ASSESSMENT OF CHESAPEAKE BAY COMMERCIAL SOFTSHELL CLAMS *Mya arenaria* and *Tagelus plebeius* WITH EMPHASIS ON ABUNDANCE AND DISEASE STATUS

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INTRODUCTION

The softshell clam, *Mya arenaria*, has supported an important commercial fishery in the Maryland portion of Chesapeake Bay since the early 1950's, when harvesting of unexploited subtidal populations by hydraulic escalator dredge began. Annual landings peaked at 680,000 bushels in 1964, remained above 500,000 bushels through 1971, subsequently falling to between 365,000 and 56,000 bushels through 1991, after which harvests declined steadily to levels less than 1% of the 1964 peak landing. (Maryland DNR Fisheries Service Statistics, Annapolis, MD). Since 1994, commercial landings have been in freefall, with harvests regularly negligible. While market dynamics have contributed somewhat to declining catches, there is no doubt that this species population has declined to "remnant" status. When American eel prices effectively removed them as blue crab bait, clammers started, in the early 1980's, to target the stout razor clam, *Tagelus plebeius,* which are marketed as bait for eel and crab pot and trotline fisheries. Over time, most of the commercial clam fleet has focused its efforts on harvesting razor clams. Although, until recently, no records were kept of razor clam landings in Maryland, conversations with commercial clammers indicate that between about 1980 and 2004, landings exceeded softshell clam landings on a regular basis.

As softshell clam harvests declined, the value, both ex-vessel and retail, has dramatically increased. With dockside values in excess of $80 to $100+ per bu during the last dozen years or so (Maryland DNR Fisheries Service Statistics, Annapolis, MD), and retail values 2-3 times greater than dockside, exploitation pressure on softshell clams persisted in spite of their declining population levels and geographical retreat. In recent years, however, only a small fraction of the commercial fleet targets this species. Although less valuable per bushel, razor clam production has increasingly replaced softshell clam harvests in terms of income. There is no daily limit for razor clams, except when prohibited or conditional areas are opened for harvest, while softshell clams are limited to either 8 bu or 15 bu dependent on the season. Until 2004, as gleaned from individuals in the razor clam industry, dockside process per bushel averaged between $20-25. Since 2004 (when the population crashed as documented in this report), prices have increased slowly to $30-35 per bu, and more recently $40+. This price increase reflects both the decline in population abundance and the demand from the blue crab industry. Not surprisingly, increased bait costs have contributed to higher blue crab prices. And, perhaps of the greatest importance, the severe decline in both of these bivalves has significantly decreased the forage base of the

Bay's ecosystem. Just about every taxonomic group preys upon softshell clams, from Nemerteans to Cetaceans. Although predator-prey interactions are poorly documented for razor clams, the softshell clam has been an important, sometimes dominant prey item for species such as blue crabs, summer and winter flounder, Atlantic croaker, and spot (Virnstein 1977; Lipcius and Hines 1986; Baker and Mann 1991; Homer and Boynton 1978; Homer and Mihursky 1991; MacKenzie 1997), just to name a few. Additionally, juvenile softshell and razor clams are prey for numerous other benthic invertebrate populations including several species of polychaetes, snails, mud crabs, and shrimp (Haven 1970; Hidu and Newell 1989; Baker and Mann 1991).

Chesapeake Bay *Mya arenaria* populations are affected by several pathological conditions that may be fatal, including disseminated neoplasia (DN) and *Perkinsus* sp. protozoan infections. Disseminated [hemic] neoplasia, first described in New England *M. arenaria* (Brown et al. 1977), was subsequently also reported as epizootic in some years among Chesapeake Bay *M. arenaria* populations (Farley et al. 1986; Farley et al. 1991). Rapidly proliferating, anaplastic and aneuploid cells come to dominate affected clam circulatory systems, displacing normal hemocyte cells and their critical physiological functions. Pathology associated with this disease has been compared to that of vertebrate leukemia (Smolowitz et al. 1989) and is fatal within 9 months of experimental transmission (House et al. 1998). With prevalences up to 58% reported in some Chesapeake Bay clam populations (Farley et al. 1991), the impact of DN disease on clam population mortality is projected to be significant.

Both *Mya arenaria* and *Tagelus plebeius* from Virginia waters were reported to be infected by the lethal protozoan oyster pathogen *Perkinsus marinus* (= *Dermocystidium marinum*) in a brief, early note (Andrews 1954). However, this parasite was not detected in four Chester River and two Eastern Bay, Maryland *M. arenaria* samples examined in 1971 (Hamons, 1971), nor in diverse Maryland *M. arenaria* samples analyzed prior to1990 (MDDNR pathologist Sara V. Otto, pers. comm.). Since 1990, *Perkinsus* sp. infections have been detected at apparent increasing frequency among Chesapeake Bay *M. arenaria* populations (McLaughlin and Faisal 2000). During 2000, *Perkinsus* sp. infections were detected at high prevalences (30-100%) and intensities in all *M. arenaria* and *T. plebeius* samples examined during a CBSAC-funded survey (Dungan et al. 2002). Mild pathology and prevalent defensive parasite encapsulation observed in some infections are interpreted to suggest only that they may compromise growth and reproduction of infected clams (McLaughlin and Faisal 1998).

However, extreme parasite densities with systemic distributions occurring in clams examined by us indicate an acute, probably lethal, disease condition (Dungan et al. 2002).

The severe decline in softshell clam populations throughout the 1990's (as evidenced by harvest reports) led to the initiation of the present study. The crash of the razor clam population in 2004 extended the effort.

Objectives.

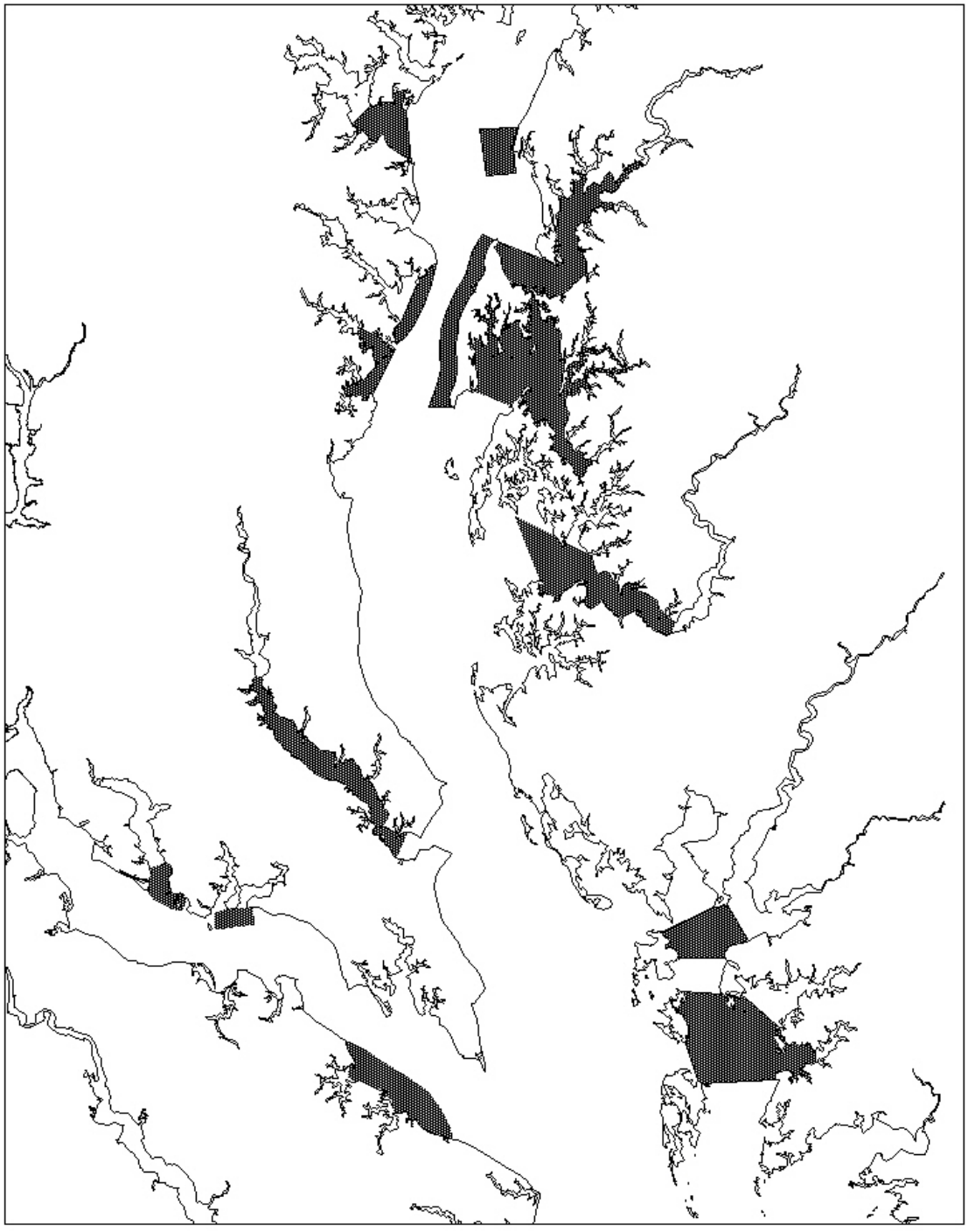
1. To characterize the present condition of softshell and razor clam populations with respect to distribution, abundance, and co-habitation.
2. Assess the status of diseases in softshell and razor clam populations.

