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PREVALENCE OF *PERKINSUS* SPP. IN CHESAPEAKE BAY SOFT-SHELL CLAMS, *MYA ARENARIA* LINNAEUS, 1758 DURING 1990–1998

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ABSTRACT Prevalence and intensity of *Perkinsus* spp. infections were determined in soft-shell clams *Mya arenaria* during 1990 to 1998 based upon incubation of rectal tissues in Ray's fluid thioglycollate medium. During the study, soft-shell clams were collected from 18 sites in the upper Chesapeake Bay in Maryland. Enlarged hypnospores were found in ~7% (114/1,705) of the soft-shell clams. Peak prevalences occurred in the fall of 1992 with ~53% (16/30) at Piney Point and 50% (15/30) at Eastern Neck, and in August 1995 with ~64% (18/28) and ~37% (11/30) at Cedar Point and Piney Point, respectively. This investigation provides evidence that *Perkinsus* spp. infections in soft-shell clams are more common than previously thought.

KEY WORDS: soft-shell clam, *Perkinsus* spp., Chesapeake Bay, Maryland, infection, intensity

INTRODUCTION

Protozoa of the genus *Perkinsus* have been associated with significant losses of feral and cultured species of bivalve mollusks worldwide. Previous reports of *Perkinsus* sp. infections in soft-shell clams *Mya arenaria* of the Chesapeake Bay have been uncommon (Andrews 1954), and its geographic distribution remains to be determined. Recently, McLaughlin and Faisal (1998a) reported the presence of *Perkinsus* spp. in Maryland soft-shell clams and described the associated histopathological alterations. Most of the infections observed were light in intensity, limited to the gills and palps, and evoked cellular host responses including encapsulation of invading parasites. As infection intensity increased, the parasite was found in nearly all tissues of the soft-shell clam, sometimes causing adverse host effects (McLaughlin and Faisal 1998a, 1999). Interestingly, two species of *Perkinsus* were recently isolated from hemolymph and gills of soft-shell clams collected from the Chesapeake Bay and propagated *in vitro* (McLaughlin and Faisal 1998b). Morphology, life cycle, and molecular characterization studies showed similarities between the soft-shell clam hemolymph isolate and *P. marinus*, and provided evidence that the gill isolate was an undescribed *Perkinsus* sp. (McLaughlin and Faisal 1998a,b, Kotob et al. 1999a,b).

In the assay routinely used for the detection and quantitation of *Perkinsus* spp. cells in bivalves, host tissues are incubated in Ray's fluid thioglycollate medium (RFTM) and enlarged hypnospores then stained with Lugol's iodine (Ray 1952). In soft-shell clams, the use of rectal tissue in thioglycollate assays was found to be effective for diagnosing advanced *Perkinsus* spp. infections (McLaughlin and Faisal 1999). A positive result implies that the infection has progressed from the early encapsulation stage within gill tissues to a more systemic infection spreading into various tissues of the infected clam. In this paper, we report infection prevalences and intensities of *Perkinsus* spp. in *Mya arenaria* collected from 18 sites in the Chesapeake Bay during 1990 to

