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A review of recent information on the Haplosporidia, with special reference to *Haplosporidium nelsoni* (MSX disease)

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**Abstract** – The current status of the Haplosporidia is reviewed as well as recent information on *Haplosporidium nelsoni*, the causative agent of MSX disease in oysters. Recent molecular phylogenetic analyses with greatly increased taxon sampling support monophyly of the Haplosporidia and hypothesize placement of the group as sister taxon to the phylum Cercozoa. Oyster pathogens in the genus *Bonamia* should be considered haplosporidians based on molecular sequence data. Thus, the group contains 4 genera: *Uropsoridium*, *Haplosporidium*, *Bonamia* and *Minchinia*. Molecular phylogenetic analyses support monophyly of *Uropsoridium*, *Bonamia* and *Minchinia*, but *Haplosporidium* forms a paraphyletic clade. Reports of haplosporidia worldwide are reviewed. Molecular detection assays have greatly increased our ability to rapidly and specifically diagnose important pathogens in the phylum and have also improved our understanding of the distribution and biology of *H. nelsoni* and *H. costale*. Much of the data available for *H. nelsoni* has been integrated into a mathematical model of host/parasite/environment interactions. Model simulations support hypotheses that recent *H. nelsoni* outbreaks in the NE United States are related to increased winter temperatures, and that a host other than oysters is involved in the life cycle. Evidence is presented that natural resistance to *H. nelsoni* has developed in oysters in Delaware Bay, USA. However, in Chesapeake Bay, USA *H. nelsoni* has intensified in historically low salinity areas where salinities have increased because of recent drought conditions. Efforts to mitigate the impact of *H. nelsoni* involve selective breeding programs for disease resistance and the evaluation of disease resistant non-native oysters.

**Key words:** Phylogeny / Diagnostics / Numerical model / Haplosporidia / *Haplosporidium nelsoni*

1 **Introduction**

The Haplosporidia constitute a small group of endoparasites, mostly of marine invertebrates (Perkins 2000), although one species is known from freshwater invertebrates. At present there are 36 recognized species in the phylum; however, numerous others have been reported, but not specifically identified, from many different invertebrate hosts. Several species have been associated with epizootic mortalities of commercially important molluscs. The most well-studied member of the group is *Haplosporidium nelsoni*, which causes MSX disease in the eastern oyster, *Crassostrea virginica*, on the east coast of North America. This parasite, along with a closely related species, *H. costale*, which causes SSO disease, also in the eastern oyster, were covered in a 1996 review (Ford and Tripp 1996) that considered history and distribution, life stages, infection and disease processes; epizootiology and environmental influences; and control/management measures, including selective breeding for disease resistance. General reviews of the phylum Haplosporidia include contributions by Perkins (1990, 1991, 2000) as well as an earlier review by Sprague (1979).

The present review will emphasize recent developments, which include research on *H. nelsoni* that has occurred since the 1996 publication. They include: 1) progress in characterizing, phylogenetically, the Haplosporidia; 2) reports of new species of Haplosporidia and new hosts; 3) development and implementation of molecular detection assays; 4) numerical modeling of *H. nelsoni*; and 5) changes in the distribution of *H. nelsoni* epizootics, including prevalence decline in Delaware Bay, and intensification of disease-caused oyster mortalities in Chesapeake Bay that have led to the testing of non-native oysters and selectively-bred native oysters.

2 **Phylogenetic position of the Haplosporidia**

2.1 **Historical perspective**

Since the discovery of the first species in the late 1800s, the Haplosporidia have been a troublesome group for
taxonomists and phylogeneticists, and there have been nu-
merous classification schemes proposed for placement of
the group within the protists. Early workers placed species
in the order Haplosporida, class Sporozoa of the phylum
Protozoa. With the advent of electron microscopy in the
1950s, the tremendous morphological diversity of single-
celled organisms became apparent and many groups of pro-
tists were elevated to phylum rank. Sprague (1979) sepa-
rated the Haplosporida and Paramyxea from other Sporozoa
by including both groups in the new phylum Ascetospora.
The phylum Ascetospora was subsequently abandoned and the
Haplosporidia and Paramyxida were each elevated to phylum
However, recently Cavalier-Smith and Chao (2003b) resur-
rected Ascetospora as a class in the phylum Cercozoa, subphy-
lum Endomyxa. In their scheme class Ascetospora includes
three orders – Haplosporida, Paramyxida and Claustrosporidiae
(but see Sect. 2.2). The Haplosporida were most recently char-
acterized morphologically as a group of parasitic protists hav-
ing multinucleate plasmodia and ovoid, walled spores lacking
polar filaments or polar tubes, and with an orifice at one pole.
The orifice is covered either externally by a hinged lid or in-
ternally by a flap of wall material (Perkins 2000). The place-
ment of the genus Bonamia in the Haplosporida (see Sect. 3.2)
muddles this definition of the group because no spore stage has
been observed in Bonamia. If a spore stage is truly lacking in
Bonamia spp. it is unclear at present what morphological char-
acters define Haplosporida.

2.2 Molecular phylogenetic analyses

First attempts to determine the relationship of the
Haplosporida to other Eukaryota using molecular sequence
data hypothesized placement of the group within the parking-
dom Alveolata (see Cavalier-Smith 1993) as a taxon of equal
rank with the other alveolate phyla – Ciliophora, Apicomplexa
and Dinoflagellata (Siddall et al. 1995; Flores et al. 1996). A
molecular phylogenetic analysis by Berthe et al. (2000) placed
the Haplosporida as sister taxon to the Dictyostelida and
also provided molecular phylogenetic support for separation
of the phylum Haplosporidia and phylum Paramyxida. Recent
molecular phylogenetic analyses using rRNA gene sequences
(Cavalier-Smith and Chao 2003a,b), and combined rRNA and actin gene sequences (Reece et al. 2004) included much
more sequence data available for a variety of eukaryote taxa.
These studies documented monophyly of Haplosporida and
hypothesized a relationship between the Haplosporida and the
Cercozoa, a relationship not previously recognized. Cavalier-
Smith and Chao (2003b) placed the Haplosporida as an order
within the phylum Cercozoa (Fig. 1), but with weak support
(bootstrap = 20 or 60 depending on sequences included). They
state that “Cercozoa comprise four major distinctly separate
subclades” – Ascetospora (actually just Haplosporida in the
analyses), the gromiid testate amoebae, the Phytomyxea, and
a very large group of classical Cercozoa including zooflagel-
lates, filose testate amoebae and chlorarachnean algae. Each
of these clades could, and perhaps should, be considered a
separate phylum as they are of equal rank in the phyloge-
netic analyses. Cavalier-Smith and Chao (2003a,b) include the
Haplosporida within the Cercozoa, rather than as a separate
phylum, primarily because they share with classical Cercozoa
an “almost unique” single nucleotide deletion – a justification
requiring further support in our opinion.