METHODS

Field Sampling

Hydraulic escalator dredge

Mya arenaria and *T. plebeius* populations were sampled by hydraulic clam dredge during the course of this study at within seven regions of Maryland's Chesapeake Bay: Chester R., Upper Bay, Eastern Bay, Choptank R., Patuxent R., Potomac R., and Tangier Sound (map below). Geographic coordinates, water temperature and salinity, and substrate characteristics were recorded at each sampling site. A quantified area of bottom sediment was excavated using a commercial hydraulic escalator dredge fitted with a 6.5cm^2-mesh retention screen, and all captured softshell and razor clams counted (the DGPS in use is accurate to ±2% linear distance in feet). Initially, timed tow collections were made (with tow length recorded), but this proved to be inefficient. Instead, later collections were conducted by segmenting long dredge tows, between 500 and 1,000 linear feet, into 100 linear foot subsamples. This modification resulted in a more efficient and consistent sampling effort.



Areas surveyed for clam populations, 2001-2008.

Data recorded from each dredge tow segment included the following: distance (area) towed, bottom type, water depth, tow time, and the number and volume of clams. Anterior-posterior shell lengths were measured and recorded to the nearest millimeter for representative subsamples of each species from the tow collective.

Sentinel locations were established for obtaining samples for disease analyses. As clam "beds" are ephemeral, fixed locations were impossible to establish. Rather, sentinel sites were set up more broadly within regions. Each sample consisted of 40 clams of either or both species and all were delivered to the Oxford Laboratory for disease analyses. In 2004, in response to widespread razor clam mortalities, the number of disease sample collection sites was greatly expanded.

Bottom grab collections

A bottom grab (sampling approximately $0.10m^2$) was used to attempt to capture young-of-the year (YOY) softshell clams in the following regions: Upper Bay, the Chester River, the Choptank River, and in Tangier Sound. Samples were collected during November 2001, March 2002, and May 2002. A total of 172 grab samples were collected. Bottom grab material was washed through a 2mm screen and all softshell clams collected, counted, and measured for shell length. Geographic position, water temperature and salinity, and substrate characteristics were recorded for each sample.

Laboratory assay procedures and analyses

RFTM dermo disease assays

Following receipt at Oxford Laboratory, clam samples were held for 24–72 h in flowing ambient Tred Avon River water to allow clams to purge entrained sand and fecal matter. Thirty live clams were selected from each sample and processed for disease analyses. Clams were measured, shucked from their shells, and their muscular mantle margins and siphons trimmed away. Laboratory procedures followed Ray (1966) and Dungan et al. (2002). Relative parasite densities in tissue macerates were categorized and recorded as absent (0), or light (1) to heavy (5) (Mackin 1961, Choi et al. 1989). For each clam sample analyzed, a *Perkinsus* sp. infection intensity index was calculated as the sum of individual infected clam categorical infection intensities, divided by the number of infected clams in the sample. Sample disease prevalences

were calculated as the percent of assayed sample clams affected. Duplicate RFTM-incubated labial palp tissues from heavily infected clams were selected as inocula for parasite in vitro isolation and propagation efforts**.**

Histopathological analyses.

Laboratory procedures follow Dungan et al. 2002. Histological sections were examined for the presence, tissue distribution, and intensity of DN disease; the presence, distribution, intensity, and host defensive response to *Perkinsus* sp. infections; and presence, intensity, tissue distribution, and pathology of other infectious or parasitic conditions. Intensity of DN disease was staged (1--5) for affected clams (Farley et al. 1986) and a DN disease intensity index was calculated for each clam sample as the sum of individual affected clam intensity stages, divided by the number of affected clams in the sample.

Pathogen isolation and propagation

Laboratory procedures followed Dungan and Hamilton (1995) and Bushek et al. (2000). Clonal parasite cultures were expanded and cryopreserved for subsequent taxonomic identification by DNA nucleotide sequencing.

RESULTS

Objective 1.

Stock Assessment

A total of 2,114 hydraulic escalator samples were taken between 2001 and 2008, from 7 regions (Table 1) with summarized and representative standing stock estimates (number per acre) given in Table 2 (*Mya arenaria*) and Table 3 (*Tagelus plebeius*).

Table 1. Total number of dredge samples collected by Region during 2001-2008.

REGION	TOTAL NUMBER OF SAMPLES COLLECTED
Upper Bay	446
Chester River	421
Eastern Bay and tributaries	581
Choptank River	232
Patuxent River	215
Potomac River	112
Tangier Sound	107
Total	2,114

Table 2. *Mya arenaria* standing stocks, number per acre, annual averages.

Region	Site	Mya arenaria, number per acre							
		2001	2002	2003	2004	2005	2006	2007	2008
Upper Bay	Swan Point	10,500		3,200	250				
	Sandy Point	18,500	1,500	750	700		100		
	Love Point	8,600	1,400	1,300	8,900				
	Bay Bridge		3,100	700					
	Hacketts Point	15,500	2,500	100	3,400		300		
	Matapeake Hill	4,800	1,200	1,100	4,200		2,700		
	Thomas Point		1,200		300		150		
	Tolly Point			3,100	100		0		
Patapsco River	Bodkins Point	7,100			175				
West River	Rock Point		1,200		3,600				
Chester River	Love Point	9,400		700					
	Piney Point	14,300	350	500	1,200	80	0	1,800	100
	Old Field	3,700	5,500	3,700	1,300	50	100		
	Buoy Rock	9,200	3,600	700	4,800	1,600	200	1,700	800
	Spaniard Point		5,600		700	800			0
	Nichols Point		10,700	1,800	2,700	1,000	0	1,600	100
Eastern Bay	Romancoke	4,400	4,500	1,500	10,900				
	Upper Hill Bar	9,000	900	500	14,200	10,400	150		
	Narrow Point	4,300	2,800	50	800	600			
	Parsons Island	5,000	400		800		5,700		
	Bodkin Island	2,300		6,800	8,800	2,100	1,000		
	Cabin Creek			0	1,400		0		
	Kent Point			1,000	8,800				
(Miles R.)	Leeds Creek	1,000	150	700	60		0		
(Wye R.)	Drum Point		150		150		0		900
Choptank River	Bolingbroke Sands	10,700	750	80	3,500	300		1,400	8,800
	Horn Point	1,400							
	Chlora Point	23,300	7,100	50	1,000	100			6,500
	Castle Haven	8,200		200		50		100	500
Patuxent River	Sandgates	400	100		2,100				0
	Broomes Isl. NOB	13,800	2,000		1,100				0
	Broomes Island	2,100	0		1,100				0
	Buzzards Island		150		0				
	Prison Point	400	0		3,100				
	Sotterly Point	400	150		8,900				
	Patterson Point	0	20		400				
	Sheridan Point		100		700				0
	Drum Cliffs	15	0		1,200				
Potomac River	Bonums Creek		1,600						
	St. Clements Bay		30						
	St. Catherines		350						
	Cobb Island		600						
Tangier Sound	Manokin R.	1,900	200	250	1,200				
	Mainstem	12,000	4,800	1,300	6,700				

