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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 *Urosporidium*, *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

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taxonomists and phylogeneticists, and there have been numerous 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 protists were elevated to phylum rank. Sprague (1979) separated 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 Paramyxea were each elevated to phylum rank (Desportes and Perkins 1990; Perkins 1990, 1991, 2000). However, recently Cavalier-Smith and Chao (2003b) resurrected Ascetospora as a class in the phylum Cercozoa, subphylum Endomyxa. In their scheme class Ascetospora includes three orders – Haplosporida, Paramyxida and Claustrosporida (but see Sect. 2.2). The Haplosporidia were most recently characterized morphologically as a group of parasitic protists having 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 internally by a flap of wall material (Perkins 2000). The placement of the genus *Bonamia* in the Haplosporidia (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 characters define Haplosporidia.

2.2 Molecular phylogenetic analyses

First attempts to determine the relationship of the Haplosporidia to other Eukaryota using molecular sequence data hypothesized placement of the group within the parvkingdom 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 Haplosporidia as sister taxon to the Dictyosteliida and also provided molecular phylogenetic support for separation of the phylum Haplosporidia and phylum Paramyxea. 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 Hapolsporidia and hypothesized a relationship between the Haplosporidia and the Cercozoa, a relationship not previously recognized. Cavalier-Smith and Chao (2003b) placed the Haplosporidia 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 Haplosporidia in the analyses), the gromiid testate amoebae, the Phytomyxea, and a very large group of classical Cercozoa including zooflagellates, 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 phylogenetic analyses. Cavalier-Smith and Chao (2003a,b) include the Haplosporidia 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 – Haplosporidia, Paramyxea and *Claustrosporidium*. The molecular phylogenetic analysis by Cavalier-Smith and Chao (2003a) places *Marteilia refringens* (Paramyxea) "well within Haplosporidia" and sister to *Haplosporidium costale*. Nonetheless, Cavalier-Smith and Chao (2003a) state, inexplicably, that Haplosporida and Paramyxida are separate orders in the phylum Cercozoa. However, if *M. refringens* is a haplosporidian, as their analyses indicate, then Paramyxea has no basis. Other molecular phylogenetic analyses have not hypothesized a close relationship between the Haplosporidia and the Paramyxea (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 Haplosporidia 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 Haplosporidia should be recognized as a phylum as well. Reece et al. (2004) found no support for inclusion of the paramyxean *Marteilia refringens* within the Haplosporidia.

3 Taxa within the phylum Haplosporidia

3.1 *Urosporidium, Haplosporidium, Minchinia*

The phylum Haplosporidia has long been recognized to contain only three genera, *Urosporidiuim*, *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 covers the spore orifice, and the characters that distinguish these two genera have been much debated. It is now generally recognized that spore ornamentation as observed with transmission 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 description. 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 separate 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

