployment.

An additional and possibly relevant environmental change has occurred in benthic community structure. As eutrophication of Chesapeake Bay has increased (Kemp et al. 2005), large, long-lived benthic fauna have declined. Smaller, shorter-lived, opportunistic species are more abundant in today's more degraded Chesapeake Bay (Holland et al. 1987, Long & Seitz 2009). The intermediate host(s) for *Haplosporidium nelsoni* that have been hypothesized to exist (Haskin & Andrews 1988) but which have not yet been identified may be among these thriving opportunists.

Besides describing an increasing trend in *Haplosporidium nelsoni* infection pressure, annual spring import deployments are illuminating in another way: they reveal the high levels that *H. nelsoni* can reach in truly susceptible oyster stocks. Maximum annual prevalence of infections may exceed 70% after only 3 mo of parasite exposure, and a quarter to a half of oysters in the susceptible population, or more, may have infections of serious moderate to heavy intensity (Figs. 2 & 3). By comparison, *H. nelsoni* infection prevalence at Wreck Shoal over the past decade has not exceeded 52% and has often been less than 30% (Fig. 4). Over the 10 yr period from 1999 to 2008, our James River survey has shown the maximum proportion of serious infections (moderate or greater intensity) at Wreck Shoal to range from 0 to 0.32, and average 0.11. The maximum proportion of serious infections in spring imports ranged from 0.25 to 0.68 (Fig. 2B), and averaged 0.48. Fewer oysters were infected, and fewer were seriously infected, at Wreck Shoal than among the susceptible spring imports. We interpret the difference in *H. nelsoni* infection and MSX disease between the *H. nelsoni*-naïve spring imports and wild oysters from sites like Wreck Shoal to reflect relative resistance to *H. nelsoni* parasitism and disease in the wild oysters.

It must be acknowledged that some observations, particularly from large-river reefs such as Wreck Shoal in the James River, might reflect suppression or expulsion of *Haplosporidium nelsoni* (or an intermediate host) by periodic low salinities. Mitigation of *H. nelsoni* activity by low salinities is well documented (e.g. Haskin & Ford 1982, Ford 1985), and the general absence of *H. nelsoni* from Wreck Shoal between the mid-1960s and mid-1980s (see Fig. 4) was probably an

example of this (Andrews 1983). It is unlikely that the oysters had any substantial resistance to *H. nelsoni* during this period, based on infection levels displayed when *H. nelsoni* re-colonized Wreck Shoal in the mid-1980s: prevalence was again high, and as during the initial colonization of Wreck Shoal by *H. nelsoni* in 1960 and 1961, it mirrored prevalence in the susceptible spring imports. *H. nelsoni* unquestionably remains under the influence of salinity in the James River. However, declining *H. nelsoni* levels in recent years must be at least partly due to resistance to the parasite. *H. nelsoni* levels in Wreck Shoal transplants during the 2006 York River study (Fig. 6) were not significantly different from those in either the disease-resistant domesticated oysters (the DEBYs) or those from the other *H. nelsoni*-enzootic location, Aberdeen Rock in the York River. Levels in all of these groups were significantly lower than in the Ross Rock transplants. The *H. nelsoni* resistance of domesticated oysters such as the DEBYs is well documented (e.g. Ragone Calvo et al. 2003) and widely appreciated by the aquaculture industry. Based on all the evidence above, the resistance of wild oysters in populations long exposed to *H. nelsoni* seems comparable.

We prefer 'resistance' rather than 'tolerance' to describe the disposition of these oysters toward *Haplosporidium nelsoni*. We follow the distinction made by Roy & Kirchner (2000): resistance strategies limit infection, while tolerance strategies reduce the fitness consequences of infection. Oysters from populations long exposed to *H. nelsoni*, like Wreck Shoal, have lower prevalences of *H. nelsoni* infection than susceptible populations do (they limit colonization of the tissues), and when infected, they more successfully localize infections to gill epithelia (they limit penetration of the epithelial basement membrane and proliferation within the tissues). Following Roy & Kirchner (2000), therefore, 'resistance' is a more appropriate descriptor for the observations.

Geographic distribution and demographics of disease resistance

We have argued that oyster populations at Wreck Shoal in the lower James River and at Aberdeen Rock in the York River are relatively resistant to *Haplosporidium nelsoni* parasitism. Yet the performance of oysters from upriver reefs in the Rappahannock River when transplanted to the disease-intense York River leaves little question that resistance to *H. nelsoni* is not universal in Chesapeake Bay. Oyster populations in the uppermost Rappahannock have been selected only weakly for *H. nelsoni* resistance, if at all. When transplanted to the York as annual spring imports, they very

rapidly develop high levels of parasitism and disease. This geographic heterogeneity contrasts with the situation in Delaware Bay (Delaware Bay Ecology of Infectious Diseases Group 2009), and probably reflects the inability of *H. nelsoni* to significantly colonize the upper parts of Virginia rivers (Andrews 1983) and exert a challenge on oysters in those waters. It raises the question of where oyster populations transition from relatively resistant to highly susceptible. Can the geographic extent of resistance to *H. nelsoni* parasitism be delineated?