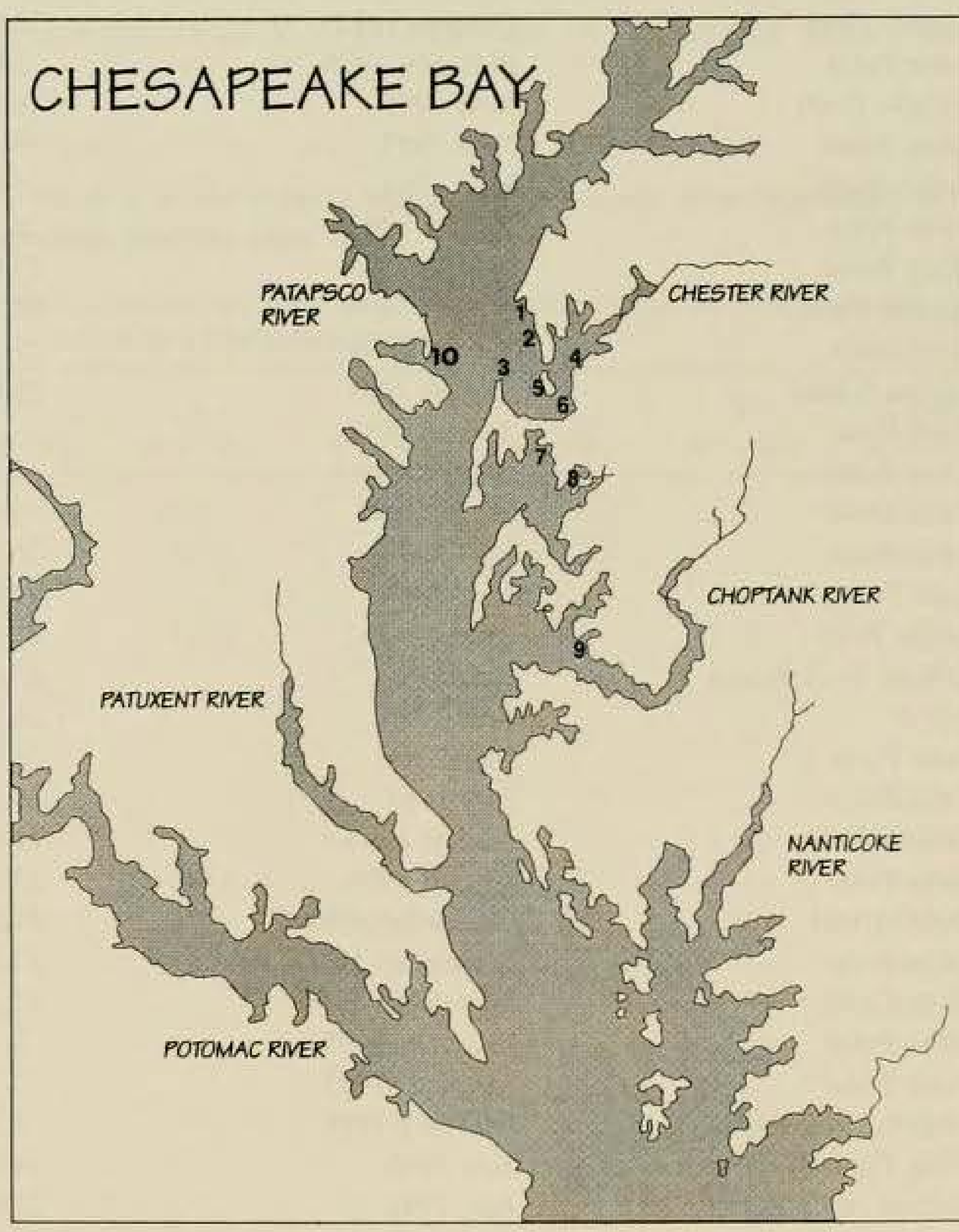

Figure. 1. Sampling sites in the upper Chesapeake Bay (1 = Swan Point; 2 = Eastern Neck; 3 = Love Point; 4 = Piney Point; 5 = Eastern Neck Island; 6= Cedar Point; 7 = Cabin Creek; 8 = Wye River; 9 = Howell's Point; 10 = Gibson Island) with *Perkinsus*-positive soft-shell clams *Mya arenaria* based upon incubation of rectal tissues in Ray's fluid thioglycollate medium (Ray 1952). Scale: 1 cm = ~6.9 miles (~11.04 km)

TABLE 1.

Prevalence (percent infected) and intensity (weighted prevalence) (Mackin 1962) of *Perkinsus* spp. in softshell clams *Mya arenaria* from sites in the upper Chesapeake Bay (n = 30).