Softshell clam stocks (Table 1) may be characterized as highly variable within a site over time and generally low, as will be shown in a later comparison of these data with survey results conducted during the 1960s and 1970s. When recruitment did occur, with few exceptions, standings stocks increased for only a short period, after which the effects of predation and disease mortality became evident. In general, softshell clam populations were highly associated with bottom type, as shown in the un-numbered table below.

	Substrate/Sediment Category			
	Hard with structure	Soft with structure	Hard (Sand, Clay)	Soft (Mud)
Percentage of softshell clams collected	71	12	12	5

As may be seen, softshell clam populations were positively associated with dense sediment areas having overburdens of shell, cobbles, and made-made materials. Such material affords refuge from predators, even where soft sediments are mixed with structure. By the end of stock estimate field operations in 2008, over 95% of softshell clams collected were from hard substrate areas with thick layers of structure covered the base sediment.

As mentioned above, contemporary abundance estimates of softshell clams were compared with similar data collected during the 1960s and 1970s. The table below gives some of the earlier estimates of softshell clam abundance. These data are from field sheets, hence no reference, and include estimates deemed credible after discussions with the lead biologist at the time. Comparisons with 2001-2008 data were made using both the Wilcoxon Rank Sum and Kruskul-Wallis Test results evaluated at $\alpha = 0.05$ (Hollander and Wolfe, 1973). Nonparametric procedures were chosen because of uncertainty regarding the underlying distribution of the earlier data. Values in bold, in the un-numbered table below, indicate statistical differences between the softshell clam data collected during the 1960s-1970s and contemporary data. Data are number of clams per acre.

		1962	1963	1968	1969	1970	1974	1975	2001-2008 Average
Upper B.	Hackett Pt						**23,000**	**39,000**	4,400
	Sandy Pt						6,300		4,000
	Matapeake						**92,000**	**74,000**	2,800
	Love Point						**46,000**		5,000
	Swan Point			**91,000**					4,700
Chester	Old Field				**156,000**				2,400
	Piney Point				**248,000**				2,300
	Buoy Rock				**262,000**				2,825
	Love Point				**54,000**				5,100
East. Bay	Romancoke					**35,000**	**104,000**	**78,000**	5,300
	Bodkin Isl.					**36,000**	**146,000**		4,200
	Upper Hill	**184,000**		**185,000**				**23,000**	5,900
	Narrow Pt.							**21,000**	1,700
(Miles R.)	Leeds Cr.	**90,000**	**153,000**	**154,000**					400
Choptank	Chlora Pt.		**238,000**						6,300
	Horn Point				**91,000**				1,400
	Castle H.				**58,000**		**153,000**		1,800
Patuxent	Sandgates						**60,000**		700
	Drum Cliffs						**8,000**		600

With one exception, Sandy Point in the Upper Bay, all comparisons gave significant differences with the earlier estimates greater than the more recent ones. (From 2003 on, even the 1974 Sandy Point data were significantly different, and greater, than the 2003-2008 estimates.). The consistent test results are overshadowed by the magnitude of the estimate differences. In general, with Sandy Point as the one exception, the 1962-1975 softshell clam abundance estimates were 1 to 2 orders of magnitude greater than those from 2001-2008.

Estimates of razor clam abundance at selected sites are given in Table 3 (note that the tabular data are expressed as thousands per acre). As the focus of this project, as originally developed, were softshell clams, many areas were surveyed where one would not expect to find razor clams. Unfortunately, there exists no historical landings records for the stout razor clam, nor were population estimate records kept during the surveys conducted during the 1960's and 1970s.

As indicated in Table 3, robust populations of *T. plebeius* were found in several areas of the Bay, particularly in Eastern Bay and its tributaries, the West River, and in the Patuxent River. Robust until severe mortality events, starting in late 2003, began decimating razor clam populations throughout the Chesapeake Bay (Table 4). A number of areas no longer support

significant populations of razor clams, although commercial abundance levels persist in the Eastern Bay tributaries, a few Patuxent River areas, and in several areas of the Choptank River.

Unlike softshell clams, razor clam boxes remain articulated for a substantial time period, a characteristic that can be exploited to obtain credible mortality rates (Table 4). Prior to the fall of 2003, no records were kept of razor clam box counts, although field notes taken in 2001 and 2002 indicate that boxes made up no more than 5-6% of razor clam populations. Large numbers of boxes were observed initially in the early fall of 2003 and, accordingly, counts were made and recorded. During the subsequent winter, we were contacted by several watermen who reported severe mortalities in the Eastern Bay area and in the Patuxent River. We refocused the current project in order to investigate these reports and found them to be accurate. By the end of 2004, we estimated that over 70% of the Bay's razor clam population had died, even in areas that supported only low levels of razor clam abundance.

Table 3. Abundance estimates of the stout razor clams in selected Bay areas. Data are given as thousands per acre.

Region	Site	*Tagelus plebeius*, thousands per acre							
		2001	2002	2003	2004	2005	2006	2007	2008
Upper Bay	Matapeake Hill	0.1	6.3	4.8	0.8		0.0		
West River	Rock Point		24.1		8.5				
Chester River	Buoy Rock	0.1	0.9	3.6	0.3	0.2	0.1	0.2	
Eastern Bay	Romancoke	7.4	77.2	34.9	34.7				
	Upper Hill Bar	3.3	68.0	29.5	31.2	12.2	0.1		
	Narrow Point	42.1	230.3	176.3	40.2	30.3	0.1		
	Parsons Island	0.3	135.0		8.2		0.0		
	Bodkin Island	0.1		0.2	0.6	0.1	0.0		
	Cabin Creek			162.4	160.1	18.5	1.6		
	Kent Point			14.7	23.0				
(Miles R.)	Leeds Creek		56.2	129.5	51.5	46.9	26.8		
(Wye R.)	Drum Point		38.1	43.4	67.8	28.9	24.2		
Choptank River	Bolingbroke Sands	3.4	1.8	1.1	0.6	0.2		1.0	6.7
	Chlora Point	6.0	5.2	6.7	1.6	4.0			57.7
	Castle Haven	8.3		0.8	0.5	9.8		101.7	8.8
Patuxent River	Sandgates		7.5		35.4				19.6
	Broomes Island		8.6		30.0				45.4
	Buzzards Island		43.9		1.9				
	Prison Point		5.9		20.8				
	Sotterly Point		8.4		9.7				
	Patterson Point		4.4		4.8				
	Sheridan Point		43.8		12.8				13.2
	Drum Cliffs		13.0		19.5				
Tangier Sound	Manokin R.	2.5	2.6	1.0	4.2				
	Mainstem	6.3	1.8	6.2	4.6				

Table 4. Razor clam observed mortality rates, with respect to data given in Table 3. Values presented are rates calculated from the number of boxes divided by the box+live total.