As noted earlier, the exposure of oyster populations to *Haplosporidium nelsoni* parasitism varies with distance up the Chesapeake Bay mainstem and its tributaries. As documented during the early years of the Delaware Bay epizootic, exposure is a function of salinity (Haskin & Ford 1982). In the lower parts of the rivers and bay, salinities are high enough to sustain chronic *H. nelsoni* activity. Where salinities are lower, *H. nelsoni* activity becomes more sporadic. Much as there is a gradient in *H. nelsoni* pressure on oyster populations, there also seems to be evidence for a gradient, rather than a sharp front or discontinuity, in *H. nelsoni* resistance. This gradient is apparent in the Fall Survey data, particularly for the Rappahannock River (see Fig. 5A), but also for the York River/Mobjack Bay system and the Piankatank and Great Wicomico Rivers (Fig. 5C). In the Rappahannock, *H. nelsoni* was observed at decreasing frequency (in fewer years) from downriver to upriver locations, but maximum prevalence during the worst year of the 1999 to 2002 epizootic showed the opposite trend: it peaked upriver, and was lowest at downriver reefs, with the mid-river reefs intermediate between the two. Similarly, *H. nelsoni* prevalence reached higher levels during periodic epizootics in the Great Wicomico River than it did in the Piankatank River and York/Mobjack systems situated further south in Chesapeake Bay. There is no reason to believe that *H. nelsoni* infection pressure was higher upriver (and up-bay) than downriver, but the capacity of the upriver (and up-bay) oyster populations for resisting *H. nelsoni* was probably lower.

The size-specific evaluation of *Haplosporidium nelsoni* parasitism, which involved sampling in the Great Wicomico River, lower Rappahannock River, Lynnhaven River, and Mockhorn Channel on the seaside Eastern Shore, provided another indication that resistance is not well developed in the Great Wicomico River. The impacts of *H. nelsoni* on oysters at Sandy Point in the Great Wicomico were modest in 2007, when prevalence peaked at 21% and the proportion of serious infections peaked at 0.04, in both cases in 76.1–100.0 mm oysters. With intensifying drought, however, *H. nelsoni* levels in the Great Wicomico in the spring of 2008 were among the highest ever recorded in Vir-

ginia. *H. nelsoni* was scarcely detectable in oysters of any size in 2008 in the Lynnhaven River, however, even though salinities almost always favor *H. nelsoni* there, and even though susceptible transplants have been found to develop intense *H. nelsoni* parasitism at high prevalence (authors' unpubl. data). It may be the case that the *H. nelsoni* pressure during 2007 and 2008 was extraordinarily high due to the drought conditions, and that even relatively resistant oysters may show elevated parasitism and disease in such years. Nonetheless, there is little indication of substantial resistance to *H. nelsoni* in the Great Wicomico.

Haplosporidium nelsoni impacts at Mockhorn Channel were similar to those in the Lynnhaven, though the significance of this is not clear. Andrews & Castagna (1978) expressed a belief that high seaside salinities may inhibit *H. nelsoni*, based on observations that '[*H.*] *nelsoni* is irregular on Seaside. Infections are sporadic from bay to bay and from one year to another; mortalities are usually light by Chesapeake Bay standards and do not follow infections with any regularity'. *H. nelsoni* activity in seaside coastal sounds, perhaps because of environmental factors, perhaps because of additional parasitism of seaside oysters by *H. costale* (Wood & Andrews 1962), remains enigmatic.

As in the Great Wicomico River, elevated *Haplosporidium nelsoni* parasitism was also noticeable by the spring of 2008 in the lower Rappahannock River, which was also influenced by the drought conditions. Yet the size-specific infection pattern differed between Broad Creek in the Rappahannock, where *H. nelsoni* is enzootic, and Sandy Point in the Great Wicomico, where *H. nelsoni* is only occasionally present. At Broad Creek, oysters of small size (and presumably young age) were much more heavily impacted by *H. nelsoni* parasitism than were larger (older) oysters. Parasite prevalence was regularly much higher through 2007 and 2008 in smaller oysters, and serious infections were much more common. At Sandy Point, the smallest oysters were least affected when the *H. nelsoni* epizootic peaked in spring 2008. We hypothesize that this difference reflects fundamental differences in the interplay between *H. nelsoni* epizootiology and oyster population biology in these systems, a hypothesis we develop further in the following paragraphs.