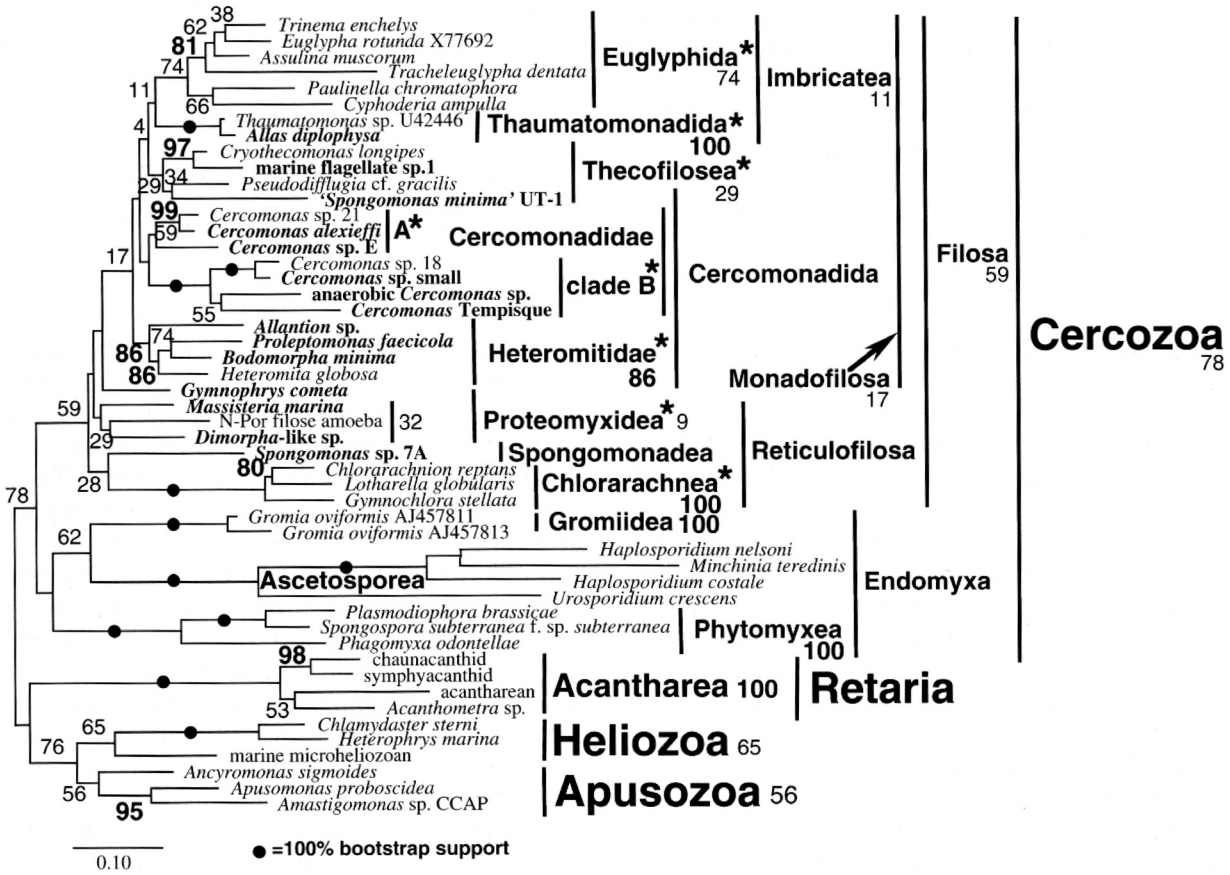

Fig. 1. Maximum likelihood tree of 50 rhizarian 18S rRNAs using 1638 positions ($\Gamma + I$ model: $\alpha = 0.55084$; $i = 0.26839$). This tree had the highest log likelihood (−25 487.62) of those yielded by 11 independent random additions of taxa. New sequences in bold. The figures are bootstrap percentages (bold if 80% or more) using the same maximum likelihood model. From Cavalier-Smith and Chao (2003b), with permission of Urban & Fischer Verlag.

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. scolopli*, 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

