2003

Perkinsus Sp Infection Risk For Manila Clams, Venerupis Philippinarum (A. Adams And Reeve, 1850) On The Pacific Coast Of North And Central America

RA Elston

CF Dungan

TR Meyers

Kimberly S. Reece
Virginia Institute of Marine Science

Journal of Shellfish Research, Vol. 22, No. 3, 661–665, 2003.

PERKINSUS SP. INFECTION RISK FOR MANILA CLAMS, *VENERUPIS PHILIPPINARUM* (A. ADAMS AND REEVE, 1850) ON THE PACIFIC COAST OF NORTH AND CENTRAL AMERICA

RALPH A. ELSTON,[1]* CHRISTOPHER F. DUNGAN,[2] THEODORE R. MEYERS,[3] AND KIMBERLY S. REECE[4]

[1]*AquaTechnics, PO Box 687, Carlsborg, Washington 98324;* [2]*Maryland Department of Natural Resources, Cooperative Oxford Laboratory, 904 S. Morris Street, Oxford, Maryland 21654;* [3]*Alaska Department of Fish and Game, PO Box 25526, Juneau, Alaska 99802;* [4]*Virginia Institute of Marine Science, PO Box 1346, College of William and Mary, Gloucester Point, Virginia 23062*

ABSTRACT Manila clams (*Venerupis philippinarum*, A. Adams and Reeve 1850) are an important aquaculture species on the west coast of North America and are also cultured in Europe, Asia, and other locations. Clams cultured on the west coast of North America are free of *Perkinsus* sp. infections, while clams from certain Asian and European sources are infected. Infection in Korean Manila clams is reportedly associated with high morbidity and mortality. We evaluated the health status of readily accessible Manila clam juveniles from Korea that were proposed for importation into Mexican waters where they would increase in size, and then be shipped into the United States, either to market destinations or to receiving waters. The examination of the clams was performed as a preliminary assessment for a producer considering the importation of Korean Manila clams. We report finding a high prevalence of a *Perkinsus* sp. causing significant tissue damage in juvenile Korean Manila clams. Parasite taxonomic verification was made using a genus-*Perkinsus* SSUrRNA gene-specific DNA probe for *in situ* hybridization. The use of this probe is validated and reported for the first time. As a result of this finding, no importation of this clam stock took place. It is urgently important to make widely known the risk of the spread of this disease into the clam stocks of the west coast of North and Central America to prevent such an introduction. In addition, we report new information regarding the prevalence and intensity of this disease in juvenile clams available for export, as well as pathologic features of the disease.

KEY WORDS: *Venerupis (Tapes) philippinarum*, juvenile clam infection, *Perkinsus* sp., DNA probe, *in situ* hybridization

INTRODUCTION

Manila clams (*Venerupis philippinarum*, A. Adams and Reeve 1850) are an important aquaculture species on the west coast of North America. More than 7 million pounds of littleneck clams, predominantly *V. philippinarum*, were produced in Washington, California, and Oregon in 2000 (Pacific Coast Shellfish Growers Association 2003), and additional production occurs in British Columbia, Canada. Although Alaska produces native littleneck clams, *Protothaca staminea* (Conrad 1837), Manila clams are exotic, and importation for aquaculture purposes is prohibited. *Venerupis philippinarum* is also an important aquaculture species in Europe and Asia, and is infected with *Perkinsus* sp. on both continents. Specifically, *Perkinsus atlanticus* occurs in Europe (Navas et al. 1992), a *P. atlanticus*-like parasite occurs in Japan (Hamaguchi et al. 1998), and *Perkinsus* sp. occurs in Korea (Choi & Park 1997) and China (Liang et al. 2001). Consistent with the close homology noted between DNA sequences at several *P. atlanticus* and *Perkinsus olseni* loci by diverse investigators, Murrell et al. (2002) assert these parasitic species to be synonymous, with taxonomic priority to the *P. olseni* name.

In contrast, clams from the west coast of North America are free of *Perkinsus* sp. infections. A survey of Manila clam health and conditions on the west coast of North America (Pacific Shellfish Institute 2001), and the required examination of over 3000 clams for health certifications from 1991 to 2002, showed no evidence of *Perkinsus* sp. infection. Moreover, such infections have not been reported elsewhere on the west coast during routine annual examinations and frequent health examinations of brood stocks and seed clams since 1985. In addition, *Perkinsus* sp. infection has not been reported in the native littleneck clam *P. staminea* or any other bivalve species from the west coasts of North or Central America.