The Great Wicomico River sub-estuary is far smaller than the Rappahannock River sub-estuary. Great Wicomico River oyster populations occur over a horizontal distance of only about 10 km, and the river is characterized by a low flushing rate that allows for retention of oyster larvae (Andrews 1979). Salinity varies little through the zone that oysters inhabit, and surveys have indicated that, when the parasite is present, *Haplosporidium nelsoni* infections are uniformly distributed through this 10 km region. There are no highly resistant

oysters in the Great Wicomico River because *H. nelsoni* epizootics occur only occasionally, for reasons that are not clear. Neither is there a substantial pool of highly susceptible oysters. The population as a whole in the Great Wicomico is moderately susceptible to disease and mortality due to *H. nelsoni* when conditions favor re-colonization of the river by the parasite. Higher levels of *H. nelsoni* in larger oysters in this system may reflect higher rates of parasite encounter (larger oysters filtering greater volumes of water than smaller oysters) and/or the intensification of chronic but subclinical infections that had been acquired over time by the larger oysters but that the smaller oysters did not possess. It is also possible that the smaller oysters represent a young cohort characterized by increased resistance to disease. This last possibility is least plausible, however. While prevalence was lower among the smaller oysters, average intensity was not. Infections were just as likely to reach serious intensities in smaller oysters as in larger individuals.

In the Rappahannock River, oysters occur over a distance of 55 km upstream from the river mouth (Whitcomb & Haven 1989). Salinity in the lower part of this range is nearly always favorable for *Haplosporidium nelsoni*. In the upper part of the range, from which spring imports originate annually, *H. nelsoni* is almost never detected because the salinity is too low. As noted earlier, this gradient in salinity has produced a gradient in *H. nelsoni* infection pressure, which we suggest has produced a gradient in disease resistance, with oysters downriver most resistant, and oysters upriver having little resistance at all. But how is it possible that resistance exists in the oyster populations downstream if disease-enzootic, higher salinity waters are primarily supported by reproductive contributions (i.e. gene flow) from oysters in upstream refugia from parasitism, as has been assumed (Mann et al. 1991)? While it is likely that larvae are transported over this distance, as physical modeling suggests (North et al. 2008), the rapid development of MSX disease in imports from the upper Rappahannock to the lower York River provides a strong indication of the fate of larvae produced in disease-free upstream waters when they settle in disease-enzootic downstream waters. Most would be expected to develop disease and mortality after a relatively short period of exposure to *H. nelsoni* (and *Perkinsus marinus*). It is precisely this dynamic that we believe we detect in the size-specific disease data for Broad Creek in the lower Rappahannock. In our study, smaller (generally younger) oysters were much more seriously affected by *H. nelsoni*: developing higher prevalences of parasitism, and far more serious infections. Larger oysters had lower prevalences of *H. nelsoni* parasitism and developed fewer advanced infections. We hypothesize that samples of smaller oysters (i.e. younger

age classes) include larger proportions of more susceptible recruits, and the individuals developing significant parasitism and disease are those more susceptible individuals advected from upstream refugia but also generated from local reproduction by susceptible oysters before they became substantially infected. Samples of increasingly larger oysters, on the other hand, include decreasing proportions of susceptible oysters as these oysters are purged from the population by disease mortalities. Central to this model is the existence and persistence of relatively resistant oysters. These must be produced in large part by oysters reproducing somewhere in the *H. nelsoni*-enzootic part of the river, where resistant oysters should theoretically be most abundant, not in refugia far upstream, where the frequency of resistant oysters must be far lower. The population biology of oysters at the scale of sub-estuaries like the Rappahannock River may be better resolved using genetic methods, which awaits future study.

The *Haplosporidium nelsoni*-oyster dynamic in Delaware Bay has been evaluated using different approaches, including a 'common garden' experiment in which progeny produced from *H. nelsoni*-exposed oysters on one hand, and oysters from an upstream refuge on the other, were compared with progeny produced from control Maine oysters never exposed to *H. nelsoni* (Delaware Bay Ecology of Infectious Diseases Group 2009). While the progeny of the Maine oysters were affected most seriously, the progeny of refuge oysters had significantly higher levels of *H. nelsoni* parasitism and mortality than the progeny of *H. nelsoni*-exposed oysters. As in Chesapeake Bay, wild oysters displaying reduced levels of *H. nelsoni* are probably the progeny of oysters selected for *H. nelsoni* resistance in disease-enzootic waters.