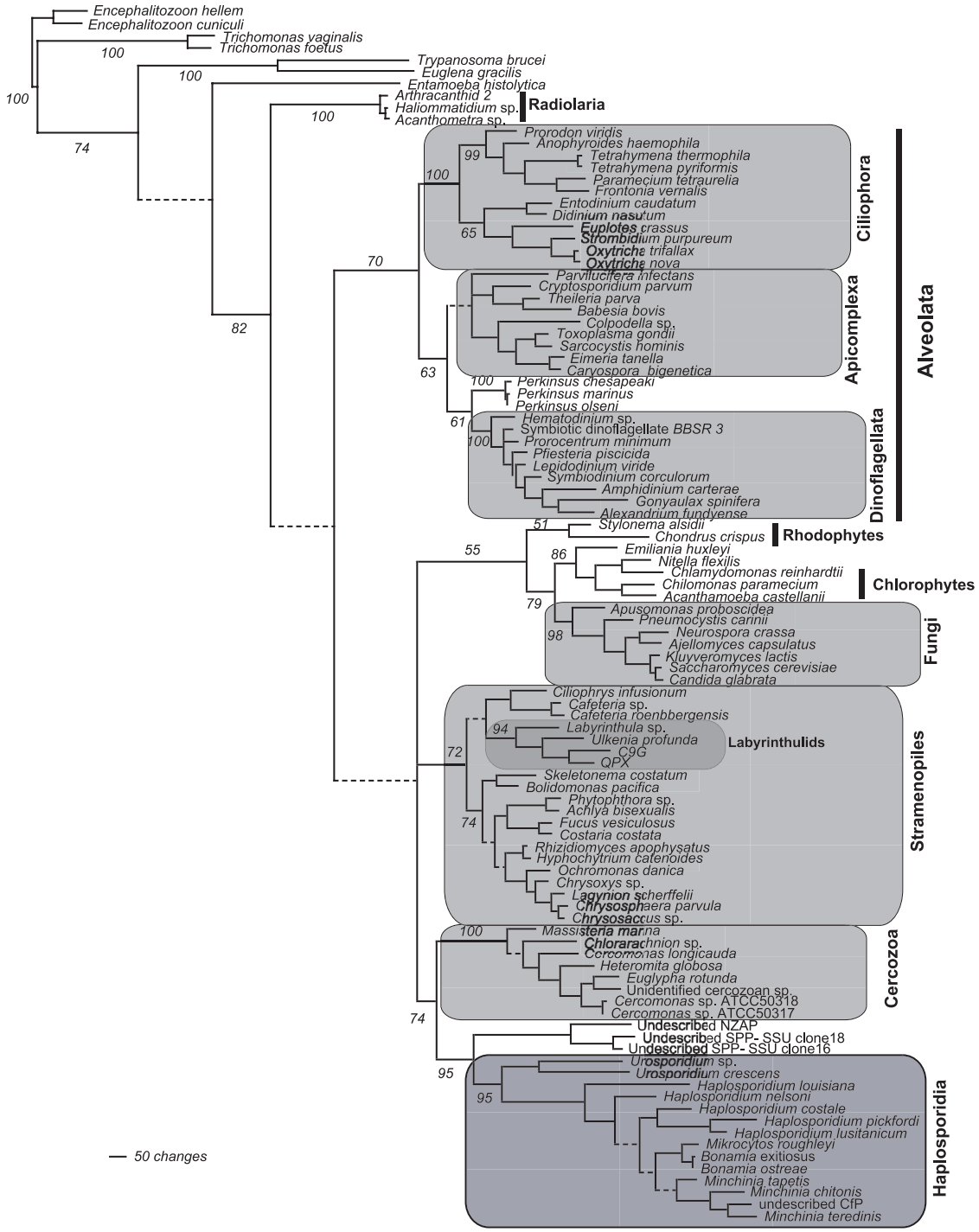

Fig. 2. Strict jackknife consensus of 4 equal length trees resulting from parsimony analysis with SSU rDNA and actin amino acid data set. Analysis was done on the complete taxonomic data set with 798 poorly aligned nucleotide position in the SSU rDNA removed. Jackknife support values are given at the nodes. Dashed lines indicate clades that did not have jackknife support values above 50. From Reece et al. (2004), with permission of the American Society of Parasitologists.

experiments with spore-forming haplosporidans 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 "microcell" 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

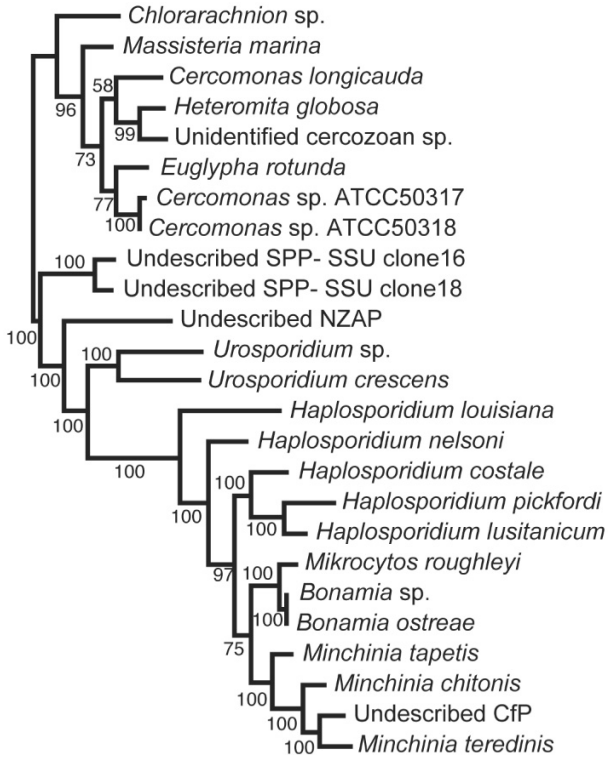

Fig. 3. Strict jackknife consensus tree of parsimony analysis of SSU rDNA sequence data to examine relationships within the Haplosporidia. Tree statistics: length (L) = 3838 nucleotide changes, consistency index (CI) = 0.600, retention index (RI) = 0.658. Jackknife support values are given at the nodes. From Reece et al. (2004) with permission of the American Society of Parasitologists.