Manila clams may be imported as a live market product from Korea, Japan, or other Asian countries into North America. In 1998, we evaluated the health status of juvenile Manila clams from Korea that had been proposed for importation into Mexican waters, where they would gain size before shipment to the United States, either to market destinations or to receiving waters for further grow out. The examination of clams was performed as a preliminary assessment for a producer considering the importation of Korean Manila clams. We report the finding of a high prevalence of a *Perkinsus* sp. causing significant tissue damage in juvenile Korean Manila clams.

As a result of this finding, no importation of this clam stock took place. It is urgently important to make widely known the risk of the spread of this disease to west coast North American clam stocks to prevent the introduction of this debilitating and lethal clam parasite. In addition, we report here new information regarding the prevalence and intensity of this disease in juvenile clams that are available for export, as well as pathologic features of the disease. Finally, a novel genus-*Perkinsus* DNA probe for *in situ* hybridization (ISH) assays on histologic samples is described.

Taxonomic references to the Manila clam (also commonly referred to as the Japanese littleneck clam) in the scientific literature are particularly confusing. We have designated the species as *V. philippinarum* in accordance with the Committee on Scientific and Vernacular Names of Molluscs within the Council of Systematic Malacologists, American Malacological Union (American Fisheries Society 1998). The common name Manila clam is also found in the literature, apparently in reference to the same species, associated with scientific designations of *Tapes philippinarum*, *Ruditapes philippinarum*, *Tapes semidecussatus*, and *Tapes japonica*.

*Corresponding author. E-mail: aquatech@olypen.com

TABLE 1.

ISH assay results with genus-*Perkinsus* SSUrRNA probe, Perksp700DIG.

Parasite	Host	Sample	± Probe Hybridization	Sample Source	Reference
Perkinsus sp.	*V. philippinarum*	98-SH14-5		R. A. Elston	this article
Perkinsus sp.	*V. philippinarum*	98051504-2		Y. Maeno	Maeno et al. 1999
P. atlanticus	*R. decussatus*	685a		C. Azevedo	Azevedo 1989
P. olseni	*H. laevigata*	ST389-35		C. L. Goggin	Goggin et al. 1989
P. chesapeaki	*M. arenaria*	CHBRMa-14		C. Dungan	Dungan et al. 2002
P. andrewsi	*M. balthica*	MB3a2		F. G. Kern	Coss et al. 2001
P. marinus	*C. virginica*	221, 556-15		K. S. Reece	Mackin et al. 1950
P. mediterraneus	*O. edulis*	08 and 016		A. Villalba	Casas et al. in press
Perkinsus sp.	*C. pacificus*	CH02882		C. L. Goggin	Goggin et al. 1989
P. qugwadi	*P. yessoensis*	6492-A5	–	S. M. Bower	Blackbourne et al. 1998
Haplosporidium nelsoni	*C. virginica*	201, 239	–	E. Burreson	Haskin et al. 1966
H. costale	*C. virginica*	196, 774	–	E. Burreson	Couch 1967
haplosporidian-like sp.	*P. platyceros*	90-568J	–	S. M. Bower	Bower & Meyer 2002
Hematodinium sp.	*C. sapidus*	98-513	–	J. D. Shields	Shields 1994
Hematodinium sp.	*N. norvegicus*	990427Nnor-1	–	G. Stentiford	Field & Appleton 1995

MATERIALS AND METHODS

A total of 64 Manila clams [16–32 mm shell length (SL)] from Inchon Bay, South Korea, were clinically examined in February 1998 and were fixed whole in Davidson's shellfish fixative (Shaw & Battle 1957). These tissues were processed for routine histologic examination.

A representative tissue section containing parasites was evaluated by ISH. The genus-*Perkinsus* DNA probe was designed to specifically target SSU rRNA sequences of *Perkinsus* species by aligning the available SSU rRNA gene sequences, while not hybridizing to the sequences of closely related parasite taxa including dinoflagellates and apicomplexans. An SSU rRNA gene sequence is not available for *Perkinsus qugwadi*. The resulting probe Perksp700DIG (5′-CGCACAGTTAAGTRCGTGRGCACG-3′) was 5′ end-labeled with digoxigenin (Sigma-Genosys, The Woodlands, TX). ISH assays were performed as previously described (Stokes & Burreson 1995, Stokes & Burreson 2001), except that 125 μg/mL pronase was used for permeablization, instead of proteinase K, for a 30-min digestion, and a probe concentration of 7 ng/μl was used for hybridization. The probe was tested on an array of *Perkinsus* sp.-infected, paraffin-embedded tissues (Table 1), including *Perkinsus marinus* in *Crassostrea virginica*, *P. atlanticus* in *Ruditapes decussatus*, *P. olseni* in *Haliotis laevigata*, *Perkinsus andrewsi* in *Macoma balthica*, *Perkinsus* sp. in *Vereurupis philippinarum* from Japan, *Perkinsus chesapeaki* in *Mya arenaria*, *Perkinsus mediterraneus* n. sp. in *Ostrea edulis* (Casas et al. in press), *Perkinsus* sp. in *Chama pacificus*, and *P. qugwadi* in *Patinopecten yessoensis*. Probe specificity was validated by testing tissue sections of the blue crab *Callinectes sapidus*, which was infected with the parasitic dinoflagellate *Hematodinium* sp. (Shields 1994), *Hematodinium* sp.-infected Norway lobster *Nephrops norvegicus* (Field & Appleton 1995), *Haplosporidum nelsoni*-infected and *Haplosporidum costale*-infected *C. virginica* oysters, and spot prawn *Pandalus platyceros*, infected by an undescribed haplosporidian-like protozoan parasite (Bower & Meyer 2002). Replicate sections of nonspecific ISH assay signal controls of each sample were tested identically, except that they received hybridization buffer without probe during the overnight hybridization step.