Cavalier-Smith and Chao (2003b) resurrect Ascetospora
to include three groups – Haplosporida, Paramyxida and
Claustrosporidiae. The molecular phylogenetic analysis by
Cavalier-Smith and Chao (2003a) places Marteilia refrin-
gens (Paramyxida) “well within Haplosporida” and sister to
Haplosporidium costale. Nonetheless, Cavalier-Smith and
Chao (2003a) state, inexplicably, that Haplosporida and
Paramyxida are separate orders in the phylum Cercozoa. How-
ever, if M. refringens is a haplosporianid, as their analyses
indicate, then Paramyxida has no basis. Other molecular phy-
genetic analyses have not hypothesized a close relation-
ship between the Haplosporida and the Paramyxida (Berthe
et al. 2000; Reece et al. 2004). Claustrosporidium is placed in
Ascetospora on the basis of organelles called haplosporosomes
in the sporoplasm (Cavalier-Smith and Chao 2003b), although
these organelles also occur in vegetative stages of Myxozoa
(Morris et al. 2000). Unfortunately no molecular data are
available for Claustrosporidium with which to evaluate this
proposed relationship (and see Sect. 3.3).

The molecular phylogenetic analysis by Reece et al.
(2004) using both rRNA and actin gene sequences supports
Haplosporida as a monophyletic clade and places the group
as sister taxon to Cercozoa (Fig. 2) with moderate support
(jackknife = 74), suggesting that if Cercozoa is recognized
as a phylum, then Haplosporida should be recognized as a
phylum as well. Reece et al. (2004) found no support for
inclusion of the paramyxean Marteilia refringens within the
Haplosporida.

3 Taxa within the phylum Haplosporida

3.1 Urosporidium, Haplosporidium, Minchinia

The phylum Haplosporidia has long been recognized to
contain only three genera, Urosporidium, Haplosporidium
and Minchinia, and about 33 species (Perkins 2000). Urosporidium is characterized by species with an internal flap
of wall material covering the spore orifice. Minchinia and
Haplosporidium both have an external, hinged lid that cov-
ers the spore orifice, and the characters that distinguish these
two genera have been much debated. It is now generally rec-
ognized that spore ornamentation as observed with transmis-
sion and scanning electron microscopy is the best character
for distinguishing species and for separating Minchinia and
Haplosporidium. Unfortunately, the spore ornamentation for
the type species of Haplosporidium, H. scolopli, is unknown
and the species has not been reported since its original de-
scription. Two attempts by the first author to find H. scolopli,
by examining hundreds of type hosts from the type locality
in France, failed. Uncertainty about the spore morphology of
the type species has hindered characterization of the genus
Haplosporidium and the identification of characters that sep-
erate it from the genus Minchinia. Ormières (1980) proposed
that species with spore ornamentation composed of epispore
cytoplasm be placed in Minchinia, and species with spore
ornamentation composed of spore wall material be placed in *Haplosporidium*. Most recent workers have accepted this convention (McGovern and Burreson 1990; Hine and Thorne 1998, 2002; Azevedo et al. 1999; Azevedo 2001; Burreson 2001). However, Perkins (2000) based generic assignment solely on whether spore ornamentation is visible with a light microscope, without regard for ontogenetic origin of the ornamentation. Thus, Perkins (2000) proposed that *Minchinia* includes species in which the ornamentation is visible with a light microscope and *Haplosporidium* includes species in which ornamentation is not visible with a light microscope.

The recent molecular phylogenetic analysis by Reece et al. (2004) supports the importance of ontogenetic origin of spore ornamentation. In their analysis (Fig. 3) the genus *Minchinia* formed a monophyletic clade, and all species of *Minchinia* have ornamentation composed of epispore cytoplasm. The genus *Haplosporidium*, however, formed a paraphyletic clade (Fig. 3), suggesting that more genera are necessary to encompass the morphological diversity of species with ornamentation derived from the spore wall. Unfortunately, new generic assignments cannot be made at the present time because of the lack of knowledge on ornamentation of the type species of *Haplosporidium*, *H. scolopii*, and of many other species presently assigned to *Haplosporidium*.

### 3.2 Bonamia

Perhaps the most interesting new finding is molecular phylogenetic support for inclusion of the genus *Bonamia* in the phylum Haplosporidia (Carnegie et al. 2000; Reece and Stokes 2003; Reece et al. 2004). *Bonamia* has long been suspected to be a haplosporidian because of the presence of organelles called haplosporosomes (Perkins 2000), but no spore stage has been observed, so the genus had previously not been assigned with certainty to any group. In a recent molecular phylogenetic analysis (Reece et al. 2004), species of *Bonamia* formed a monophyletic clade nested within the traditional haplosporidian taxa, as sister taxa to *Minchinia* spp., not as a basal clade (Fig. 3). This alignment as sister taxon to a spore-forming genus suggests that *Bonamia* does form spores, so perhaps the stages observed to date are intermediate life cycle stages and spores are formed in some other, as yet unidentified, organism. Alternatively, it is possible that spores have been lost in the *Bonamia* lineage. Loss of spores is supported by the observation that *Bonamia ostreae* can be transmitted directly between oyster hosts in the laboratory via cohabitation (Elston et al. 1986) or by inoculation of purified intrahemocyte stages (Hervio et al. 1995). With the possible exception of *H. pickfordi* (Barrow 1961), direct transmission...
experiments with spore-forming haplosporidians have been unsuccessful (Ford and Tripp 1996), and it is widely believed that an intermediate host is a necessary component of the life cycle in those species that form spores (Andrews 1984; Haskin and Andrews 1988; Powell et al. 1999). If Bonamia spp. truly lack spores, it makes morphological definition of the Haplosporidia problematic because the group can no longer be defined as organisms that contain spores with an orifice at one pole.

Molecular sequence analyses (Fig. 3) and ultrastructure data also suggest that another “microciliate” parasite, Mikrocytos roughleyi, is a species of Bonamia (Cochennec-Laureau et al. 2003; Reece et al. 2004). Mikrocytos roughleyi is a parasite of the Sydney rock oyster Saccostrea glomerata in
Australia (Farley et al. 1988). *Mikrocytos roughleyi* parasitizes oyster hemocytes, as do *Bonamia* species, and it is sister taxon to *Bonamia* spp. in molecular phylogenetic analyses (Cochennec-Laureau et al. 2003; Reece et al. 2004). A second species of *Mikrocytos, M. mackini*, which parasitizes vesicular connective tissue cells in *Crassostrea gigas* in British Columbia, Canada (Farley et al. 1988) and Washington, USA, apparently is not related to *Bonamia* and it is not a member of the Haplosporida (Hine et al. 2001a; Carnegie et al. 2003).

### 3.3 *Claustrosporidium*

Larsson (1987) established the genus *Claustrosporidium* and included two species, *C. gammari* and *C. aselli*, both of which had originally been placed in *Haplosporidium*. He also erected the family *Claustrosporidiidae* containing the single genus *Claustrosporidium* and included it in the Haplosporida. The sporoplasm of *Claustrosporidium gammari* does contain haplosporosomes, but the spore does not have an orifice at one pole and spore wall formation is not the same as in the typical haplosporidians. For these reasons, Perkins (2000), in a thorough discussion, did not accept the placement of *Claustrosporidium* spp. in the Haplosporida. Unfortunately, no molecular sequence data are available for *Claustrosporidium* spp., so phylogenetic analyses that include the genus have not been possible. Although *Claustrosporidium* was not included in their phylogenetic analyses, Cavalier-Smith and Chao (2003b), because of the presence of haplosporosomes, include it in a separate order *Claustrosporida* within the class *Ascetospora* equal in rank to the order Haplosporida.