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 Haplosporidia (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 Haplosporidia. 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 Haplosporidia. 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 Haplosporidia

The 36 recognized species in the Haplosporidia 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 Haplosporidia. 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 Haplosporidia

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 (Figueras 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 Azevedo et al. (2003). Plasmodial stages of an unidentified haplosporidian were implicated in high mortality of cultured abalone in New Zealand during the austral summer 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 Haplosporidia 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.

Species	Host	Location	Comments*/References
Urosporidium			
U. fuliginosum Caullery and Mesnil 1905	Polychaete *Syllis gracilis*	English Channel, France	Type species of *Urosporidium.* No EM. Caullery and Mesnil (1905)
U. pelseneeri (Caullery and Chappellier 1906)	Trematode sporocysts in clams *Donax vittatus, Barnea candida*	English Channel, France	No EM. Caullery and Chappellier (1906); Dollfus (1925)
U. crescens DeTurk 1940	Trematode metacercariae of *Spelotrema nicolli* in the blue crab *Callinectes sapidus*	East coast of USA	TEM, SEM, DNA. DeTurk (1940); Perkins (1971)
U. fauricum Zaika and Dolgikh 1963	Trematode in the mollusk *Rissoa splandida*	Ukraine	No EM. Zaika and Dolgikh (1963)
U. constantae Howell 1967	Trematode sporocysts of *Bucephalus longicornutus* in the oyster *Ostrea lutaria*	New Zealand	No EM. Howell (1967)
U. jiroveci Ormières et al. 1973	Trematode sporocysts of *Gymnophallus nereicola* In the clam *Abra ovata*	Mediterranean Sea, France	TEM. Ormières et al. (1973)
U. spisuli Perkins et al. 1975	Nematode in surf clam *Spisula solidissima*	East coast of USA	TEM, SEM. Perkins et al. (1975); Perkins et al. (1977)
U. cannoni Anderson et al. 1993	Polyclad flatworm *Stylochus* sp.	Moreton Bay, QLD, Australia	TEM, SEM. Anderson et al. (1993)
Haplosporidium			
H. scolopli Caullery and Mesnil 1899	Polychaete *Scoloplos mülleri.*	Cap de la Hague, France	Type species of *Haplosporidium.* No EM. Caullery and Mesnil (1899)
H. heterocirri Caullery and Mesnil 1899	Polychaete *Heterocirrus viridis*	Cap de la Hague, France	No EM. Caullery and Mesnil (1899)
H. marchouxi Caullery and Mesnil 1905	Polychaete *Salmacina dysteri*	Cap de la Hague, France	No EM. Caullery and Mesnil (1905)
H. potamillae Caullery and Mesnil 1905	Polychaete *Potamilla torelli*	Cap de la Hague, France	No EM. Caullery and Mesnil (1905)
H. vejdovskii Caullery and Mesnil 1905	FW oligochaete *Mesenchytraeus flaviduus*	Czech Republic	No EM. Caullery and Mesnil (1905)
H. limnodrili Granata 1913	FW oligochaete *Limnodrilus udekemianus*	Florence, Italy	No EM. Granata (1913)
H. nemertis Debaisieux 1920	Nemertean *Lineus bilineatus*	Plymouth, UK	No EM. Debaisieux (1920)
H. caulleryi Mercier and Poisson 1922	Polychaete *Nereilepas fucata*	Luc-sur-Mer, Calvados, France	No EM. Mercier and Poisson (1922)
H. ascidiarum Duboscq and Harant 1923	Tunicates *Sydnium elegans, Ciona intestinalis*	Mediterranean Sea	TEM. Duboscq and Harant (1923); Ormières and de Puytorac (1968); Ciancio et al. (1999)
H. cernosvitovi Jírovic 1936	FW oligochaete, *Opistocysta flagellum*	Misiones Province, Argentina	No EM. Jírovec (1936)
H. pickfordi Barrow 1961	FW snails *Physella parkeri, Lymnaea stagnalis, Heliosoma companulatum*	Michigan, USA	TEM, SEM, DNA. Barrow (1961); Burreson (2001)
H. costale Wood and Andrews 1962	Oyster *Crassostrea virginica*	East coast of North America	TEM, DNA. Wood and Andrews (1962); Perkins (1969)
H. Louisiana (Sprague 1963)	Mudcrab *Panopeus berbstii*	East and Gulf of Mexico coasts of USA	TEM, SEM., DNA. Sprague (1963); Perkins (1975)

Table 1. Continued.