RESULTS

Histologic Evaluation of Infected Clams

The prevalence of juvenile clams infected with the presumptive *Perkinsus* sp., was 59 of 64 (92%), based on histologic examination. The protozoa were systemically distributed in a variety of organs, most typically in subepithelial areas of the gills, and fre-

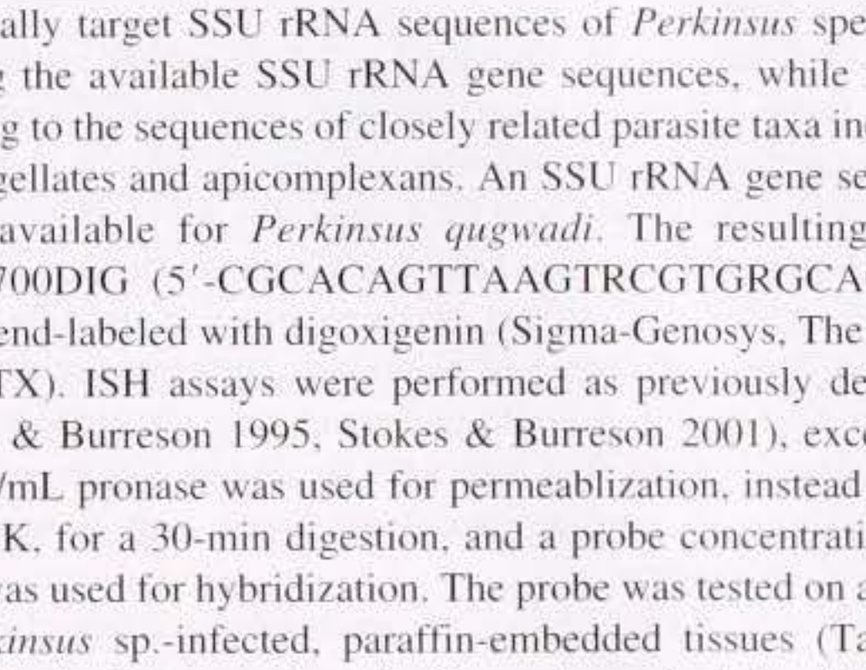

Figure 1. Gill tissue of a juvenile Korean Manila clam infected with *Perkinsus* sp. (arrows). Note the dense cellularity (hemocytosis) in the vicinity of the parasites. Bar, 10 μm, H&E.