### 3.4 Currently recognized Haplosporida

The 36 recognized species in the Haplosporida are listed in Table 1. Many species listed have not been reported since the original, often brief, description, and all may not be valid species. For example, it seems unlikely that the five species of *Haplosporidium* reported from polychaetes in northern France (Caullery and Mesnil 1905; Mercier and Poisson 1922) are all distinct species. Also, the spore ornamentation is unknown for many species of *Haplosporidium* listed in Table 1 and some of them may be transferred to *Minchinia* or synonymized with other species when more is known about their morphology.

The unnamed haplosporidians listed in Table 2 all seem to be correctly assigned to the phylum Haplosporida. The species from *Ostrea angasi* in NW France is probably *H. armoricanum*, but there is not enough information available for the other species listed to assign them to existing species or to describe them as new species.

### 4 Recent reports of Haplosporida

Haplosporidia continue to be discovered in new hosts and habitats. Only one species is known from freshwater, *Haplosporidium pickfordi* from snails in USA lakes (Burreson 2001), but two other infections in freshwater hosts recently have been reported. Amphipods of the genus *Diporeia* are infected with a haplosporidian in Lake Michigan and Lake Huron in the USA (Messick and Nalepa 2002), and zebra mussels, *Dreissena polymorpha*, have been observed to harbor haplosporidian infections in Europe (D. P. Molloy, personal communication).

Haplosporidians have also recently been reported in new marine hosts. Mussels, *Mytilus edulis*, were found infected with spores of an unidentified haplosporidian in Maine, USA (Figuera et al. 1991) and also during a long-term monitoring program in Atlantic Canada (Stephenson et al. 2002). *Mytilus galloprovincialis* was found infected with *Minchinia* sp. in the Mediterranean Sea off France (Comps and Tigé 1997). Another unidentified haplosporidian was found in cultured bay scallops, *Argopecten irradians*, in China (Chu et al. 1996). Spores of a haplosporidian parasite were observed in the cockle *Cerastoderma edule* in Spain (Carballal et al. 2001), and the parasite recently was described as *Haplosporidium edule* by Azvedo et al. (2003). Plasmodial stages of an unidentified haplosporidian were implicated in high mortality of cultured abalone in New Zealand during the austral summers of 2000 and 2001 (Diggles et al. 2002; Hine et al. 2002). Heavy systemic infections of plasmodia were observed in moribund animals, but no spores were present. The parasite has not been observed in wild abalone of the same species. This parasite groups with the Haplosporida in molecular phylogenetic analyses (Reece and Stokes 2003; Reece et al. 2004).
Table 1. Accepted species in the Haplosporidia. Host names are those reported in the original description.

<table>
<thead>
<tr>
<th>Species</th>
<th>Host</th>
<th>Location</th>
<th>Comments*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Urosporidium</em></td>
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<tr>
<td><em>U. fuliginosum</em></td>
<td>Polychaete <em>Syllis gracilis</em></td>
<td>English Channel, France</td>
<td>Type species of <em>Urosporidium</em>, No EM. Caullery and Mesnil (1905)</td>
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<td></td>
<td><em>U. pelseneeri</em> (Caullery and Chappellier 1906)</td>
<td>Trematode sporocysts in clams <em>Donax vittatus</em>, <em>Barnea candida</em></td>
<td>English Channel, France</td>
<td>No EM. Caullery and Chappellier (1906); Dollfus (1925)</td>
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<td><em>U. crescens</em></td>
<td>DeTurk 1940</td>
<td>East coast of USA TEM, SEM, DNA.</td>
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<td>DeTurk (1940); Perkins (1971)</td>
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<td><em>U. fauricum</em></td>
<td>Zaika and Dolgikh 1963</td>
<td>Trematode in the mollusk <em>Rissost spondida</em></td>
<td>Ukraine</td>
<td>No EM. Zaika and Dolgikh (1963)</td>
</tr>
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<td></td>
<td><em>U. constantae</em> Howell 1967</td>
<td>Trematode sporocysts of <em>Gymnophallus nereicola</em> in the clam <em>Abra ovata</em></td>
<td>New Zealand</td>
<td>No EM. Howell (1967)</td>
</tr>
<tr>
<td><em>U. jiroveci</em></td>
<td>Ormières et al. 1973</td>
<td>Trematode sporocysts of <em>Bucephalus longicornatus</em> in the clam <em>A. ovata</em></td>
<td>Mediterranean Sea, France TEM. Ormières et al. (1973)</td>
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<td><em>U. spisuli</em></td>
<td>Perkins et al. 1975</td>
<td>Nematode in surf clam <em>Spisula solidissima</em></td>
<td>East coast of USA TEM, SEM, Perkins et al. (1975); Perkins et al. (1977)</td>
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<td><em>U. cannoni</em></td>
<td>Anderson et al. 1993</td>
<td>Polyclad flatworm <em>Stylochus</em></td>
<td>Moreton Bay, QLD, Australia TEM, SEM. Anderson et al. (1993)</td>
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<td><em>Haplosporidium</em></td>
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<tr>
<td><em>H. scolopi</em></td>
<td>Polychaete <em>Scoloplos mulleri</em>.</td>
<td>Cap de la Hague, France</td>
<td>Type species of <em>Haplosporidium</em>. No EM. Caullery and Mesnil (1899)</td>
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<td></td>
<td><em>H. heterocirri</em> Caullery and Mesnil 1899</td>
<td>Polychaete <em>Heterocirrus viridis</em></td>
<td>Cap de la Hague, France</td>
<td>No EM. Caullery and Mesnil (1899)</td>
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<td><em>H. marchoxi</em></td>
<td>Caullery and Mesnil 1905</td>
<td>Polychaete <em>Salmacina dysteri</em></td>
<td>Cap de la Hague, France</td>
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<td><em>H. potamiliae</em></td>
<td>Caullery and Mesnil 1905</td>
<td>Polychaete <em>Potamilla torelli</em></td>
<td>Cap de la Hague, France</td>
<td>No EM. Caullery and Mesnil (1905)</td>
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<td><em>H. vejdvoskii</em></td>
<td>Caullery and Mesnil 1905</td>
<td>FW oligochaete <em>Mesenchytraeus flavidus</em></td>
<td>Czech Republic</td>
<td>No EM. Caullery and Mesnil (1905)</td>
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<td><em>H. limnodrili</em></td>
<td>Granata 1913</td>
<td>FW oligochaete <em>Linnodrilus udekemianus</em></td>
<td>Florence, Italy</td>
<td>No EM. Granata (1913)</td>
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<td><em>H. nemertis</em></td>
<td>Debaiseaux 1920</td>
<td>Nemertean <em>Lineus bilineatus</em></td>
<td>Plymouth, UK</td>
<td>No EM. Debaiseaux (1920)</td>
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<td><em>H. caulleryi</em></td>
<td>Mercier and Poisson 1922</td>
<td>Polychaete <em>Nereilepas faceta</em></td>
<td>Luc-sur-Mer, Calvados, France</td>
<td>No EM. Mercier and Poisson (1922)</td>
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<td><em>H. asciheram</em></td>
<td>Duboscq and Harant 1923</td>
<td>Tunicates <em>Sydnium elegans</em>, <em>Ciona intestinalis</em></td>
<td>Mediterranean Sea</td>
<td>TEM. Duboscq and Harant (1923); Ormières and de Puytorac (1968); Ciancio et al. (1999)</td>
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<td><em>H. cernosvitovi</em></td>
<td>Jirovec 1936</td>
<td>FW oligochaete, <em>Opistocysta flagellum</em></td>
<td>Misiones Province, Argentina</td>
<td>No EM. Jirovec (1936)</td>
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<td><em>H. pickordi</em></td>
<td>Barrow 1961</td>
<td>FW snails <em>Physella parkeri</em>, <em>Lymnaea stagnalis</em>, <em>Helisoma canaeneratum</em></td>
<td>Michigan, USA</td>
<td>TEM, SEM, DNA. Barrow (1961); Burreson (2001)</td>
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<td><em>H. Louisiana</em></td>
<td>(Sprague 1963)</td>
<td>Mudcrab <em>Panopeus herbstii</em></td>
<td>East and Gulf of Mexico coasts of USA</td>
<td>TEM, SEM, DNA. Sprague (1963); Perkins (1975)</td>
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Table 1. Continued.