Region	Site	*Tagelus plebeius*, observed mortality, %							
		2001	2002	2003	2004	2005	2006	2007	2008
Upper Bay	Matapeake Hill	NA	NA	46	93		100		
West River	Rock Point		NA		56				
Chester River	Buoy Rock	NA	NA	10	86	82	90	80	
Eastern Bay	Romancoke	NA	NA	10	16				
	Upper Hill Bar	NA	NA	30	40	76	99		
	Narrow Point	NA	NA	40	64	25	99		
	Parsons Island	NA	NA		53		100		
	Bodkin Island	NA		20	40		100		
	Cabin Creek			5	20	81	82		
	Kent Point			68	50				
(Miles R.)	Leeds Creek		NA	15	14	63	34		
(Wye R.)	Drum Point		NA	10	23	60	36		
Choptank River	Bolingbroke Sands		NA	20	79	90		52	26
	Chlora Point		NA	40	86	68		44	35
	Castle Haven			35	99	59		13	48
Patuxent River	Sandgates		NA		36				21
	Broomes Island		NA		44				7
	Buzzards Island		NA		68				
	Prison Point		NA		37				
	Sotterly Point		NA		38				
	Patterson Point		NA		39				
	Sheridan Point		NA		71				38
	Drum Cliffs		NA		46				
Tangier Sound	Manokin R.	NA	NA	5	41				
	Mainstem	NA	NA	10	68				

Of importance is the aversion of razor clams to benthic areas overburdened with structure. During the course of the present study, 90% of the razor clams collected were from bare substrates, primarily sandy areas (un-numbered table below). This is in direct contrast with the distribution of softshell clams which were collected mostly from areas where structure existed. This is of note, as razor clams are not afforded predator refuge from habitat characteristics and must rely on their burrowing ability and thick foot to avoid predators.

	Substrate/Sediment Category			
	Hard with structure	Soft with structure	Hard (Sand, Clay)	Soft (Mud)
Percentage of razor clams	3	7	70	20

Special Tasks.

Sampling techniques.

Sampling protocols evolved over time, with changes specifically designed where practical for compatibility with methods used for previously collected data. Initially, samples were obtained through discrete hydraulic dredge tows, where tow length and time were recorded. An optimal tow length was established and a modified sampling design was employed, which consisted of a variable number of segmented tows of 100 linear feet (~25 m^2). Where exceedingly dense substrates that severely impeded tow speed were encountered, tow segments were limited to no more than 6 minutes, regardless of tow length. In all cases, both the lengths and durations of segments were recorded.

The number of tows constituting a sample set varied from 3-14 segments, with an average of approximately 7 segments per set. ow number for a given set was dependent on catch rate in the following ways.

1. Where no clams were collected after several tows, the set was fixed at 3-5 segments.
2. Where clams were very abundant, 8-14 segments constituted the sample set.
3. During periods of high razor clam mortalities, 8-12 segments were run.

Under Condition-2 (above), clams were collected from the escalator until a sufficient number were obtained for shell length measurements and disease analyses. At that point, clams were counted as they passed by on the escalator, keeping segment data discrete.
Condition-3 (above) was a special case. The unprecedented razor clam mortalities during 2004 necessarily shifted projected efforts towards documenting what turned out to be a major Chesapeake Bay bivalve mortality event. The razor clam mortality event was so intense and widespread that data acquisition by established methods and protocols was prohibitively inefficient. A more time-efficient, but accurate (and precise) sampling procedure for estimating razor clam mortality was quickly established and tested. This method consisted of operating the dredge on a site, allowing the escalator to clear, establishing a beginning point on the escalator, raising the escalator, and finally counting the number of live and dead razor clams as the escalator cleared. This technique was evaluated by comparing results with those of the standard sampling technique. No differences were found in the estimation of razor clam mortality

between the two methods. The new method took approximately 20% of the time it took to collect the same information as did the conventional technique.

Establish a young-of-the-year index.

An attempt was made to establish a method for creating a softshell clam YOY index, using a standard Petersen Grab. Accordingly, a total of 89 samples were collected over a 2-year period in 3 Chesapeake Bay regions. Samples were preserved in the field and later examined microscopically to identify and enumerate all molluscs captured in the grab.

Given the time-consuming nature and expense of the pilot effort and the lack of significant results with respect to YOY softshell clams, this task was terminated early in 2003. An expansion of this effort from Pilot to Project was judged to be an inefficient expenditure of resources, in light of its marginal anticipated information returns. In addition, as softshell clam escalator dredge catch data were accumulated, it was clear that scheduling sites for grab sample collections would be impossible given the increasingly ephemeral nature of softshell clam distribution.

Objective 2.

***Perkinsus chesapeaki* infections** occur among at least 6 species of clams in Chesapeake Bay and Delaware Bay (Reece et al. 2008). Infections occurred during 2000-2009 at variable mean summer-fall prevalences, which ranged at 26-83% and 13-100% respectively among commercially harvested *M. arenaria* and *T. plebeius* clam populations in Maryland waters of Chesapeake Bay (Fig. 1). In general, mean summer-fall infection prevalences varied similarly between years for both species, suggesting that common environmental or other forces had similar effects on the epizootiology of *P. chesapeaki* infections in both clam species. Among several years when winter-spring clam samples were collected, mean cool-season infection prevalences were consistently and dramatically lower than warm-season prevalences among *M. arenaria* softshell clams. In contrast, mean cool season infection prevalences were similar to warm-season prevalences for *T. plebeius* razor clams; even exceeding the mean warm-season prevalence during 2009 (Fig. 2). These apparent contrasting observations on different seasonal dynamics of *P. chesapeaki* infections among two species of sympatric clams suggest that *P. chesapeaki* is physiologically active and virulent at low seasonal water temperatures; but that the

defensive capabilities of *M. arenaria* clams may surpass those of *T. plebeius* clams in extirpating *P. chesapeaki* infections during winter-spring periods of low water temperatures. Mean annual infection prevalences were calculated from results of Ray's fluid thioglycollate medium (RFTM, Ray 1966) assays from 4,796 clams during 2000-2009.

Fig. 1. Mean summer-fall *P. chesapeaki* infection prevalences among two Chesapeake Bay clam species: 2000-2009