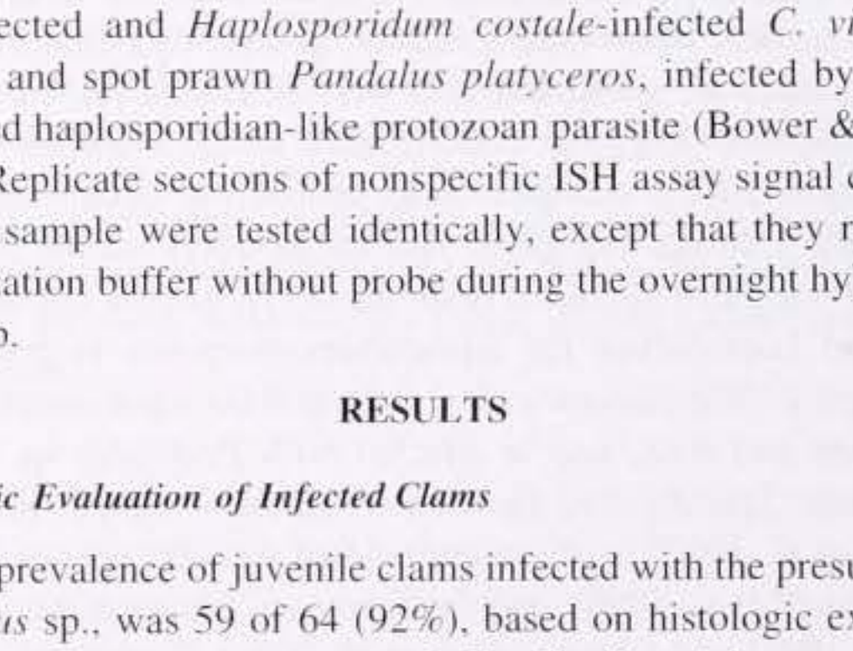

Figure 2. Higher magnification of a cyst of *Perkinsus* sp. trophozoites in the gill tissue of the Manila clam (arrow). Bar, 10 μm, H&E.

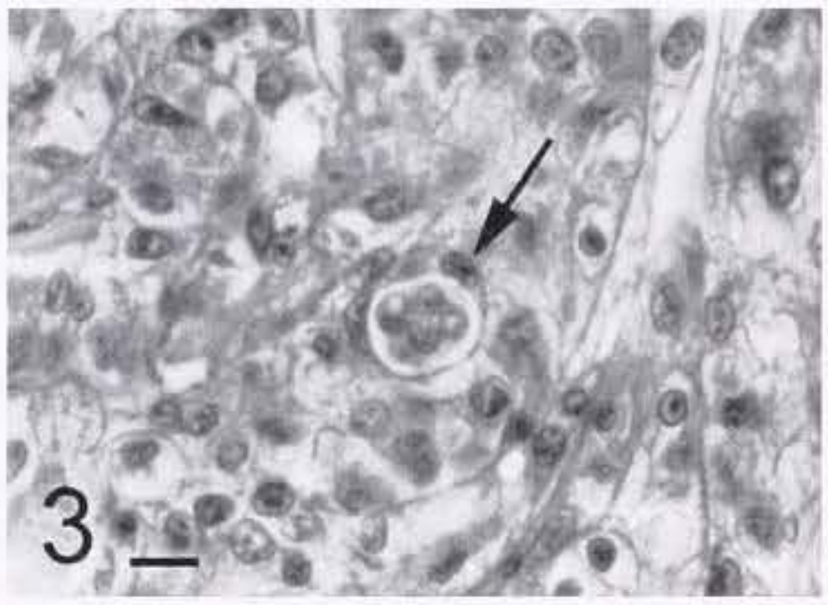

Figure 3. Cyst of *Perkinsus* sp. trophozoites encapsulated by a hemocyte within the gill of a Manila clam. Bar, 10 µm, H&E.

quently in the mantle and labial palps. Parasites were often associated with tissue hemocytosis (Fig. 1) and occurred as single or multiple trophozoites (Fig. 2). In severe infections, the parasites were more abundantly distributed in the tissues, including the vascular sinuses around the digestive diverticula. Broad areas of the subepithelial connective tissues were composed of solid masses of parasite cysts in the most severe infections. In many cases, the parasites were contained within a thin-walled cyst formed by one to several host cells (Fig. 3). Such encapsulations contained up to 10 protozoan cells and associated hemocytosis. The parasites were often characterized by the presence of an eccentric vacuole (Fig. 1 and 3), characteristic of *Perkinsus* sp. trophozoites.

Confirmation of Perkinsus *sp. by ISH*

The genus-*Perkinsus* SSUrRNA gene probe Perksp700DIG demonstrated strong hybridization to *Perkinsus* sp. cells in all of the tissue sections, except those of *P. qugwadi* infecting *P. yessoensis* (Table 1 and Fig. 4A–I). No hybridization to parasite cells of other genera was observed. ISH of parasite cells in tissue sections of infected Korean Manila clams with this genus-*Perkinsus* probe confirmed the genus level affiliation of the parasites in our sample of juvenile Korean Manila clams (Fig. 5).

DISCUSSION

We report the confirmation by ISH assays and histology of *Perkinsus* sp. infections in Manila clam seed proposed for the introduction into Mexican waters and the subsequent transport to growout sites on the Pacific coast of the United States. This is the first confirmation by a molecular diagnostic probe of *Perkinsus* sp. infection of Korean Manila clams As a result of these findings, the plan for importation of these clams was rejected by the shellfish producer, and no Korean seed clams were imported to the west coasts of Mexico or the United States. However, the ready avail-