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<th>Species</th>
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<td><em>H. nelsoni</em></td>
<td>Oyster <em>Crassostrea virginica</em>, <em>C. gigas</em></td>
<td>East coast of North America, California, USA; Japan; Korea;</td>
<td>TEM, DNA. Haskin et al. (1966); Perkins (1968)</td>
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<td>(Haskin et al. 1966)</td>
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<td><em>H. tumefaciens</em></td>
<td>Mussel <em>Mytilus californianus</em></td>
<td>California, USA</td>
<td>No EM. Taylor (1966)</td>
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<td>Taylor 1966</td>
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<td><em>H. armoricanum</em></td>
<td>Oysters <em>Ostrea edulis</em>, <em>Ostrea angasi</em></td>
<td>Europe</td>
<td>TEM. SEM. Van Banning (1977); Azevedo et al. (1999)</td>
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<td>Mudcrab <em>Rhithropanopeus harrisi</em></td>
<td>Caen, Calvados, France</td>
<td>TEM. Similar to <em>H. Louisiana</em>. Marchand and Sprague (1979)</td>
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<td>Mediterranean Sea, France</td>
<td>TEM. Ormières (1980)</td>
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<td>Azevedo 1984</td>
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<td><em>H. comatulae</em></td>
<td>Crinoid <em>Oligometra serripinna</em></td>
<td>Lizard Island, QLD, Australia</td>
<td>TEM. La Haye et al. (1984)</td>
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<tr>
<td>La Haye et al. 1984</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>H. edule</em></td>
<td>Cockle, <em>Cerastoderma edule</em></td>
<td>NW Spain</td>
<td>TEM, SEM. Azevedo et al. (2003)</td>
</tr>
<tr>
<td>Azevedo et al. 2003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bonamia</strong></td>
<td></td>
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</tr>
<tr>
<td><em>B. ostreae</em></td>
<td>Oyster <em>Ostrea edulis</em></td>
<td>California, Maine, USA; Europe</td>
<td>Type species of <em>Bonamia</em>. TEM, DNA. Pichot et al. (1979)</td>
</tr>
<tr>
<td>Pichot et al. 1979</td>
<td></td>
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<tr>
<td><em>B. exitiosa</em></td>
<td>Oyster <em>Ostrea chilensis</em></td>
<td>New Zealand</td>
<td>TEM, DNA. Hine et al. (2001)</td>
</tr>
<tr>
<td>Hine et al. 2001</td>
<td></td>
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<tr>
<td><em>B. roughleyi</em></td>
<td>Oyster <em>Saccostrea commercialis</em></td>
<td>NSW, Australia</td>
<td>TEM, DNA. Farley et al. (1988); Cochenne-Laurleau et al. (2003)</td>
</tr>
<tr>
<td>(Farley et al. 1988)</td>
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</tr>
<tr>
<td><strong>Minchinia</strong></td>
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<td></td>
</tr>
<tr>
<td><em>M. chitonis</em></td>
<td>Chiton <em>Lepidochitona cinereus</em></td>
<td>English Channel, UK and France</td>
<td>Type species of <em>Minchinia</em>. TEM, SEM, DNA. Lankester (1885); Ball (1980)</td>
</tr>
<tr>
<td>(Lankester 1885)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. dentali</em></td>
<td>Scaphopod <em>Dentalium entale</em></td>
<td>Mediterranean Sea, France</td>
<td>TEM. Arvy (1949); Desportes and Nashed, (1983)</td>
</tr>
<tr>
<td>(Arvy 1949)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. tapetis</em></td>
<td>Clam <em>Ruditapes decussatus</em></td>
<td>Portugal, NW Spain</td>
<td>TEM, SEM, DNA. Vilela (1950); Azevedo (2001)</td>
</tr>
<tr>
<td>(Vilela 1951)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. teredinis</em></td>
<td>Shipworms <em>Teredo spp.</em></td>
<td>East Coast of USA</td>
<td>TEM, SEM, DNA. Hillman et al. (1990); McGovern and Burreson (1990)</td>
</tr>
<tr>
<td>Hillman et al. 1990</td>
<td></td>
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</tr>
</tbody>
</table>

*No EM = no electron microscopy performed; TEM = transmission electron microscopy performed, SEM = scanning electron microscopy performed; DNA = some DNA sequence information available.

In addition to reports of new or unidentified species, known species have been confirmed in other hosts or locations by DNA-based assays (see Sect. 5). *Haplosporidium nelsoni* has been confirmed in the oyster *Crassostrea gigas* in California, USA; Korea; Japan and France (Burreson et al. 2000; Renault et al. 2000; Kamaishi and Yoshinaga 2002), and in the oyster *Crassostrea virginica* in Atlantic Canada (Stephenson et al. 2003). *Haplosporidium costale* has been reported in the oyster *Crassostrea virginica* from Long Island Sound, New York, USA (Sunila et al. 2002).

The lists of named species or recent reports of hapsporidian in Tables 1 and 2 suggest that hapsporidians are widely distributed around the world in both marine and freshwater environments. Unfortunately, the prevalence of infection is often extremely low and spores are often not present in
Table 2. Reports of unnamed haplosporidians.