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

*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 haplosporidians in Tables 1 and 2 suggest that haplosporidians 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.

Genus	Host	Location	Comments/References
Not designated	Oyster *Crassostrea gigas*	California, USA	Katkansky and Warner (1970)
Not designated	Oyster, *Ostrea lurida*	Oregon, USA	Mix and Sprague (1974)
Not designated	Clam, *Tresus capax*	Oregon, USA	Armstrong and Armstrong (1974)
Not designated	Blue crab, *Callinectes sapidus*	Virginia, North Carolina, USA	Newman et al. (1976)
Haplosporidium sp.	Oyster, *Ostrea angasi*	NW France	Probably *H. armoricanum*. Bachere et al. (1987)
Not designated	Shrimp, *Penaeus vannamei*	Nicaragua or Cuba	Dyková et al. (1988)
Not designated	Mussel *Mytilus edulis*	Maine, USA	Figueras et al. (1991)
Haplosporidium sp.	Oyster *Crassostrea gigas*	Mediterranean Sea, France	Comps and Pichot (1991)
Not designated	Scallop, *Argopecten irradians*	China	Chu et al. (1996)
Minchinia sp.	Mussel *Mytilus galloprovincialis*	Mediterranean Sea, France	Comps and Tigé (1997)
Haplosporidium sp.	Pearl oyster *Pinctada maxima*	Western Australia	Hine and Thorne (1998)
Haplosporidium sp.	Rock oyster *Saccostrea cucullata*	Western Australia	Hine and Thorne (2002)
Not designated	Abalone, *Haliotis iris*	New Zealand	Diggles et al. (2002), Hine et al. (2002), Reece and Stokes (2003)
Not designated	Freshwater amphipod, *Diporeia* sp.	Michigan, USA	Messick and Nalepa (2002)
Not designated	Mussel, *Mytilus edulis*	Nova Scotia, Canada	Stephenson et al. (2002)
Not designated	Zebra mussel, *Dreissena polymorpha*	France, Germany	D. P. Molloy, Pers. Comm.