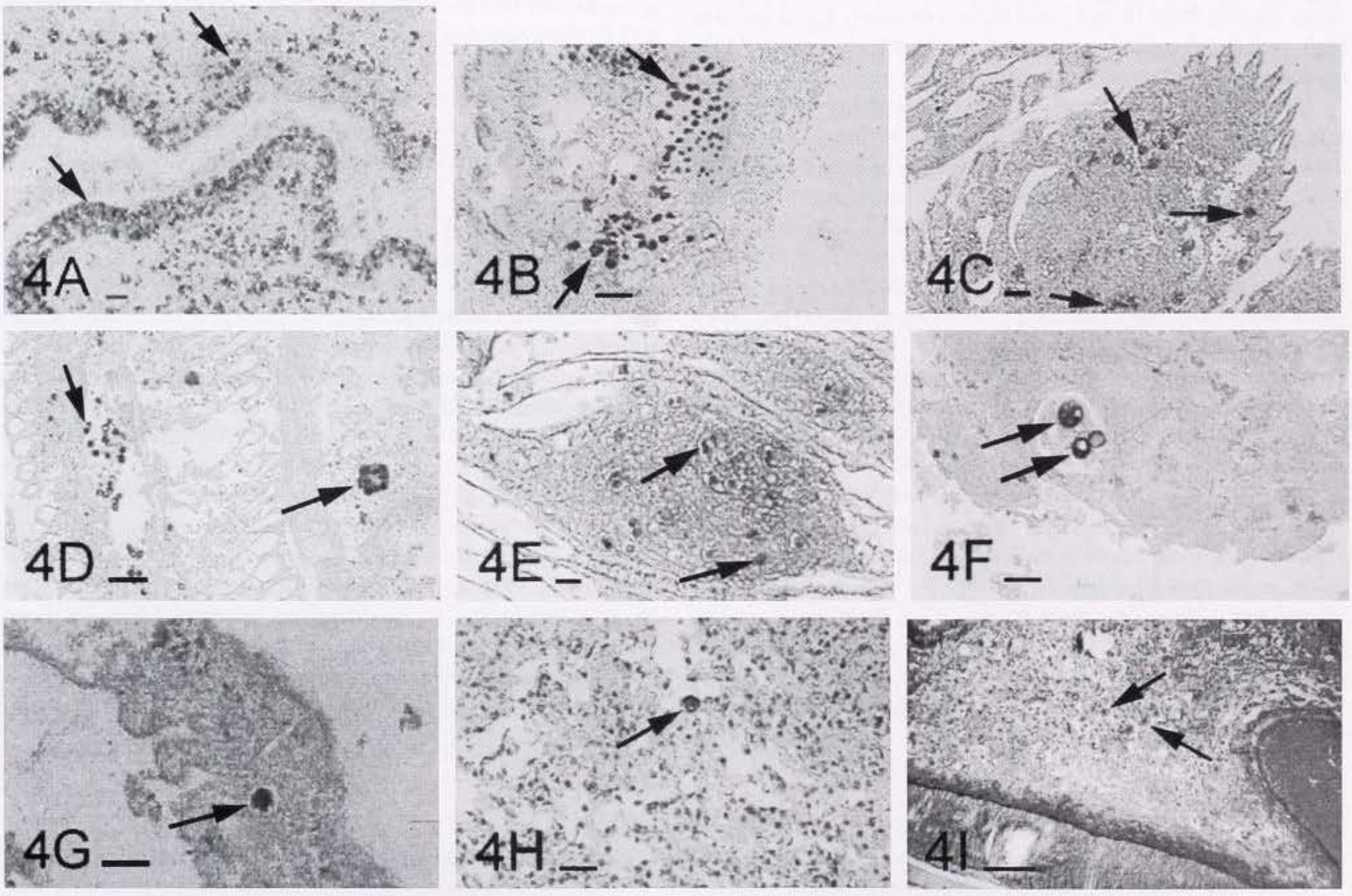

Figure 4. Tissues sections of host tissues reacted with the genus-*Perkinsus* probe Perksp700 by ISH. Positively stained *Perkinsus* sp. parasites are shown by arrows. (A) *P. marinus* in *C. virginica* intestine (bar, 10 µm). (B) *Perkinsus* sp. in *C. pacificus* (bar, 10 µm). (C) *P. atlanticus* in *R. decussatus* (bar, 10 µm). (D) *P. olseni* in *H. laevigata* gill and mantle (bar, 25 µm). (E) *Perkinsus* sp. in *M. balthica* (bar 25 µm). (F) *Perkinsus* sp. in Japanese *V. philippinarum* (bar, 10 µm). (G) *P. chesapeaki* in *M. arenaria* (bar 10 µm). (H) *P. mediterraneus* n. sp. in *O. edulis* (bar, 10 µm). (I) *P. qugwadi* in *P. yessoensis* (no hybridization observed) (bar, 25 µm).