Location	Date	Temperature (° C)	Salinity (ppt)	Percent Infected	Weighted Prevalence
Swan Point	December 1990	9.0	7.5	7	0.07
Swan Point	March 1991	8.0	5.0	0	0.00
Swan Point	July 1991	27.5	10.5	0	0.00
Howell's Point	August 1991	20.0	9.2	10	0.27
Wye River	August 1991	25.4	12.5	3	0.03
Swan Point	October 1991	20.0	12.5	23	0.33
Swan Point	January 1992	5.0	14.5	3	0.03
Little Choptank	March 1992	—	—	0	0.00
Love Point	April 1992	7.7	11.5	3	0.03
Swan Point	April 1992	5.0	10.0	0	0.00
Piney Point	April 1992	9.9	9.3	0	0.00
Bishop Head Point	April 1992	—	—	0	0.00
Sandy Point	April 1992	12.8	3.8	0	0.00
Gibson Island[1]	April 1992	8.0	12.0	0	0.00
Cabin Creek	June 1992	24.9	13.1	7	0.06
Swan Point	July 1992	3.6	11.0	0	0.00
Love Point	July 1992	25.8	10.8	0	0.00
Sandy Point	July 1992	23.8	10.0	0	0.00
Wye River	July 1992	23.8	13.0	0	0.00
Swan Point	September 1992	0	12.0	7	0.10
Piney Point	September 1992	22.6	5.0	53	1.17
Gibson Island	October 1992	15.9	16.3	26	0.40
Eastern Neck	October 1992	13.6	14.0	50	1.50
Swan Point	February 1993	3.0	7.0	0	0.00
Nichols Point	June 1993	20.4	4.0	0	0.00
Piney Point	June 1993	19.1	4.2	0	0.00
Cedar Point[2]	June 1993	17.0	4.2	0	0.00
Swan Point	August 1993	25.1	10.0	17	0.02
Piney Point	August 1993	25.0	9.8	10	0.30
Eastern Neck	August 1993	25.1	10.0	10	0.20
Love Point	August 1993	25.3	11.0	0	0.00
Gibson Island	August 1993	26.0	8.2	0	0.00
Rock Point	August 1993	—	—	0	0.00
Swan Point	October 1993	13.7	14.0	3	0.03
Swan Point	March 1994	4.4	5.0	0	0.00
Swan Point	June 1994	26.6	3.5	0	0.00
Love Point	June 1994	22.0	8.0	0	0.00
Sandy Point	June 1994	25.0	4.9	0	0.00
Eastern Neck Island	June 1994	25.0	5.5	0	0.00
Pier 1	June 1994	26.2	5.0	0	0.00
Love Point	July 1994	26.6	5.0	0	0.00
Swan Point	July 1994	26.6	3.5	0	0.00
Eastern Neck	August 1994	25.2	6.5	0	0.00
Swan Point	August 1994	25.6	7.1	0	0.00
Huntingfield	September 1994	22.2	10.0	0	0.00
Swan Point	September 1994	22.2	9.0	0	0.00
Swan Creek	September 1994	22.2	8.0	0	0.00
Swan Point	February 1995	2.2	6.0	10	0.10
Piney Point	February 1995	3.1	6.0	3	0.03
Eastern Neck Island	February 1995	3.1	6.0	3	0.03
Swan Point	July 1995	24.2	6.0	0	0.00
Eastern Neck Island	July 1995	25.0	10.0	0	0.00
Cedar Point[1]	August 1995	23.5	14.0	64	1.57
Piney Point	August 1995	23.6	13.5	37	1.33
Swan Point	August 1995	23.0	12.5	13	0.20
Swan Point	October 1996	15.6	2.2	13	0.20
Swan Point	July 1998	25.6	4.0	7	0.10

[1] n = 28.
[2] n = 29.
Prevalence and intensity were based upon incubation of rectal tissues in Ray's fluid thioglycollate medium (Ray 1952).