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

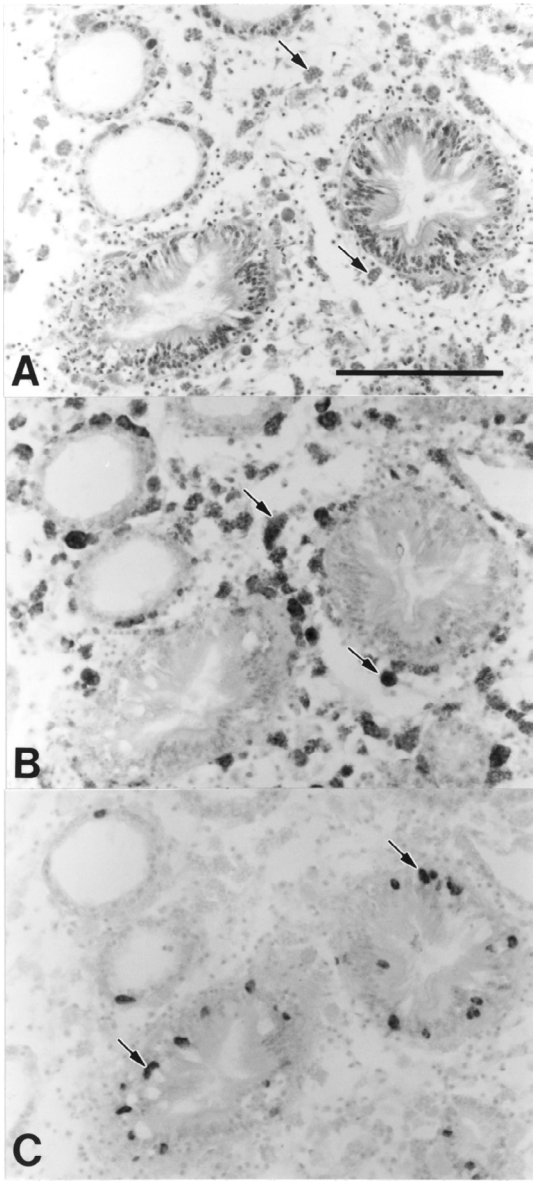

Fig. 4. In situ hybridization (ISH) of consecutive histological sections of an oyster, *Crassostrea virginica*, with a mixed infection of *Haplosporidian nelsoni* and *H. costale*. A. Hematoxylin and eosin stain; two of the many obvious plasmodia in connective tissue indicated by arrows. Scale bar = 100 μm and applies to all figures. B. ISH with *H. costale* DNA probe of same area shown in A. Note *H. costale* plasmodia in connective tissue (two indicated by arrows), but not in epithelium. C. ISH with *H. nelsoni* DNA probe of same area shown in A and B. Note *H. nelsoni* plasmodia in epithelium (two indicated by arrows), but not in connective tissue. From Stokes and Burreson (2001), with permission of Journal of Shellfish Research.

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

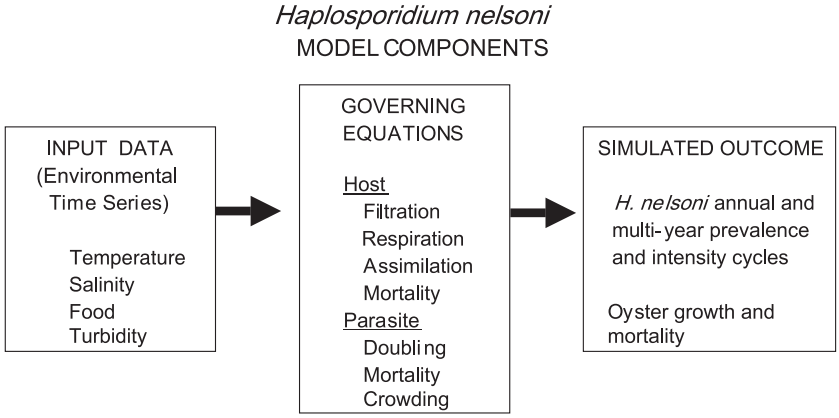

Fig. 5. Conceptual model of *Haplosporidium nelsoni – Crassostrea virginica* interactions showing elements of its three principal components.

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

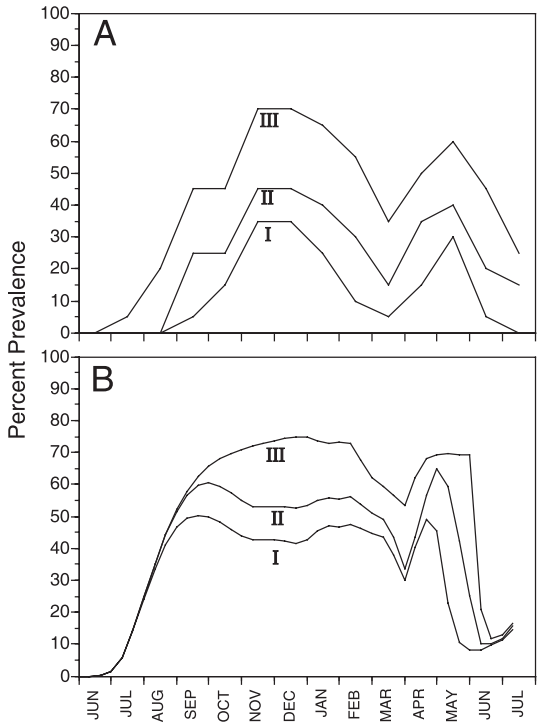

Fig. 6. Typical observed annual cycle of *Haplosporidium nelsoni* infection prevalence (A) and model simulation of the annual cycle (B). Roman numerals designate increasingly advanced infection categories: I – Epithelial infections; II – Subepithelial, local infections; III – Systemic infections.