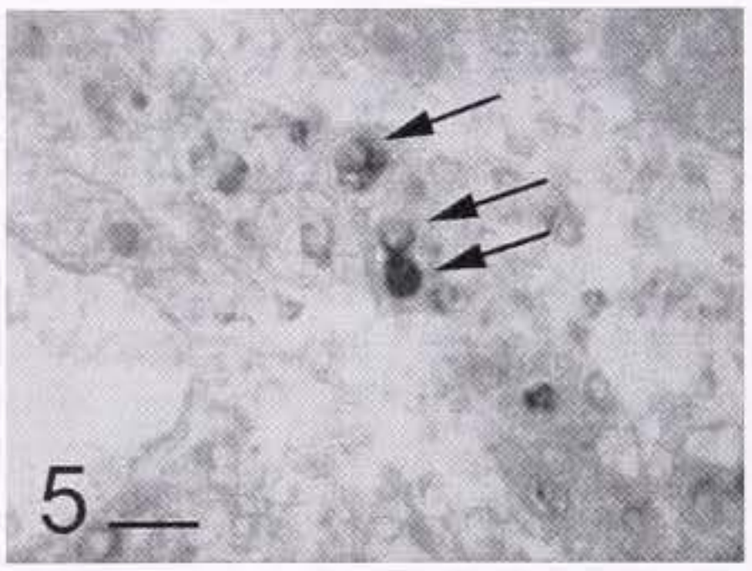

Figure 5. Tissue section of Korean Manila clam reacted with genus-*Perkinsus* probe Perksp700DIG by *ISH*. Positively stained *Perkinsus* sp. parasites are shown by arrows. Bar, 10 µm.

ability of such infected seed clams from Korean or Japanese producers requires vigilance to ensure that no such importations take place into areas that are free of the pathogen, such as the west coasts of North and Central America. Reports of lethal *Perkinsus* sp. infections in European and eastern Asian Manila clams from latitudes as far north as that of northern Oregon, confirm the high likelihood that such infections, if introduced, could persist and be transmitted, with damaging results to both wild and cultured clam stocks along the Pacific coasts of North and Central America.

This study demonstrated that infection prevalence in seed clams ranging from 16 to 32 mm SL can be nearly 100% and that high parasite intensities cause significant histologic damage to the organs of infected clams, particularly the gills.

Choi and Park (1997) studied five species of Korean clams for infections by *Perkinsus* sp. using Ray's fluid thioglycollate medium (Ray 1966) and found infected Manila clams along the south coast of Korea. While no infection occurred in clams of <15 mm SL, nearly 100% infection prevalence occurred in clams of >20 mm SL. Park et al. (1999) reported mass mortality of Manila clams along the west and south coasts of Korea over a period of several years, which was associated with *Perkinsus* sp. infections. They reported 100% infection prevalence in 142 clams from Komsoe Bay on the west coast of Korea with moderately severe mean parasite intensities of 2.87 based on the infection intensity scale of Choi et al. (1989). A negative correlation was found between the intensity of *Perkinsus* sp. infections and the clam condition index, while clam size was positively correlated with infection intensity.

Maeno et al. (1999) reported *Perkinsus* sp. parasites in Manila clams from an inner bay of the western part of Japan in April 1998, using genus-*Perkinsus*-specific antibodies. These authors concluded that the parasites were *Perkinsus* sp. based on a positive reaction with both single and clustered trophozoites. Hamaguchi et al. (1998) have reported the first detection of *Perkinsus* sp. in Japanese Manila clams. Anecdotal information that we received from the Korean supplier of the seed clams and their Japanese customers indicated that the Manila clam seed had been transported from the Korean source to Japan for at least 20 y with no unusual mortalities or loss of growth reported. This anecdotal report and the multiple reports of the *Perkinsus* sp. parasite occurring about 1997 or 1998 in Japan and Korea suggest that it could have been a new introduction to the Korean clams, as well as the Japanese clams, at about this time.

Manila clams and other bivalve species from Europe reportedly have been infected with *Perkinsus* sp., as follows: *P. atlanticus* from the Mediterranean coast of Spain (region of the Ebro Delta, Tarragona, Spain) infected *R. philippinarum* (Sagrista et al. 1996); Manila clams from the Lagoon of Venice in northeast Italy infected with a *Perkinsus* sp. (DaRos et al. 1998); and *P. atlanticus* infected the carpet shell clam (*R. decussatus*) from European locations (Ordas et al. 2000). Villalba et al. (2000) reported a significant correlation between the SL of *R. decussatus* and *P. atlanticus* infection intensity. No clams of <20 mm SL were infected, and the highest seasonal parasite intensities occurred in spring and late summer to early autumn.