1998. Prevalences and intensities were determined using rectal tissues in thioglycollate assays.

MATERIALS AND METHODS

Clam Collections

More than 1,700 soft-shell clams were collected in 57 samples (~30 clams/sample) from 18 sites in the Chesapeake Bay (Fig. 1) from 1990 to 1998 by hydraulic escalator dredge. Clams from each sampling site were held on ice and transported immediately to the wet lab facility at the Cooperative Oxford Laboratory (COL), Oxford, Maryland. Clams were held in 76-L glass aquaria supplied with Tred Avon River water or artificial seawater at temperatures between 8 and 20 °C until processed (1–2 days). Salinities were adjusted to approximate those of the collection sites (5–16 ppt).

Ray's Fluid Thioglycollate Assays

Pieces of rectum were excised from each clam and incubated in RFTM (Ray 1952) for 5–7 days. Incubated tissues were subsequently macerated on glass slides, stained with Lugol's iodine, and examined for spherical blue-black bodies characteristic of *Perkinsus* spp. (Ray 1952). Infection intensity was estimated using a semiquantitative scale from 0 (negative) to 7 (extremely heavy infection), modified from Ray (1954) and Mackin (1962). Weighted prevalences were determined by adding the individual assigned intensities and dividing by the number of clams sampled (Mackin 1962).

RESULTS

Perkinsus spp. infections were found in ~7% (114/1,705) of soft-shell clams examined. Infected clams were found at 10 of the 18 sites surveyed (Fig. 1). Temperatures and salinities at sites positive for *Perkinsus* spp. ranged from 2.2 to 25.4 ° C and from 2.2 to 16.3 ppt (Table 1). Peak prevalences of soft-shell clam *Perkinsus* spp. usually occurred in the late summer and fall when salinities and temperatures were highest. A peak in prevalence occurred in the fall of 1992 with ~53% (16/30) at Piney Point and 50% (15/30) at Eastern Neck (Table 1). Prevalence also peaked in August 1995 with ~64% (18/28) and ~37% (11/30) at Cedar Point and Piney Point, respectively. As shown in Table 2, intensities of infections in the rectal tissues ranged from extremely light (stage 1) to heavy (stage 5). Extreme cases (stages 6 and 7) were observed in less than 1% (9/1705) of the soft-shell clams examined. The maximum weighted prevalences observed were 1.17 at Piney Point in September 1992 and 1.57 at Cedar Point in August 1995.

DISCUSSION

Previous reports of *Perkinsus* spp. infections in soft-shell clams are rare. In Virginia, *Perkinsus* sp. was reported to occur infrequently in soft-shell clams and the low infection intensities observed were not associated with histopathological lesions or mortalities (Andrews 1954). Similarly, histological examination of over 3,500 soft-shell clams collected from 20 sites in Maryland during 1969 to 1989 revealed only occasional occurrences of *Perkinsus* spp. (Sara V. Otto, Maryland Department of Natural Re-

TABLE 2.

Infection intensities of *Perkinsus* spp. in softshell clams, *Mya arenaria*, from sites in the upper Chesapeake Bay based upon incubation of rectal tissues in Ray's fluid thioglycollate medium (Ray 1952).