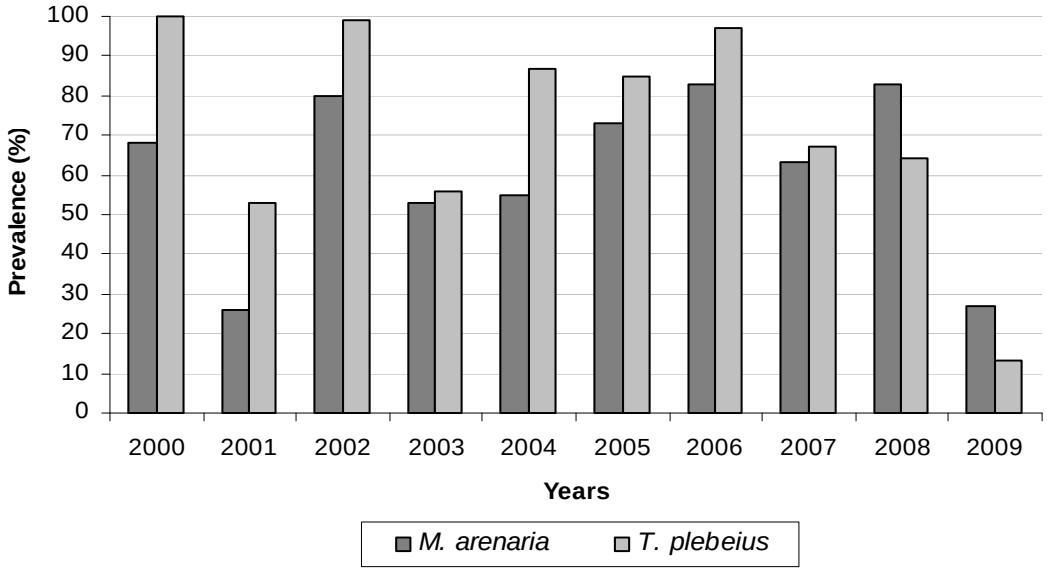
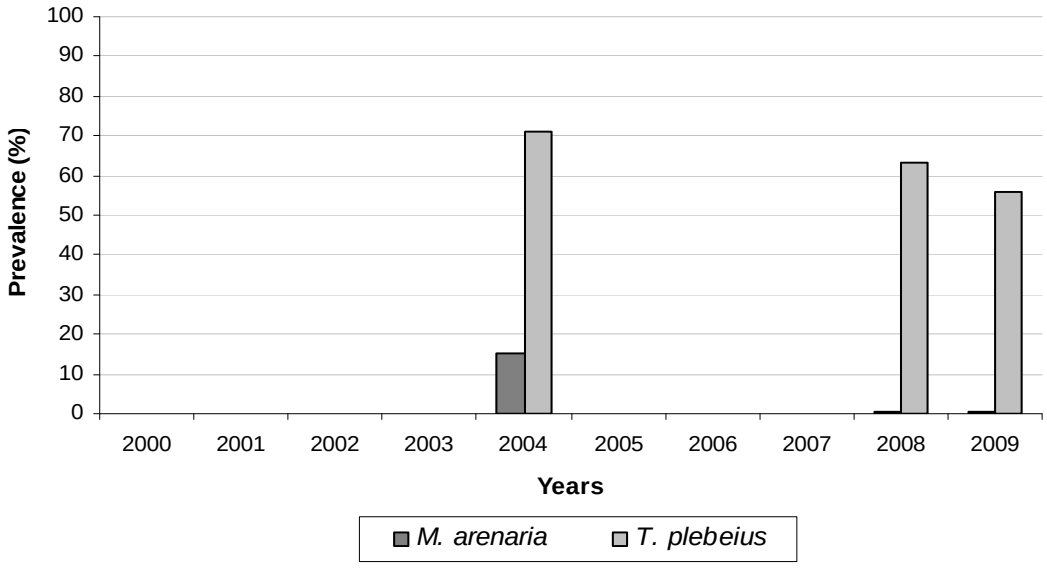
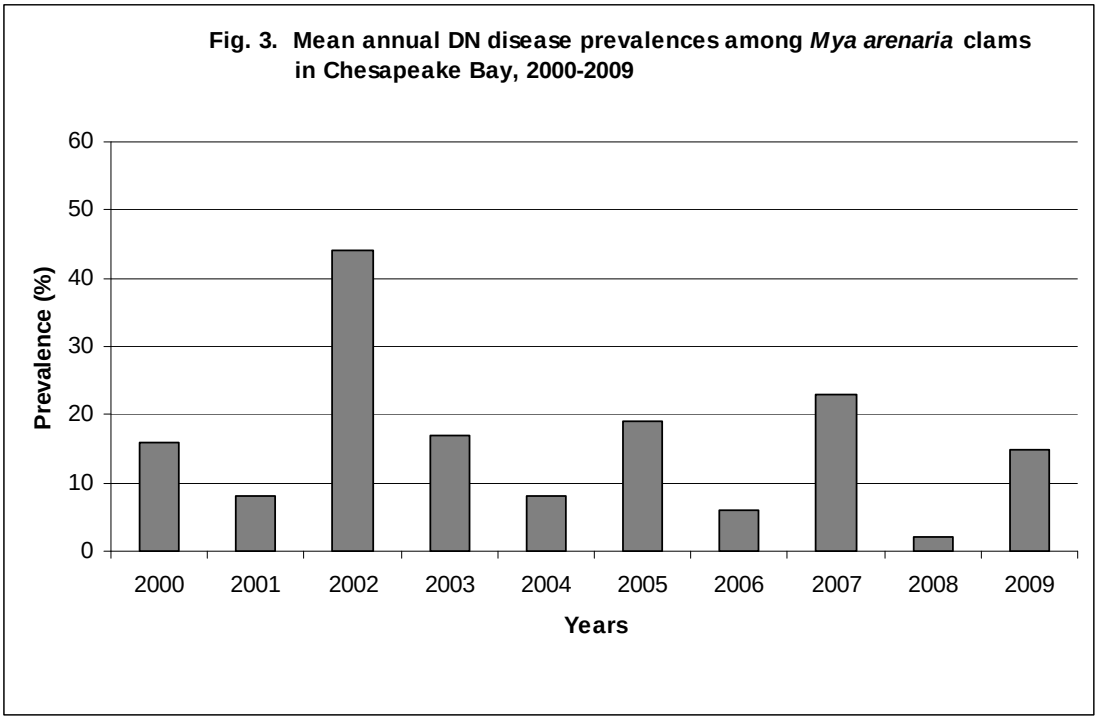


Fig. 2. Mean winter-spring *P. chesapeaki* infection prevalences among two Chesapeake Bay clam species: 2004, 2008, 2009



Extreme razor clam mortalities that occurred during the winter-spring months of 2004 coincided with a mean *P. chesapeaki* infection prevalence of 71% (sample range 7-98%) among *T. plebeius* razor clam samples from that period (Fig. 2). As in all years when clam samples were collected and analyzed for diseases during winter-spring seasons (2004, 2008, and 2009), high prevalences of *P. chesapeaki* infections uniquely persisted among *T. plebeius* razor clam populations during those annual cool seasons. These data suggest that prevalent *P. chesapeaki* infections may cause *T. plebeius* razor clam mortalities during colder, winter-spring months. Pathogen cells dispersed during springtime as a consequence of death and decomposition of infected razor clams may infect sympatric *Mya arenaria* clams at the beginning of the warm water season, when *P. chesapeaki* pathology is most severe among softshell clams. If the latter inference has merit, then infected *T. plebeius* razor clams may function as reservoirs of *P. chesapeaki* cells in benthic Chesapeake Bay clam habitats shared by both clam species, and as potential vectors for early dispersal of infectious pathogen cells as estuarine waters warm during spring and early-summer.

Disseminated neoplasias (DN disease) occurred among *Mya arenaria* clams at variable mean annual prevalences of 2-44% (decadal mean = 16%) among 1,853 softshell clams that were analyzed histologically during 2000-2009 (Fig. 3). Prevalences of DN disease among softshell clams in individual samples from that decade ranged between 0-100%. High prevalences of a lethal neoplastic disease among Chesapeake Bay clams are alarming, due to the possibility that they may reflect the environmental presence of carcinogens in benthic estuarine habitats. The cause of DN disease in softshell clams remains uncertain, although there is some evidence for a potentially infectious retroviral agent (House et al. 1998), and the disease has been characterized as transmissible among *Mya arenaria* clams in Chesapeake Bay, where high DN disease prevalences in softshell clam samples have been commonly documented at 30-90% since 1983 (Farley et al. 1991, Dungan et al. 2002). Periodically or locally significant softshell clam mortalities from DN disease in Chesapeake Bay are likely, especially when DN disease compromises defensive capabilities among clams that are coincidentally infected by *P. chesapeaki* or other microbial pathogens.



Gill epithelial cell nuclear hypertophy (GENH) disease is a previously unrecognized virus disease that was found to be prevalent among Chesapeake Bay *Mya arenaria* clams during the current investigation (Dungan et al. 2007). GENH virus infections of the nuclei of gill epithelial cells cause pathological hypertrophy of the nuclei of infected gill epithelial cells, which may functionally compromise infected cells. As genomic controls and functions of gill cells are commandeered for virus replication and packaging (Fig. 4), the disease may impair the critical feeding and respiratory functions of *Mya arenaria* gill tissues.