The relationship of *Perkinsus* sp. in European waters to the *Perkinsus* sp. found in Korea and Japan is unknown at this time. Nonetheless, this and other studies cited in this report indicate the presence of this damaging parasite in Korean and Japanese Manila clams, confirmed first in this study by histology and then definitively by the *Perkinsus* sp.-specific probe presented for the first time in this article. This knowledge can be used to prevent the unintentional introduction of this parasite to west coast of North and Central America. We urge that the science presented in this article be applied by shellfish growers, and by natural resource and conservation managers to prevent such a damaging introduction.

ACKNOWLEDGMENTS

N. A. Stokes, K. L. Hudson, K. Apakupakul, and R. M. Hamilton provided expert technical assistance in the performance of ISH assays. *Perkinsus* sp.-infected mollusc histologic samples were generously provided by C. Azevedo, S. M. Bower, E. M. Burreson, C. L. Goggin, F. G. Kern, and Y. Maeno. Parasitic dinoflagellate-infected crustacean tissue samples were provided by J. D. Shields and G. D. Stentiford. This work was supported in part by National Oceanic and Atmospheric Administration (NOAA) Sea Grant funding of project NA86RG0037 to CFD. This work is also a result of research sponsored in part by NOAA Office of Sea Grant, U.S. Department of Commerce, under grant No. NA96RG0025 to the Virginia Graduate Marine Science Consortium and the Virginia Sea Grant College Program, and under grant No. NA016RG2207 to the Maryland Graduate Marine Science Consortium and the Maryland Sea Grant College Program. The U.S. Government is authorized to produce and distribute reprints for governmental purposes, notwithstanding any copyright notation that may appear hereon. VIMS contribution #2575.

LITERATURE CITED

American Fisheries Society. 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: Mollusks. 2nd ed. American Fisheries Society Special Publication 26. Bethesda, MD.

Azevedo, C. 1989. Fine structure of *Perkinsus atlanticus* n. sp. (Apicomplexa, Perkinsea) parasite of the clam *Ruditapes decussatus* from Portugal. *J. Parasitol.* 75:627–635.

Blackbourne, J., S. M. Bower & G. R. Meyer. 1998. *Perkinsus qugwadi* sp. nov. (incertae sedis), a pathogenic protozoan parasite of Japanese scallops, *Patinopecten yessoensis*, cultured in British Colombia, Canada. *Can. J. Zool.* 76:942–953.

Bower, S. M. & G. R. Meyer. 2002. Morphology and ultrastructure of a protistan pathogen in the haemolymph of shrimp (*Pandalus* spp.) in the northeastern Pacific Ocean. *Can. J. Zool.* 80:1055–1068.

Casas, S. M., A. Grau, K. S. Reece, K. Apakupakul, C. Azevedo & A. Villalba. 2003. *Perkinsus mediterraneus* n. sp. a protistan parasite of the European flat oyster *Ostrea edulis* (L.) from the coast of Balearic Islands, Mediterranean Sea. *Dis. Aquat. Org.* in press.

Choi, K. S. & K. I. Park. 1997. Report on the occurrence of *Perkinsus* sp.in the Manila clams, *Ruditapes philippinarum* in Korea. *(Korean) J. Aquaculture* 10:227–237.

Choi, K.-S., E. A. Wilson, D. H. Lewis, E. N. Powell & S. M. Ray. 1989. The energetic cost of *Perkinsus marinus* parasitism in oysters: quantification of the thioglycellate method. *J. Shellfish Res.* 8:125–131.

Coss, C. A., J. A. F. Robledo & G. R. Vasta. 2001. Description of *Perkinsus andrewsi* n. sp. isolated from the Baltic clam (*Macoma balthica*) by characterization of the ribosomal RNA locus, and development of a species-specific PCR-based diagnostic assay. *J. Eukaryot. Microbiol.* 48:52–61.

Couch, J. A. 1967. Concurrent haplosporidian infections of the oyster, *Crassostrea virginica* (Gmelin). *J. Parasitol.* 53:248–253.

DaRos, L., M. G. Marin, N. Nesto & S. E. Ford. 1998. Preliminary results on a field study on some stress-related parameters in *Tapes philippinarum* naturally infected by the protozoan *Perkinsus* sp. *Mar. Env. Res.* 46:249–252.

Dungan, C. F., R. M. Hamilton, K. L. Hudson, C. B. McCollough & K. S. Reece. 2002. Two epizootic diseases in Chesapeake Bay commercial clams, *Mya arenaria* and *Tagelus plebeius*. *Dis. Aquat. Org.* 50:67–78.

Field, R. H. & P. L. Appleton. 1995. A *Hematodinium*-like dinoflagellate infection of the Norway lobster *Nephrops norvegicus*: observations on pathology and progression of infection. *Dis. Aquat. Org.* 22:115–128.