Implications for oyster conservation and restoration

The implications of these observations for management of *Haplosporidium nelsoni* parasitism in wild populations are profound. We have gained an appreciation that, while upstream refugia may contribute to larval recruitment downriver in disease-enzootic areas (North et al. 2008), the contribution is not so significant that an evolutionary response to *H. nelsoni* parasitism among downriver oysters is precluded. On the contrary: in Delaware Bay (Haskin & Ford 1979, Delaware Bay Ecology of Infectious Diseases Group 2009) but also in Chesapeake Bay, exposure to *H. nelsoni* over many oyster generations has produced substantial resistance on the part of the oyster hosts. Capitalizing on this resistance will best be accomplished by increasing the chance that resistant oysters will reproduce. Protection of oysters for this purpose in sanctuaries from har-

vest has been advocated for years. In the late 1990s, a committee of regional oyster experts identified 2 'essential components of oyster restoration efforts': 'three-dimensional reefs, standing substantially above the bottom', and 'permanent reef sanctuaries' that 'permit the long-term growth and protection of large oysters that provide increased fecundity and [which] may lead to the development of disease resistant oysters' (Chesapeake Research Consortium 1999, p. 1). A decade later, this concept is finally beginning to be embraced by resource managers and the public. Giving resistant oysters more opportunities to spawn before they eventually succumb to *H. nelsoni* (as even some more resistant individuals will) or *Perkinsus marinus* should theoretically increase the proportion of long-lived, resistant individuals in the population. This itself should increase the average size of oysters, as oysters surviving to greater ages will generally be larger (Galtsoff 1964). It should also yield benefits for shell budgets. Oysters tend to be smaller today than they were centuries ago (Harding et al. 2008), and smaller oyster shells deteriorate more rapidly (Ford et al. 2006). When oyster recruitment rates are low, there is an overall loss of shell (i.e. calcium carbonate) from even the healthiest Chesapeake Bay system, the James River (Mann et al. 2009). Increasing the size of oysters in (and by means of) sanctuaries may slow rates of shell loss, making positive shell budgets more likely even without marked increases in oyster recruitment.

Our work provides insight into where such sanctuaries should be sited. Recent larval transport modeling has identified putative sources and sinks within the oyster reef networks of Chesapeake Bay tributaries. A study that modeled the larger Virginia tributaries placed source reefs, which are net exporters of larvae, near the saline heads of the estuaries. Sink reefs, net recipients of larvae, were located near the river mouths (see Fig. 9 in North et al. 2008). A second study modeled the much smaller Lynnhaven River system, but similarly placed source reefs deep in the interior of the system, and sinks near the mouth (Lipcius et al. 2008). The authors of both studies have argued that such modeling should be used to determine which oyster reefs should be conserved or restored (the sources), and which should be targeted for exploitation (the sinks). What both studies lack, however, is perspective on the biology of the systems. In the Lynnhaven River, for example, there are relatively few oysters inhabiting the theoretical 'source' areas. Before constructing reefs in such areas, we should understand what limits oyster recruitment and/or survival in these putative source regions.

Our appreciation for how biology may contravene physical modeling is clearer where the larger western Chesapeake Bay tributaries are concerned. While the larval transport models may locate source reefs far

upriver in the James, York, and Rappahannock Rivers (North et al. 2008), we know from our results that recruits exported from these areas may be heavily impacted by *Haplosporidium nelsoni* (and *Perkinsus marinus*) parasitism in the middle and lower parts of the rivers where salinity favors the parasites. Simply considering disease dynamics alone, this aspect of the biology of the system suggests that mid- to lower-river reefs may be more vital than those far upriver for the function and stability of the systems. While the net flow of passive particles may be from up- to downriver, net oyster dispersal (net gene flow) may occur, perhaps in stepwise fashion, in the opposite direction.

Considering other influences on oyster survival and reproduction—predation compounding disease mortality in higher salinities downriver, and freshwater kills and suppression of reproduction and recruitment by lower salinities nearer the heads of estuaries—we may hypothesize that mid-river oyster reefs are most critical. Oysters historically have been most abundant in the middle reaches of the large Virginia rivers (Haven et al. 1978), and a long-term analysis of Delaware Bay oyster populations indicated periodic population expansions from, and contractions to, a core refuge in the middle region of the bay (Powell et al. 2008). The importance of mesohaline to polyhaline components of oyster populations with respect to oyster conservation and restoration in these systems clearly must not be discounted, despite the presence of *Haplosporidium nelsoni* and *Perkinsus marinus*.

In summary, multiple lines of investigation have converged on a single essential finding: impacts of *Haplosporidium nelsoni* are waning in lower Chesapeake Bay, despite the continued presence of the parasite, in oyster populations that have been exposed to the most persistent and acute parasite pressure. This is clear evidence of increasing resistance to this introduced pathogen. We suggest that the most important oyster reefs for conservation and restoration are those that harbor these resistant oysters in upper mesohaline to polyhaline waters, despite the presence of parasitic disease.

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