		Intensity of Infection (Stages 1–7)							
Site (n = 30)	Date (month & year)	1 # (~%)	2 # (~%)	3 # (~%)	4 # (~%)	5 # (~%)	6 # (~%)	7 # (~%)	Total # (~%)
Swan Point	December 1990	2 (7)							2 (7)
Howell's Point	August 1991	1 (3)	1 (3)		1 (3)				3 (10)
Wye River	August 1991	1 (3)							1 (3)
Swan Point	October 1991	4 (13)	1 (3)		1 (3)			1 (3)	7 (23)
Swan Point	January 1992	1 (3)							1 (3)
Love Point	April 1992	1 (3)							1 (3)
Cabin Creek	June 1992	2 (7)							2 (7)
Swan Point	September 1992	1 (3)	1 (3)						2 (7)
Piney Point	September 1992	8 (27)	3 (10)	3 (10)		1 (3)		1 (3)	16 (53)
Gibson Island	October 1992	6 (20)	1 (3)		1 (3)				8 (27)
Eastern Neck	October 1992	6 (20)	2 (7)	1 (3)	1 (3)	2 (7)	2 (7)	1 (3)	15 (50)
Eastern Neck	August 1993		3 (10)						3 (10)
Swan Point	August 1993	4 (13)	1 (3)						5 (17)
Piney Point	August 1993	1 (3)	1 (3)					1 (3)	3 (10)
Swan Point	October 1993	1 (3)							1 (3)
Swan Point	February 1995	3 (10)							3 (10)
Piney Point	February 1995	1 (3)							1 (3)
Eastern Neck Island	February 1995	1 (3)							1 (3)
Cedar Point[1]	August 1995	5 (18)	5 (18)	3 (11)	2 (7)	3 (11)			18 (64)
Piney Point	August 1995	6 (20)	3 (10)	1 (3)			1 (3)		11 (37)
Swan Point	August 1995	2 (7)	2 (7)						4 (13)
Swan Point	October 1996	1 (3)			1 (3)			2 (7)	4 (13)
Swan Point	July 1998	2 (7)							2 (7)

[1] n = 28.

Stage 1 = extremely light, 2 = very light, 3 = light, 4 = moderate, 5 = heavy, 6 = very heavy, 7 = extremely heavy.

sources, Oxford, Maryland, pers. comm.). McLaughlin and Faisal (1999) demonstrated that positive thioglycollate assays using rectal tissue denote advanced, generalized infections of *Perkinsus* spp. in soft-shell clams. This observation was verified in histologic preparations. Hence, the findings from our present study suggest that *Perkinsus* spp. infections may be increasing in soft-shell clams of the Chesapeake Bay. Indeed, soft-shell clams have been shown to host more than one species of *Perkinsus*, one closely related to *P. marinus* and the other an undescribed *Perkinsus* sp. (McLaughlin and Faisal 1998a,b; Kotob et al. 1999a,b). The lack of an effective tool for distinguishing between species of *Perkinsus* within a host limits our ability to attribute the increased prevalence to one or both soft-shell clam *Perkinsus* species.

This apparent elevation in *Perkinsus* spp. infections in soft-shell clams of the Chesapeake Bay parallels increased prevalences and range extensions of *P. marinus* infections in the eastern oyster. Increased occurrences of the oyster parasite were attributed to high salinities from successive droughts during the 1980s, concurrent mild winter temperatures, and movement of infected oysters (Burreson and Calvo 1996). Range extensions of *P. marinus* parasites were further hypothesized to be associated with genetic changes in the host or parasite and/or environmental parameters (Ford 1996). Coincidental increases in soft-shell clam *Perkinsus* spp. in the Chesapeake Bay may be attributable to similar factors. For example, the extension of oyster *P. marinus* into all productive oyster grounds in the Chesapeake Bay in the late 1980s and early 1990s (Burreson and Calvo 1996) coincided with the increased occurrence of soft-shell clam *Perkinsus* spp. observed in this study. Indeed, *P. marinus* was first reported in oysters in Swan Point, Chester River, in 1987 (Burreson and Calvo 1996) less than 3 years before *Perkinsus* spp. infections were observed in soft-shell clams from the same site. Similarly, reduced infection levels of *P. marinus* in Chesapeake Bay oysters during 1994 (Burreson and Calvo 1996) were concurrent with reduced soft-shell clam *Perkinsus* spp. prevalences in the same year.

The high prevalences of soft-shell clam *Perkinsus* spp. at some sites in Maryland during 1992 coincided with the lowest recorded catch of soft-shell clams since 1962 (Connie Lewis, Maryland Department of Natural Resources, Annapolis, Maryland, pers. comm.). The continued low harvests of soft-shell clams in the Chesapeake Bay suggest further investigations on impacts of the parasite to *M. arenaria* fisheries are warranted.

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