Goggin, C. L., K. B. Sewell & R. J. G. Lester. 1989. Cross-infection experiments with Australian *Perkinsus* species. *Dis. Aquat. Org.* 7:55–59.

Hamaguchi, M., N. Suzuki, H. Usuki & H. Ishioka. 1998. *Perkinsus* protozoan infection in short-necked clam *Tapes* (=*Ruditapes*) *philippinarum* in Japan. *Fish Pathol.* 33:473–480.

Haskin, H. H., L. A. Stauber & J. G. Mackin. 1966. *Minchinia nelsoni* n. sp. (Haplosporida, Haplosporidiidae): causative agent of the Delaware Bay oyster epizootic. *Science* 153:1414–1416.

Liang, Y.-B., X.-C. Zhang, L.-J. Wang, B. Yang, Y. Zhang & C.-L. Cai. 2001. Prevalence of *Perkinsus* sp. in the Manila clam *Ruditapes philippinarum* along northern coast of Yellow Sea in China. *Oceanol. et Limnol. Sinica* 32:502–511.

Mackin, J. G., M. Owen & A. Collier. 1950. Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). *Science* 111:328–329.

Maeno, Y., T. Yoshinaga & K. Nakajima. 1999. Occurrence of *Perkinsus* species (Protozoa, Apicomplexa) from Manila clam *Tapes philippinarum* in Japan. *Fish Pathol.* 34:127–131.

Murrell, A., S. N. Kleeman, S. C. Barker & R. J. G. Lester. 2002. Synonymy of *Perkinsus olseni* Lester & Davis, 1981 and *Perkinsus atlanticus* Azevedo, 1989 and an update on the phylogenetic position of the genus. *Perkinsus*. *Bull Eur. Assoc. Fish Pathol.* 22:258–265.

Navas, J. I., M. C. Castillo, P. Vera & M. Ruiz-Rico. 1992. Principal parasites observed in clams, *Ruditapes decussates* (L.), *Ruditapes philippinarum* (Adams et Reeve), *Venerupis pullastra* (Montagu), and *Venerupis aureus* (Gmelin), from the Huelva coast (S.W. Spain). *Aquaculture* 107:193–199.

Ordas, M., A. Ordas, C. Beloso & A. Figueras. 2000. Immune parameters in carpet shell clams infected with *Perkinsus atlanticus*. *Fish Shellfish Immunol.* 10:597–609.

Pacific Coast Shellfish Growers Association. 2003. West Coast shellfish production. Olympia, WA: Pacific Coast Shellfish Growers Association. Available at: www.pcsga.org.

Park, K. I., K. S. Choi & J. W. Choi. 1999. Epizootiology of *Perkinsus* sp. found in the Manila clam, *Ruditapes philippinarum* in Komsoe Bay, Korea. *J. Korean Fish. Soc.* 32:303–309.

Pacific Shellfish Institute. 2001. Manila Clam Mortality and Health Evaluation. Final report to Saltonstall-Kennedy Program, National Marine Fisheries Service, U.S. Department of Commerce on Grant No. Grant Number: NA96FD0194. Olympia, WA: Pacific Shellfish Institute.

Ray, S. M. 1966. A review of the culture method of detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proc. Natl. Shellfish Assn.* 54:55–69.

Sagrista, E., M. Durfort & C. Azevedo. 1996. Ultrastructural data on the life cycle of the parasite, *Perkinsus atlanticus* (Apicomplexa), on the clam, *Ruditapes philippinarum*, in the Mediterranean. *Sci. Mar. Barc.* 60:283–288.

Shaw, B. L. & H. I. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster, *Crassostrea virginica* (Gmelin). *Can. J. Zool.* 35:325–347.

Shields, J. D. 1994. The parasitic dinoflagellates of marine crustaceans. *Ann. Rev. Fish. Dis.* 4:241–271.

Stokes, N. A. & E. M. Burreson. 1995. A sensitive and specific DNA probe for the oyster pathogen *Haplosporidium nelsoni*. *J. Eukaryot. Microbiol.* 42:350–357.

Stokes, N. A. & E. M. Burreson. 2001. Differential diagnosis of mixed *Haplosporidium costale* and *Haplosporidium nelsoni* infections in the eastern oyster, *Crassostrea virginica*, using DNA probes. *J. Shellfish Res.* 20:207–213.

Villalba, A., S. M. Casas, M. J. Carballal & C. Lopez. 2000. Effects of perkinsiosis on the clam *Ruditapes decussatus* industry of Galicia (NW Spain). *J. Shellfish Res.* 19:649.