<table>
<thead>
<tr>
<th>Genus</th>
<th>Host</th>
<th>Location</th>
<th>Comments/References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not designated</td>
<td>Oyster <em>Crassostrea gigas</em></td>
<td>California, USA</td>
<td>Katkansky and Warner (1970)</td>
</tr>
<tr>
<td>Not designated</td>
<td>Oyster, <em>Ostrea lurida</em></td>
<td>Oregon, USA</td>
<td>Mix and Sprague (1974)</td>
</tr>
<tr>
<td>Not designated</td>
<td>Blue crab, <em>Callinectes sapidus</em></td>
<td>Virginia, North Carolina, USA</td>
<td>Newman et al. (1976)</td>
</tr>
<tr>
<td>Not designated</td>
<td>Shrimp, <em>Penaeus vannamei</em></td>
<td>Nicaragua or Cuba</td>
<td>Dyková et al. (1988)</td>
</tr>
<tr>
<td>Not designated</td>
<td>Mussel <em>Mytilus edulis</em></td>
<td>Maine, USA</td>
<td>Figueras et al. (1991)</td>
</tr>
<tr>
<td><em>Haplosporidium</em> sp.</td>
<td>Oyster <em>Crassostrea gigas</em></td>
<td>Mediterranean Sea, France</td>
<td>Compas and Pichot (1991)</td>
</tr>
<tr>
<td>Not designated</td>
<td>Scallop, <em>Argopecten irradians</em></td>
<td>China</td>
<td>Chu et al. (1996)</td>
</tr>
<tr>
<td><em>Haplosporidium</em> sp.</td>
<td>Pearl oyster <em>Pinctada maxima</em></td>
<td>Western Australia</td>
<td>Hine and Thorne (1998)</td>
</tr>
<tr>
<td><em>Haplosporidium</em> sp.</td>
<td>Rock oyster <em>Saccostrea cucullata</em></td>
<td>Western Australia</td>
<td>Hine and Thorne (2002)</td>
</tr>
<tr>
<td>Not designated</td>
<td>Freshwater amphipod, <em>Diporeia</em> sp.</td>
<td>Michigan, USA</td>
<td>Messick and Nalepa (2002)</td>
</tr>
</tbody>
</table>

infected hosts, making it difficult to obtain sufficient material for species descriptions.

5 Development and implementation of molecular detection assays

5.1 General considerations

Molecular detection assays for aquatic pathogens are being developed at an increasingly rapid rate. Unfortunately, the assays often have not been validated against traditional techniques, and most of these assays have not been thoroughly tested for inclusivity (Do they detect all strains of the pathogen?) or specificity (Do they cross react with any other organism?). The basic problem is that molecular detection assays too often are developed from a few sequences from a limited geographic range of the pathogen without a good understanding of the overall sequence variability within the species, and they are often not sufficiently tested for specificity. Thus, assays may not detect all genetic strains of the species throughout its range or they may cross react with other species. In addition, it is important to realize that the polymerase chain reaction (PCR) detects DNA and not necessarily a viable pathogen. To confirm the presence of a viable pathogen, PCR should be used in conjunction with other methods that allow visualization of the pathogen in tissue, such as histology or in situ hybridization with DNA probes.

Nonetheless, the development of sensitive and specific molecular detection assays has greatly increased our ability to rapidly and specifically diagnose important pathogens in the
phylum Haplosporidia. The use of the assays has significantly improved our understanding of the distribution and biology of pathogenic members of the phylum.

5.2 Specific assays

As might be expected, the first molecular assays were developed for *Haplosporidium nelsoni*, the causative agent of MSX disease in oysters along the east coast of North America. The assays target variable regions of the small subunit rRNA gene. A DNA probe sequence for *H. nelsoni* was identified by Fong et al. (1993), and it was tested on *H. nelsoni* cells in hemolymph smears. PCR primers (Stokes et al. 1995a) and a DNA probe (Stokes and Burreson 1995) for *H. nelsoni* were tested for sensitivity and specificity and have been used by various researchers to identify *H. nelsoni* in oysters. The presence of *H. nelsoni* in *Crassostrea gigas* was verified using these molecular detection assays (see Sect. 4). These molecular diagnostic tools have more recently been used to verify *H. nelsoni* as the cause of epizootic oyster mortality in Nova Scotia, Canada (Stephenson et al. 2003). In addition, the primer sequences identified by Stokes et al. (1995a) have been used by others to develop a competitive, quantitative PCR assay for *H. nelsoni* (Day et al. 2000) and a multiplex PCR assay (Penna et al. 2001; Russell et al. 2004) that detects *H. nelsoni*, *H. costale* and *Perkinsus marinus*.

DNA-based diagnostic assays have also been developed for other haplosporidians. Specific PCR primers and a DNA probe have been developed for *Minchinia teredinis*, a parasite of shipworms, *Teredo* spp. along the east coast of North America (Stokes et al. 1995b). Molecular diagnostic assays have also been developed for *Bonamia* spp. These are discussed in detail in the paper by Carnegie and Cochennec-Laureau in this issue of Aquatic Living Resources entitled: Microcell parasites of oysters: recent insights and future trends.

5.3 Discrimination of *H. nelsoni* and *H. costale*

Haplosporidian species are very difficult to identify in histological sections if only plasmodia are present. Host and location can be a good guide, but host and geographic range often overlap among species. Identification of the oyster pathogens *Haplosporidium nelsoni* and *H. costale* has been particularly problematic in the absence of spores. These two species parasitize oysters along the east coast of North America and they overlap in areas where salinity is consistently greater than about 25 ppt. The plasmodia stages of these two species cannot be reliably distinguished in histological sections. The SSU rRNA gene for *H. costale* was first characterized by Ko et al. (1995), who identified, but did not test, potential PCR primer sequences. More recently, PCR primers that target a region of the SSU rRNA gene different from that of Ko et al. have been identified and tested (Stokes and Burreson 2001). These assays have been used in conjunction with molecular assays for *H. nelsoni* to differentially diagnose the two species (Fig. 4). Interestingly, the use of DNA probes for both species on the same oyster sample revealed mixed infections of *H. nelsoni* and *H. costale* that were not detected using histology (Stokes and Burreson 2001). In addition, the molecular tools have been used to verify the presence of *H. costale* in Long Island Sound, New York (Sunila et al. 2002). The molecular tools were also used to identify plasmodia in oysters sampled in October in Virginia and Long Island sound. Because of the seasonal timing of the infection, the parasite...
was thought to be *H. nelsoni*. However, DNA probes revealed that the plasmodia were *H. costale* (Stokes and Burreson 2001; Sunila et al. 2002). The presence of *H. costale* plasmodia in October is unprecedented and challenges historical criteria for the seasonality and epizootiology of this pathogen. Earlier studies on the epizootiology of *H. costale* had established the annual cycle as very predictable with clinical plasmodial infections appearing in spring, and sporulation in May and June. New infections occur before August 1st, but remain subclinical and undetectable until the following spring (Couch and Rosenfield 1968; Andrews and Castagna 1978). Numerous samples of oysters from coastal Virginia collected from summer through winter over many years revealed no *H. costale* infections (Andrews and Castagna 1978). It is unclear whether this apparent change in seasonality is real or simply the result of improved diagnostic sensitivity.

### 6 Numerical modeling of *Haplosporidium nelsoni*

#### 6.1 Overview

The review of *H. nelsoni*-caused MSX disease in 1996 (Ford and Tripp 1996) presented a large body of information concerning infection cycles, the influence of environment on prevalence and intensity, and the disease process. Many of these data have since been integrated into a mathematical model of host-parasite-environment interactions (Ford et al. 1999a; Paraso et al. 1999; Powell et al. 1999). The model is based on one developed earlier for the other major eastern oyster pathogen, *Perkinsus marinus* (cause of Dermo disease) (Hofmann et al. 1995; Powell et al. 1996). Both models simulate infection cycles within the oyster and in oyster populations under different environmental conditions, and forecast conditions that can initiate and end epizootics in oyster populations.