Since the first recognition and partial characterization of this new virus disease during 2005, it has been consistently diagnosed at high prevalences among *Mya arenaria* clams in samples from all Chesapeake Bay clam habitats (Fig. 5). Mean annual prevalences for GENH virus disease among 1,934 softshell clams that were analyzed during 2000-2009 ranged from 18–90% (decadal mean = 67%), and prevalences among individual samples from that decade ranged between 0-100%. A recent trend of elevated annual means for GENH virus infection prevalences among *Mya arenaria* clams is apparent during 2006-2009, suggesting that recent impacts of that disease have been broadly distributed and prevalent among diminished softshell clam populations in the Maryland portion of Chesapeake Bay

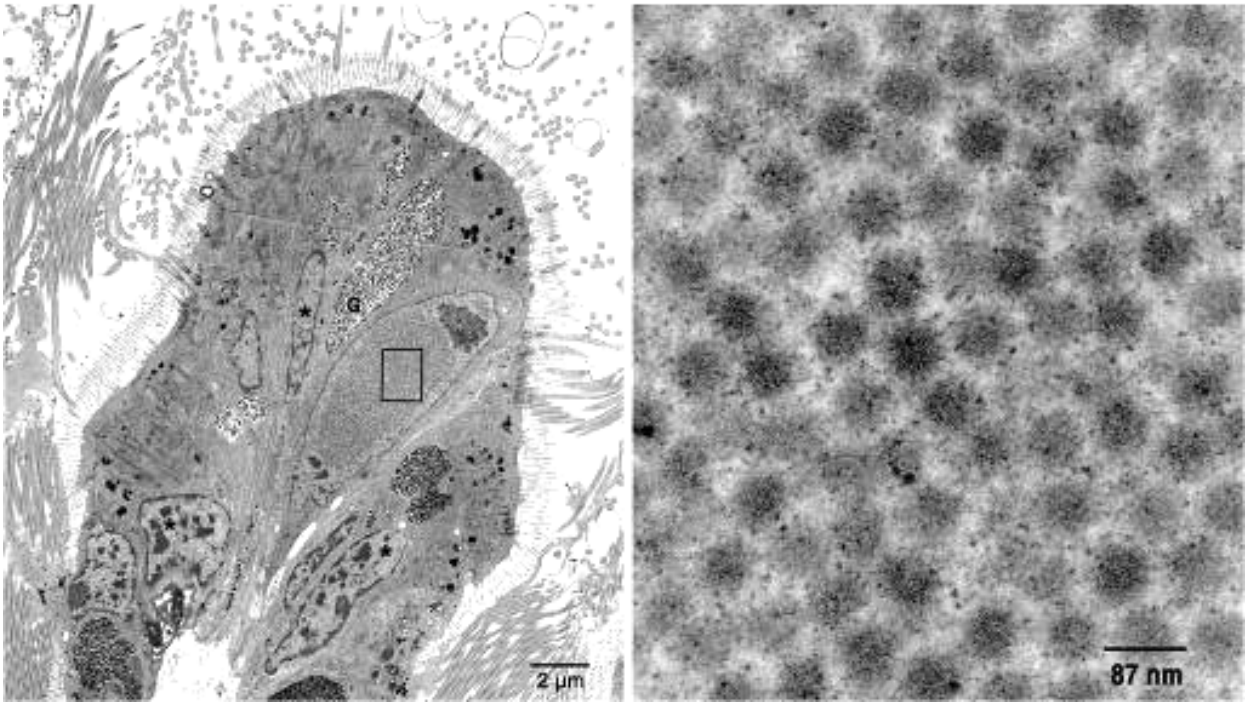
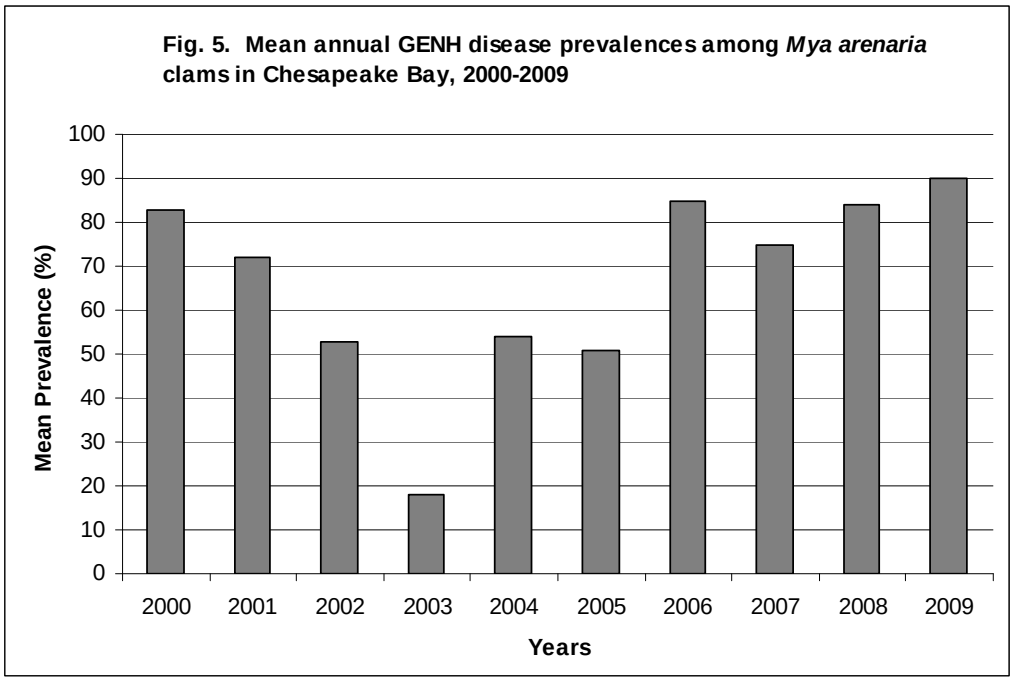
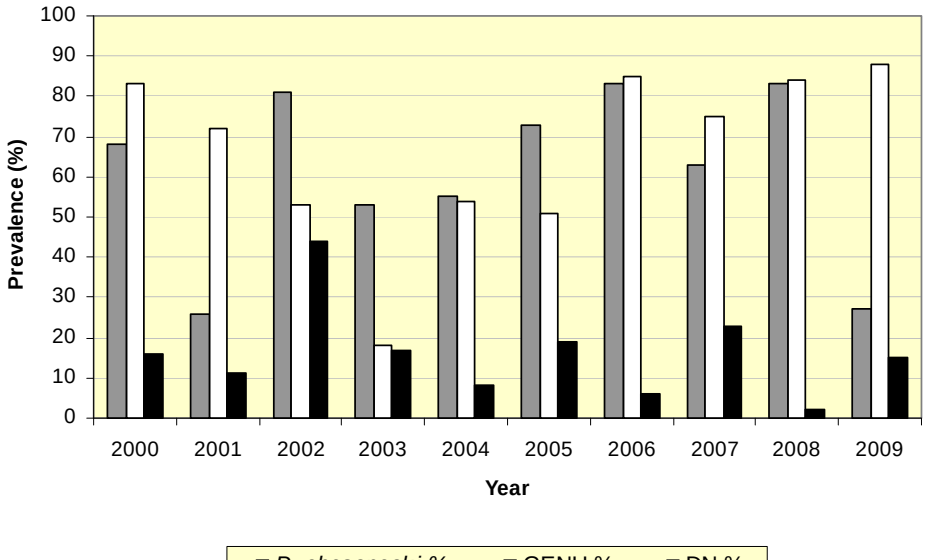


Fig. 4. Transmission electron micrographs of ciliated epithelial cells at the tip of a gill filament of a *Mya arenaria* clam bearing a GENH virus infection (left). Normal, mottled nuclei of 6 adjacent cells (asterisks) surround the hypertrophic, virus-infected central nucleus containing an inset-rectangle. At higher magnification (right), the inset area shows close–packed virus particles that have been replicated and assembled within the infected nucleus.