regimes (Paraso et al. 1999). Simulations using time series collected along the salinity gradient in Delaware Bay reproduced the observed increased and decreased *H. nelsoni* infection levels under low and high freshwater runoff conditions, respectively. It has long been known that spring freshwater runoff is an important control on *H. nelsoni* infection levels in many areas (Andrews 1964; Haskin and Ford 1982; Andrews 1983). Thus, it was of interest to simulate hypothetical shifts in timing of the runoff. In Delaware Bay, a shift from March to either February or May affected the mid-Bay only, where salinity remained between 13 and 19 ppt (Paraso et al. 1999). A February runoff reduced the spring prevalence peak and caused a complete loss of systemic infections. In contrast, a May discharge occurred too late to affect parasite proliferation in the spring. In the upper Bay, the spring runoff eliminated the spring peak, and in the lower Bay, it had almost no effect at all. Shifting the timing of the runoff made no difference in these regions.

The model was constructed to reflect year-to-year fluctuation in *H. nelsoni* prevalence, associated with winter temperatures, that have been documented in Delaware Bay (Ford and Haskin 1982). This permitted simulations to be run under various long-term climate change scenarios (Hofmann et al. 2001). One such set of simulations was run for a site in upper Chesapeake Bay. The environmental time series from this site was experimentally modified to simulate short and long periods of the highest and lowest recorded temperatures. Model runs indicated that at sites where the parasite is already prevalent, relatively short periods of high temperature resulted in only minor deviations from the average pattern because average temperatures are already high enough to support parasite development. In contrast, the measured low temperature conditions, applied for a single year, caused a dramatic reduction in prevalence, which extended over a two-year period. 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" terms 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.

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 the *P. marinus* 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 5×10^5 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 7×10^5 parasites g^{-1} wet weight. The resemblance of threshold values 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 10^4 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 upestuary during a drought in the mid 1960s (Andrews and Wood 1967; Farley 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; Matthiessen 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 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). In 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 measurably 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

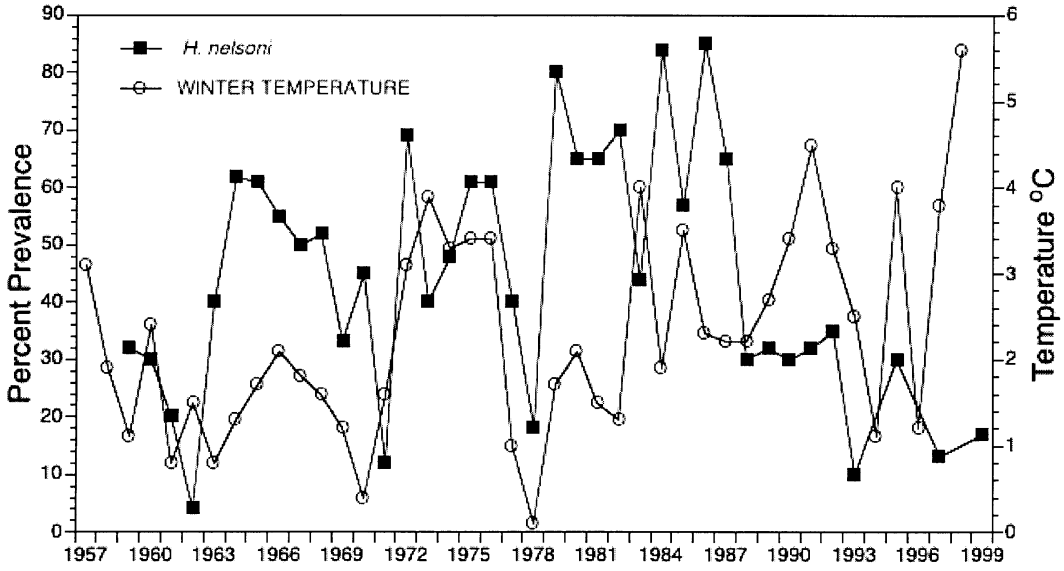

Fig. 7. Mean autumn prevalence of *Haplosporidium nelsoni* (considered to be a measure of the infection pressure experienced by oysters over the summer) in lower Delaware Bay and mean winter (December – March) air temperature at nearby Millville, New Jersey, USA. Note the persistence of prevalences of 30% or less since 1988, despite high temperatures.

record. Annual mortalities over most of the upper Bay for the two-year period of 1985-86 were two or more times that of the preceding years and the heaviest since the initial mortalities in 1957-59 (Haskin and Ford 1986).

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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