The *H. nelsoni* model, like that of *P. marinus*, has three components (Fig. 5). The core consists of a body of “governing equations” developed from observational and experimental data: for instance the relationship of body size or temperature to oyster respiration rates, the effect of salinity on parasite doubling times, or the effect of parasite burden on oyster filtration rates. Input data, or “forcing functions,” consisting of environmental time series are then inserted into the equations. The forcing functions for the oyster-parasite models are temperature, salinity, food, and turbidity. The model then generates a series of simulations, based on the environmental time series, which depict annual and multi-year prevalence and intensity cycles of the parasites, and cumulative oyster growth and mortality. The simulations are compared with the same parameters actually observed under those conditions. The model can be considered to be “validated” if the simulations generated using input data independent from those used to construct the equations can reproduce the pattern and general levels observed in the field. It should be noted that construction of the model required that numerous assumptions be made about biological relationships when direct experimental or observational data were unavailable. Some assumptions were made based on other biological systems or on general physiological principles; others may be proxies for the real mechanism, which provide the same answer simply by chance or because they operate by a similar mechanism.

#### 6.2 Temperature and salinity effects

Model simulations using a temperature and salinity time series from lower Delaware Bay (where salinity is always high enough to favor *H. nelsoni* activity) reproduced the observed annual infection cycle at that location, indicating that under favorable salinity regime, the annual temperature cycle is the primary influence on seasonal prevalence patterns (Fig. 6) (Ford et al. 1999a). One exception to this finding was that simulated *H. nelsoni* doubling rates did not diminish in the autumn to the same degree as was observed in the field. Thus, a “crowding factor” was required to limit *H. nelsoni* doubling after a certain parasite density was reached. Interestingly, the *P. marinus* model required a similar modification. In both cases, the modification was rationalized by supposing that at some point the resources provided by the host to the parasites would become limiting – as occurs in in vitro culture.

Although temperature was the dominate controlling factor in model runs under high salinity conditions, salinity became an increasingly important factor when simulations were made with data from a region that encompassed varied salinity...
Thus, it was of interest to simulate hypothetical shifts in timing of the runoff (Andrews 1964; Haskin and Ford 1982; Andrews 1983). Simulations using progressive cooling or warming conditions indicated that winter temperatures consistently lower than the 3 °C could limit the long-term development of *H. nelsoni* infections. These simulations support the hypothesis that recent outbreaks of MSX disease in the northeastern United States (Barber et al. 1997; Sunila et al. 1999) and eastern Canada (Stephenson et al. 2003) may be related to the trend towards warmer winters recorded over the last decade and a half (Cook et al. 1998).

### 6.3 Spores and transmission

One of the intriguing aspects of the *H. nelsoni* model was the need to simulate the production of two life forms – the plasmodial stage, which is most prevalent, and the spore stage, which develops almost exclusively in juvenile oysters (Barber et al. 1991; Burreson 1994). Spore formation was modeled by hypothesizing that plasmodia produce spores only when certain required factors are present in the environment within the host oyster. This element of “environmental quality” was modeled as function of a third parameter, oyster food availability. Spore production was related to a threshold environmental quality, which occurs only in small oysters because of their high growth efficiency. Whether relative growth efficiency has anything to do with spore formation in juvenile oysters and its rarity in adults is purely speculative. An alternative argument, that some type of chemical or physical signal triggers sporulation, could equally well be made. Nevertheless, as pointed out by Ford et al. (1999a) “the concept of a threshold of some factor or factors remains a biologically defensible generalization for the fact that *H. nelsoni* can complete its life cycle in small oysters, but rarely in large ones”.

The model was also used to investigate transmission (Powell et al. 1999). The actual mode of transmission is unknown, as is the infective stage of *H. nelsoni*, and the model does not assume that the spores produced in juvenile oysters are directly infective to other oysters. Nevertheless, it provides certain insights into likely characteristics of transmission because of the manner in which it had to be constructed to fit field observations. For example, simulations based on in vitro, salinity-caused mortality of *H. nelsoni* plasmodia (Ford and Haskin 1988) resulted in prevalences in low salinity sites that were greater than field observations (Paraso et al. 1999). Since this suggested that mortality of plasmodia within infected oysters was not sufficient to explain the observed relationship between infection levels and salinity in the field, an “infectivity” term was added to the model, which made the infection decrease with decreasing salinity such that at 15 ppt, the “efficiency of infection” is about 40% of that at 25 ppt. The need for this element may truly reflect the fact that, at low salinity, fewer successful infections result from contacts between infective particles and the oyster. It might equally reflect a reduced density of infective stages with lowered salinity.

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![Graph](image-url)
Simulations also needed to replicate the observation that changes in *H. nelsoni* prevalence occur rapidly and over large areas of estuaries and that these changes occur independently of local salinity. To reproduce this observation, the model employs bay-wide oscillations in infective particle availability that are tied to multi-year salinity fluctuations. Simulations mirrored long-term prevalence time series in both Delaware and Chesapeake Bays (Powell et al. 1999). Since the model does not connect oyster infection levels with subsequent transmission, the linking of infective particle availability to long-term salinity change suggests that a non oyster reservoir for infective stages itself is influenced by salinity, or that salinity is a surrogate for some other parameter such as river flow, water residence time, or dilution.

Because attempts to demonstrate direct transmission of *H. nelsoni* between oysters have consistently failed, speculation has persisted that another host exists, acting either as a reservoir for infective stages or as an intermediate host for transmission (Burreson 1988; Haskin and Andrews 1988; Ford and Tripp 1996). The modeling exercise highlighted the characteristics of a potential host: 1) it must be capable of releasing large number of infective particles rapidly and continuously during the warm months; 2) normal temperature and salinity variation cannot affect it; 3) it must be affected by cold winters, but capable of recovery within a year or two; 4) it must produce infective particles independently of *H. nelsoni* levels in the oyster population; and 5) it must exist at relatively high salinity (Powell et al. 1999). These characteristics are similar to those proposed by Haskin and Andrews (1988) based on field data.