The combined prevalences of three diseases among *Mya arenaria* clams in Maryland Chesapeake Bay bottoms during 2000-2009 show that those diseases occurred at mean annual prevalences that varied differently for each disease between years, but which occurred together during all years (Fig. 6). All three diseases occurred simultaneously among individual clams. Generally, DN disease was the least prevalent of the 3 diseases, with a long-term prevalence mean of 16% for ten years with annual prevalence means that ranged between 2-44%. Mean annual *Perkinsus chesapeaki* infection prevalances varied widely between 26-83% (decadal mean = 61%) for *M. arenaria* clams, and between 13-100% for *T. plebeius* clams (decadal mean = 72%, Fig. 1). Retrospective analyses of archived histological samples revealed that GENH virus gill lesions did not occur among *T. plebeius* razor clams, but consistently occurred among *Mya arenaria* softshell clams at mean annual prevalences of 18-90% (decadal mean = 67%). Mean annual prevalences of the 3 diseases appear to vary independently between years, but all co-occurred at relatively high prevalences during 2002. Potential interaction effects between the three diseases are uncertain, but anecdotal observations suggest that DN disease effects may enhance the intensities and impacts of *P. chesapeaki* infections by compromising hemocyte-mediated softshell clam defensive functions that may moderate the pathological effects of such infections.

Fig. 6. Mean annual prevalences of 3 diseases among *Mya arenaria* clams in Maryland Chesapeake Bay waters, 2000-2009



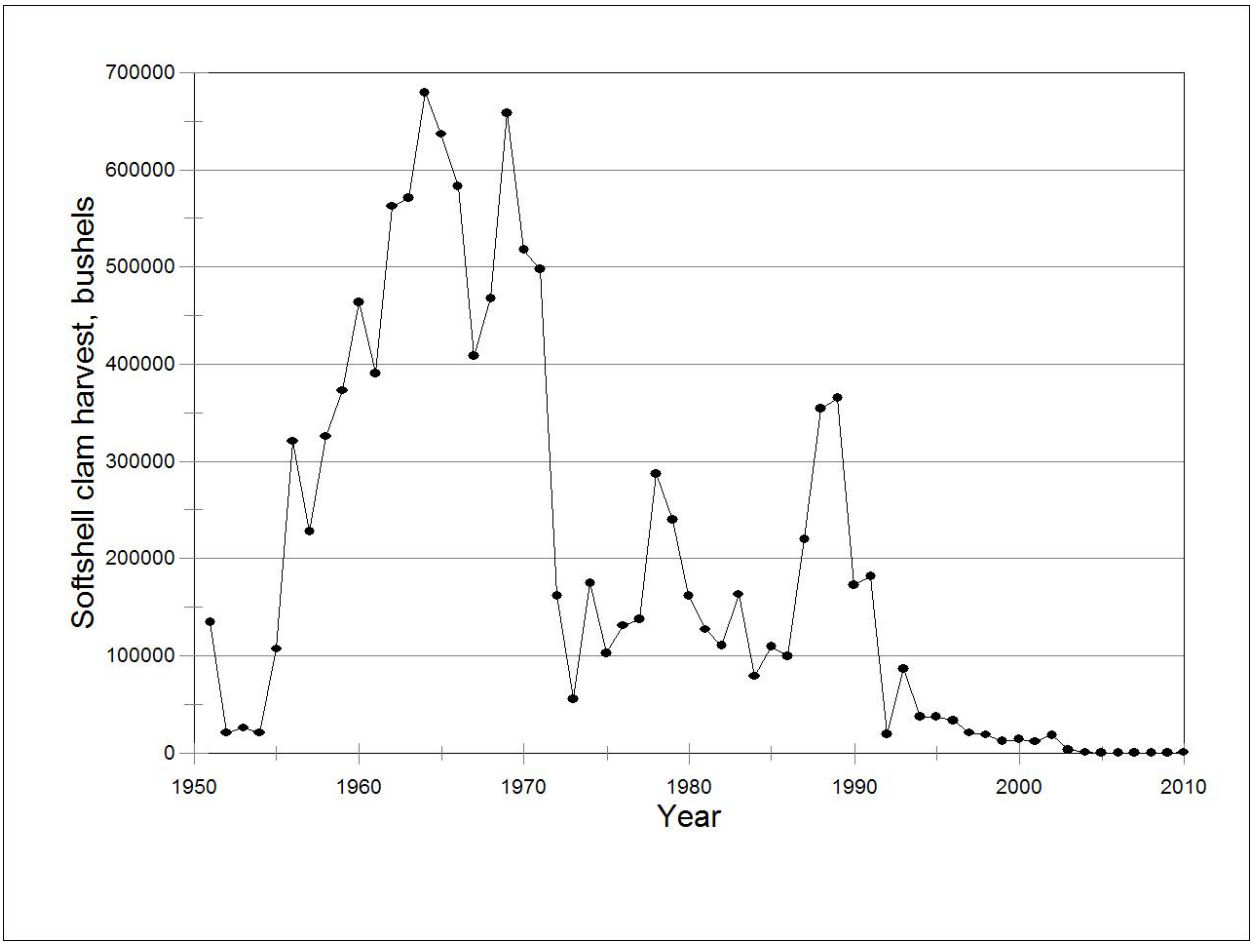
Special Task.
Establish sentinel sites for developing a consistent set of data with respect to clam diseases.

The development of sentinel sites for the purposes of acquiring disease data was given a high priority from the beginning of this project. From data collected in 2000 (DNR funded pilot study) and discussions with commercial clammers, sentinel sites were established for the initial grant period in 2001. As the project expanded geographically, sentinel disease sites were established for all Chesapeake Bay regions. Over time, however, some failures occurred in the rates of sentinel clam recruitments, and softshell clams disappeared from some sites. Subsequent efforts were made to locate and sample populations of softshell clams that were proximate to depleted sentinel sites.

During winter-spring months during 2004, catastrophic razor clam mortalities were observed and reported by harvesters throughout Maryland's Chesapeake Bay. This caused further erosion in the established sentinel disease data set, since razor clams suffered more than 90% mortality in some areas. Nevertheless, an impressive set of disease data with some temporal gaps has been consolidated by this project.

SUMMARY & DISCUSSION

Given below is a graphic depiction of the softshell clam harvest record in Maryland. Below the figure is a time line listing of events that have had significant effects on both the fishery and the clam population.


Softshell clam harvest, bushels
700000
600000
500000
400000
300000
200000
100000
0
1950
1960
1970
1980
1990
2000
2010
Year

Timeline of events significant to the softshell clam fishery and population in Maryland Chesapeake Bay waters.

1951	Hydraulic escalator dredge first used to harvest softshell clams
1952-1958	Various regulatory restrictions, including shoreline distances, exclusion of charted oyster bars, etc.
1954	*Perkinsus* sp. reported to infect Virginia softshell clams
1965	Major mortality event in Potomac River
1968	Collapse of the Virginia fishery
1971	Major mortality event in Maryland's Chesapeake Bay
1971	Unknown hyperplasia found in clam gill tissue
1971	Bruce Decision , overturning the regulation that restricted watermen from working in county waters other than that in which they resided
1971	Daily catch limit reduced from 40 to 25 bushels; cull size increased from 2 to 2.25 inches
1972	Tropical Storm Agnes floods Chesapeake Bay with freshwater, sewage, and sediments
1972	Fishery closed from June 1972 until June 1973, closed again in June 1973, and re-opened in September 1973
1973	Daily catch limit reduced to 15 bushels
1975	Cull size reduced to 2 inches
1984	Disseminated neoplasia (DN disease) found in Maryland softshell clams
1990	*Perkinsus* sp. found in Maryland softshell clams
2002	Major mortality event in Maryland

After the hydraulic escalator dredge was developed and first used to harvest softshell clams in 1951, landings rapidly increased. From 1955 through 1971, annual harvests of softshell clams averaged about 460,000 bushels (370-460 million clams per year). The effect of a series of fishery regulatory restrictions (1952-1958) is not documented, but conversations with several clammers who were active during the first 2 decades of the fishery indicated that without these restrictions, harvest totals could have been 3-4 times greater. Nevertheless, it is apparent that softshell clam populations were substantial and widespread, particularly from the Potomac River

north to the Upper Bay. In hindsight, several events foreshadowed later catastrophes. In 1954 a parasite, *Perkinsus* sp., was reported to be infecting softshell clams in Virginia; in 1965, a major mortality event occurred in the Potomac drainage, essentially ending commercial harvesting in that area; in 1968, the Virginia fishery (albeit modest with respect to Maryland's) collapsed; and in 1971 widespread mortalities occurred in Maryland, coincident, although not necessarily correlated with, the report of an unknown hyperplasia found in the gill tissue of softshell clams in Maryland. It is widely assumed that TS Agnes in 1972 caused the subsequent, severe declines in softshell clam landings, and inability of populations to return to pre-1972 levels. The report in 1971 of an unknown hyperplasia, combined with what we now know about DN disease and its devastating effects on clam populations, does suggest an alternative or companion causal factor to TS Agnes effects.

Numerous factors shaped the post-1972 fishery, including the reduction of the daily limit from 40 to 25 to 15 bushels, market forces (as most softshell clams caught in Maryland are sold in New England, there has been a long standing attempt on the part of NE states to restrict imports in order to protect local fisheries), the establishment of the razor clam fishery in the early 1980s, and the possibility that disease was eroding population levels. After TS Agnes, the fishery averaged about 165,000 bushels per year from 1974 through 1983. In 1984, DN disease was documented in Maryland softshell clam populations, coincident with a sharp decline in landings. Harvests picked up briefly from 1987 through 1991, averaging 260,000 bushels per year. In 1990, *Perkinsus* sp. infections were found in Maryland populations, harvests crashed in 1992 and since then have averaged less than 17,000 bushels per year and less than 4,000 bushels per year over the last decade.

During the course of the present study, numerous aspects of the present status of softshell clams in Maryland have been well documented and insights have been developed regarding past events and conditions. As compared to abundance estimates from surveys conducted between 1962 and 1975, current softshell clam populations have declined by over 90%. There has been a significant retraction in the range of habitats populated by this species, with most softshell clams now found only in areas with structural overburden. In more exposed areas, we found recently recruited clams to rapidly disappear, presumably to predation. Tabulated below are examples from two areas with little substrate structure, Upper Hill and Bodkins Point in Eastern Bay and one with significant structure (oyster shell), Buoy Rock in the Chester River. With disease

pressures similar among the areas, the data point to predation as the primary difference between the sites.

Abundance of sublegal (<50mm) softshell clams, number per acre, on two sites in Eastern Bay and one in the Chester River in 2004					
Location	May	June	July	August	November
Upper Hill	104,000	20,000	16,000	5,000	2,000
Bodkins Point	61,000	12,000	6,000		1,000
Buoy Rock	22,000	18,000		16,000	14,000

We have estimated that at one time, softshell clams constituted, numerically, at least 35% of Maryland's *large* bivalve population (oysters, softshell, razor, and hard clams). The loss of this population as a forage species, the magnitude of the resuspension of sediments via their burrowing, and filtration capacity (it's estimated that an individual softshell clam filters as much water as does an oyster) has had to have had a profound effect on the Bay's ecosystem.

During the present study, a substantial database was created regarding *Perkinsus chesapeaki* infections and levels of DN disease. In 2005, a previously unrecognized viral disease, gill epithelial cell nuclear hypertophy (GENH), was discovered in Maryland softshell clam populations. We were able to correlate the 2002 mortality event with disease analyses, and reasonably conclude that the greatest impediment to even modest increases in *Mya arenaria* abundance are a suite of diseases that show no sign of diminishing.

Unfortunately, there are no landing records nor are there previous survey abundance estimates of the stout razor clam, *Tagelus plebeius*. Initially, this species was a secondary focus of the present project, but several findings and events elevated the project status of razor clams. In 2000, the presence of *P. chesapeaki* infections was discovered in razor clams and in 2001, DN disease was found in this species. It was not until late 2003, however, when large numbers of razor clam boxes were observed in several areas, that consideration was given to redirect some of this project's resources to an expansion of razor clam surveys. After reports came in from watermen in early 2004, that a major razor clam mortality event was occurring, it was decided to focus on razor clam populations, beginning in March 2004. During the course of the 2004 surveys, it was determined that indeed a catastrophic mortality event had occurred, decimating razor clam populations throughout the Bay. With few exceptions, mortality rates of razor clams exceeded 50%, and it was estimated that by the end of 2004, over 70% of the entire *T. plebeius*

Maryland population had died. Some areas not as hard hit in 2004, succumbed in 2005, and it was estimated that between late 2003 and through 2005, razor clam abundance in Maryland declined by 60+%. Regional mortality estimates are given below for 2004 and indicate no area of the Bay was spared.

Razor clam regional mortality estimates, percentage of population							
	Region						
Year	Upper Bay	Chester River	Eastern Bay	Miles/Wye Rivers	Choptank River	Patuxent River	Tangier Sound
2004	75	85	75	25	85	55	55

The relatively low rates in the Eastern Bay tributaries in 2004, climbed to about 60% from data collected in 2005. Later surveys conducted between 2006 and 2008, indicated that high mortality rates persisted in razor clam populations, although surveys were geographically less extensive.

It was apparent that disease was the causal agent, although how each disease factored into the extraordinary mortality rates is unknown. What is known, however, is that both diseases persist in razor clams and that even as *T. plebeius* populations re-establish in some areas and recruit in new areas, they are susceptible to disease acquisition.

There are few records of razor clams in published accounts of food habit studies conducted within their geographic range, nor is there agreement on how they feed, that is, are they particulate or filter feeders. Without this information, it is difficult to plug them into the Bay's ecosystem and reasonably conclude what effect their steep decline in abundance has had on system functionality. It is, however, not unreasonable to conclude that a species with formerly high densities in the Bay that has suffered catastrophic and persistent mortalities has had a significant effect on system dynamics, be they energy transfers through predator-prey relationships and/or geochemical processes associated with feeding activity and burrowing.

In the course of the present study, it has been documented that softshell clam populations in Maryland's Chesapeake Bay are but remnants of what they once were, and that the primary reason for their decline and lack of ability to re-establish, is a suite of diseases. Although

placement of structure in carefully chosen areas could result in establishing pockets of softshell clams, it would be expensive and it isn't possible to predetermine rates of success. Enhancing either commerce or populations through aquaculture is not feasible given the virtual impossibility of off-bottom culture (surface waters regularly attain lethal levels in the Chesapeake Bay) and planting small clams in suitable bottom would simply provide a feeding bonanza for a myriad of predators. The remnant fishery that currently operates in Maryland has little known impact on softshell clam populations. This species does not appear to be heading towards extinction in the Chesapeake Bay region, although, as it is at the southernmost limit of its range, climate changes could result in an eventual deletion from the Bay's species list.

The present study also documented the crash of a heretofore healthy and thriving razor clam population. As with softshell clams, there aren't any remedial or stabilizing techniques that could be construed to be productive for razor clams and their disease status is chronic. Unlike softshell clams, however, there is a degree of resilience in razor clam populations as evidenced by both data collected during the present study and the continuing, although reduced, commercial fishery.

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