### 6.4 Comparisons between *H. nelsoni* and *P. marinus*

The data used to construct the *P. marinus* and *H. nelsoni* models, as well as the models themselves, provide interesting comparisons between the two parasites. Both models operate by causing parasites to multiply or to die in vivo and thus require quantitative data on parasite abundance rather than the semiquantitative staging systems routinely used to assess infection intensity of both parasites. A conversion between *H. nelsoni* infection stages and parasite abundance was developed using a process that frees the parasites from host tissues so their densities can be determined (Choi et al. 1989). Because *H. nelsoni* plasmodia are more fragile and would not survive a similar treatment, densities of this parasite were estimated by counting parasites in a known volume (area counted x section thickness) of representative tissue sections and extrapolating those concentrations to the density of plasmodia per unit weight (Ford et al. 1999a). On the other hand, both parasites can be obtained in hemolymph samples and their concentrations determined directly (Burreson et al. 1988; Ford and Kanaley 1988). For both parasites, average maximum densities in the hemolymph are in the range of $5 \times 10^3$ to $10^6$ ml$^{-1}$ and those estimated for the soft tissue are on the order of $10^6$ parasites g$^{-1}$ wet weight, which also seems to be the lethal level as higher densities are rarely found in living oysters. As mentioned earlier, models for both parasites require a “crowding factor”, which slows the replication rate when parasite densities become high. The parasite density at which crowding begins to influence *P. marinus* growth, obtained from field and experimental data (Saunders et al. 1993; Ford et al. 1999b), is similar to that estimated for *H. nelsoni* by fitting model simulation to disease prevalence and intensity: 1 to $7 \times 10^5$ parasites g$^{-1}$ wet weight. The semblance of thresholds suggests fundamental similarities in the per-parasite use of nutrients from, and the damage caused to, the host oyster by each parasite. Interestingly, the limit of consistently reliable detection for *P. marinus*, using the standard Ray/Mackin method of incubating tissues in Fluid Thioglycollate Medium, is estimated to be $10^3$ to $10^2$ parasites g$^{-1}$ wet weight (Choi et al. 1989; Bushek et al. 1994), which is similar to that calculated for *H. nelsoni*, using tissue section histology (Ford et al. 1999a).

In the model itself, the in vivo proliferation rate of *H. nelsoni* is based on a Q$_{10}$ of 3.2. This value was required to match proliferation rates at elevated temperature, inferred from prevalence increases. It is unusually high and implies that *H. nelsoni* is very sensitive to temperature change. By comparison, a more typical Q$_{10}$ of 2 provided adequate doubling in *P. marinus* simulations (Hofmann et al. 1995). Thus, under condition of rising temperature, *H. nelsoni* proliferation rates should increase faster than those of *P. marinus* and under falling temperatures, they should decrease faster. When superimposed, however, the modeled doubling times for the two parasites indicate that *H. nelsoni* has the higher proliferation rate across the entire temperature range over which both co-exist, approximately 0 to 35 °C. These comparisons are consistent with field observations showing that when oysters are exposed to both parasites in the field, *H. nelsoni* typically begins killing before *P. marinus* does (Andrews 1967; Chintala et al. 1994). A similar observation would result from a relatively higher dose of *H. nelsoni*, and although densities of *P. marinus* have been measured in the water and dose-response curves generated (Ragone Calvo et al. 2003), comparable information is unavailable for *H. nelsoni*.

### 7 Recent changes in the distribution and intensity of MSX disease outbreaks

#### 7.1 History of MSX disease outbreaks

The first recorded disease outbreak caused by *H. nelsoni* in eastern oysters occurred in the spring of 1957 in Delaware Bay, New Jersey, USA (Haskin et al. 1966). In 1959, *H. nelsoni* began causing mortalities in Mobjack Bay, a subestuary of lower Chesapeake Bay, and the parasite subsequently spread up estuary during a drought in the mid 1960s (Andrews and Wood 1967; Fairley 1975). The parasite was found in oysters along the Atlantic coasts of New Jersey, Maryland, and Virginia in 1958 and 1959, and in 1960 it was reported on the Connecticut shore of Long Island Sound (Haskin and Andrews 1988). In 1965, it was found in Great South Bay on the south shore of Long Island, New York (Andrews and Wood 1967; Haskin and Andrews 1988) and in 1967 in Wellfleet Harbor, on the north side of Cape Cod, Massachusetts (Krantz et al. 1972). In the 1980s, the reported range of the parasite was extended along the entire east coast of the United States, from Maine to...
Florida (Haskin and Andrews 1988; Hillman et al. 1988; Kern 1988; Lewis et al. 1992). More importantly, epizootics with severe mortality occurred in Oyster Bay on the north shore of Long Island, New York and in Southern Massachusetts during this decade (Haskin and Andrews 1988; Matthissen et al. 1990). Between 1984 and 1987, oyster production from the Connecticut shore of Long Island Sound dropped from 244,000 bushels to 70,000, suggesting that the Long Island area epizootic may not have been localized to Oyster Bay (Sunila et al. 1999). At the same time, H. nelsoni infections spread and intensified in Chesapeake and Delaware Bays (Haskin and Ford 1986; Burreson and Andrews 1988). In the 1990s, further epizootics with heavy mortalities occurred in southern Maine (Barber et al. 1997) and Long Island Sound (Sunila et al. 1999), and in 2002 H. nelsoni caused localized heavy mortalities in the Bras d’Or Lakes region of Nova Scotia, Canada (Stephenson et al. 2003). In Chesapeake Bay, the decade of the 1990s has seen continued spread of both H. nelsoni and P. marinus into regions of the upper Bay and tributaries where they have infected susceptible oysters and caused heavy mortalities (Tarnowski 2002; Ragone Calvo and Burreson 2003)

The demonstration by molecular detection that H. nelsoni is present in the Pacific oyster, C. gigas in Asia and in the western United States (Burreson et al. 2000; Kamaishi and Yoshinaga 2002) indicates that H. nelsoni was introduced from the Pacific; however, neither the mechanism nor the timing is known. It is usually inferred that the parasite entered the United States in shipments of infected C. gigas made by oyster growers or scientists. Deliberate introductions might well have been the source, but other possibilities must be considered. Particularly noteworthy is the great increase in ship transit between Pacific and Atlantic ports that occurred during and after World War II. Shipping could have introduced H. nelsoni via infected C. gigas attached to ship’s hulls or via release of H. nelsoni spores in the discharge of ballast water. The spore is a thick-walled stage in the life cycle of H. nelsoni. Its role in transmission is not known, but the spore in other species is typically a transmission stage that can remain “dormant” for long periods and that is highly tolerant of environmental extremes. Further, it is often concluded that H. nelsoni was introduced into Delaware Bay and then “spread” to Chesapeake Bay and other areas. However, the time required for an epizootic to occur after an introduction has taken place is unknown and the finding of H. nelsoni from Long Island Sound to Chesapeake Bay within the space of 3 years, makes it difficult to ascertain where the “first” introduction occurred, or even if there was a single introduction only. Certainly, the parasite must have been present for some time before it caused epizootics. In fact, it was not until the mid 1980s, more than 20 years after it was first detected in Long Island Sound, that epizootic mortalities were recorded in the region.

7.2 Climate-related intensification and spread of MSX disease outbreaks

Changes in climate are sometimes linked to disease outbreaks (Harvell et al. 1999; Harvell et al. 2002), including the range extension of Dermo disease epizootics into the northeastern United States (Ford 1996). Given the known sensitivity of H. nelsoni to salinity and temperature, it is reasonable to examine the role of these parameters in the apparent northward “spread” of MSX disease outbreaks of the 1980s and 1990s, as well as the intensification of the disease in Chesapeake Bay. In the Chesapeake Bay and its tributaries, salinity gradients are strong and large areas were formerly protected from high H. nelsoni infection levels by freshwater runoff that kept salinities low (Andrews 1968). Since the early 1980s, however, a series of extreme, multi-year droughts has increased salinities and permitted the spread of H. nelsoni, as well as P. marinus, into new areas of the estuary (Burreson and Andrews 1988; Smith and Jordan 1993; Burreson and Ragone Calvo 1996; Tarnowski 2002; Ragone Calvo and Burreson 2003). The result has been widespread and heavy oyster mortalities, and a severe loss of production of this commercially important species. In Delaware Bay, too, H. nelsoni also spread upbay during a severe drought in the mid-1980s (Haskin and Ford 1986), but with apparently different consequences (see below).

Most of the other oyster-growing waters of the northeast, salinities are at least 20 ppt, so that low salinity should not have been a factor limiting H. nelsoni proliferation, although drought-associated lack of flushing during recent periods of low river flow might allow concentration of infective stages.

Alternatively, a change in temperature regime might explain the northern MSX disease outbreaks, as suggested by the mathematical modeling exercise described above (Hofmann et al. 2001). Clearly, temperatures have been increasing in this area over the past two decades and it is particularly noticeable in higher winter temperatures (Karl et al. 1996; IPCC 2001), which would relax the control that cold winters appear to have on H. nelsoni. Hofmann et al. (2001), however, pointed out an inconsistency in the argument that low temperature had been the mechanism preventing MSX disease outbreaks in the north. If this were true, why have there been no outbreaks in the southeastern United States, where the parasite is present, but at relatively low prevalence and not associated with large-scale mortalities (Lewis et al. 1992; Bobo et al. 1996). Perhaps prolonged high temperatures play a role (Ford and Haskin 1982), but there is no evidence that elevated temperature inhibits H. nelsoni. Alternatively, some condition other than a direct temperature effect is unfavorable or perhaps a second host is scarce in this region.

7.3 Decline in MSX disease prevalence in Delaware Bay associated with natural resistance

The epizootic of 1957-1959 killed about 90–95% of all oysters in lower Delaware Bay, where salinities are nearly always favorable for H. nelsoni, and mortalities were estimated to be 50–60% in the lower-salinity beds (Haskin et al. 1966). This tremendous selective mortality resulted in measurable increased survival of the native Delaware Bay oyster population, which was comparable to that after one generation of selective breeding (Haskin and Ford 1979). After the initial improvement, however, no further change was documented for nearly 30 years because little or no additional selective mortality occurred on the upbay beds where most of the oysters were located. In the mid 1980s, drought allowed H. nelsoni to penetrate far upbay. Prevalences reached up to 80%, the highest on
Fig. 7. Mean autumn prevalence of *Haplosporidium nelsoni* (considered to be a measure of the infection pressure experienced by oysters over the summer) in lower Delaware Bay and mean winter (December – March) air temperature at nearby Millville, New Jersey, USA. Note the persistence of prevalences of 30% or less since 1988, despite high temperatures.

From the onset of the *H. nelsoni* epizootic in the late 1950s through the late 1980s, *H. nelsoni* infection pressure, as measured by autumn infection prevalence in downbay oysters, showed a cyclic pattern in which the years with lowest prevalence tended to follow cold winters (Fig. 7). During this period, autumn prevalence ranged from 50 to 90%. After 1989, however, prevalence rarely exceeded 30%, even during a period of above-average temperatures. An initial hypothesis that this change was linked to the onset of a Dermo disease epizootic in the oysters in 1990 was weakened by the knowledge that both parasites were simultaneously heavy in Chesapeake Bay and Long Island Sound (Sunila et al. 1999; Ragone Calvo and Burreson 2003; Ragone Calvo et al. 2003). An alternative explanation, that the heavy mortalities in 1985-86 further increased resistance to MSX disease in the native Delaware Bay oysters, is supported by two pieces of evidence: 1) imported susceptible stocks became heavily infected with *H. nelsoni*, whereas nearby wild oysters had few infections and 2) PCR-based molecular detection demonstrated the presence of *H. nelsoni* in or on gills (the initial infection site) of oysters throughout the Bay even though few infections become histologically detectable (Ford 2002). Although these results are consistent with the argument that native Delaware Bay oysters have developed a very high degree of resistance to the proliferation of *H. nelsoni* (although not necessarily to infection itself) and consequently to the development of MSX disease, the data are scattered among various types of studies. The standardized and consistent testing that documented the “first” step in the development of resistance (Haskin and Ford 1979) has yet to be done.

7.4 Selective breeding for dual disease (MSX and Dermo) resistance

The spread and intensification of both MSX and Dermo disease outbreaks during the past decade, and the finding that strains selected for resistance to MSX disease (Haskin and Ford 1979) were not resistant to Dermo disease (Burreson 1991), has driven several, ongoing, programs to develop dual disease-resistant oysters. The programs have relied on selective breeding: oysters have been exposed to natural infections and the survivors used to produce the following generation (DeBrosse and Allen 1966; Ragone Calvo et al. 2002; Guo et al. 2003). All of the projects have employed oysters that had first undergone extensive selection by *H. nelsoni*-caused mortality, either as wild stocks or in a selective breeding program, and were subsequently exposed to *P. marinus* infection. Results indicate that the oysters have become more resistant to *P. marinus*, observed mostly as a delay in the development of advanced infections, while retaining a high degree of resistance to the development of *H. nelsoni* infections.

7.5 Testing of non native oyster species for resistance to *H. nelsoni* infection in Chesapeake Bay

Over the past two decades, intensification of *H. nelsoni*, and particularly *P. marinus*, infection pressure in Chesapeake Bay has lead to a decline of over 90% in the production of *C. virginica* (United States National Marine Fisheries Service 2003). The loss of the native oyster both to the fishery and for the ecological services it provides (e.g., water filtration and habitat), has led to interest in the possible introduction of a non native oyster that could survive in the face of the two diseases. Two species, both from the Asian Pacific, have been tested in separate trials: *C. gigas* and *C. ariakensis*. Both were...
deployed at duplicate low (<15 ppt), medium (15–25 ppt), and high (>25 ppt) salinity sites in lower Chesapeake Bay and along the Atlantic coast of Virginia. Growth, survival, and infection levels were compared with those of *C. virginica* deployed at the same sites (Calvo et al. 1999; Calvo et al. 2001). To minimize the potential for unintended reproduction, only triploid non natives, which are largely sterile, were used in the tests. *Crassostrea gigas* grew faster and survived better than *C. virginica* at the high salinity sites, performed similarly at the medium salinity sites, and did less well at the low salinity locations (Calvo et al. 1999).

*Crassostrea ariakensis* outperformed the *C. virginica* at all locations (Calvo et al. 2001). At high salinity sites in both trials, *C. virginica* became heavily infected with *P. marinus* (up to 100%) and to a considerably lesser degree (maximum of 16 to 25%) with *H. nelsoni*. Both *C. ariakensis* and *C. gigas* also acquired *P. marinus* infections (up to 60–67%, respectively), but the infections remained mostly light and non lethal. No *H. nelsoni* infections were detected in either of the non native oysters. It should be recalled that *H. nelsoni* has been detected in *C. gigas* in the Pacific region, but always with very large sample sizes to detect prevalences that averaged <1% (Kern 1976; Kang 1980; Burreson et al. 2000).

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