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Testing of Great Bay Oysters for Two Protozoan Pathogens

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TESTING OF GREAT BAY OYSTERS FOR TWO PROTOZOAN PATHOGENS

A Final Report to

Piscataqua Region Estuaries Partnership

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Executive Summary

Two protozoan pathogens, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo) are known to be present in Great Bay oysters. With funds provided by the Piscataqua Region Estuaries Partnership (PREP), the Marine Fisheries Division of the New Hampshire Fish and Game Department (NHFG) continues to assess the presence and intensity of both of these disease conditions in oysters from the major natural beds within the Great Bay estuarine system. Histological examinations of Great Bay oysters have also revealed other endoparasites.

Introduction

The American oyster, *Crassostrea virginica*, can be invaded by a variety of parasites. Two particularly damaging protozoan parasites, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo), have caused high mortalities of American oysters all along the Southern and Middle Atlantic Coasts, and have been seen continuously in New Hampshire waters since the mid 1990's.

MSX was first recognized as a serious oyster pathogen in Delaware Bay in 1957 (Haskin and Andrews, 1988). Having since become widespread, it is now reported along the Atlantic Seaboard from Florida all the way to Nova Scotia. The presence of MSX in New England was initially detected in oysters taken at Milford, Connecticut in 1960 (Sindermann and Rosenfield, 1967). Later, in 1967, oysters from Wellfleet, Massachusetts were also found to contain the pathogen (Krantz et al., 1972). The presence of MSX in oysters from the Piscataqua River (Maine and New Hampshire) was discovered in 1983, although unspecialized haplosporidian plasmodia had been seen by Maine Department of Marine Resources' scientists in 1979 (S. Sherburne, Maine Department of Marine Resources, per com.). Following this, MSX was not recorded again until 1994, when Spinney Creek Shellfish, Inc. (a Maine-based aquaculture operation) learned that oysters in the Piscataqua River contained the pathogen. When oysters from these same beds were examined a year later (1995), MSX was again found, this time more prevalent than the previous year (Ken LaValley, University of New Hampshire Cooperative Extension, per. com.).

In response to the test results from Spinney Creek Shellfish, Inc., and to anecdotal information from recreational oyster harvesters (in New Hampshire) of many boxed and/or gaping oysters, three major beds in Great Bay (New Hampshire) were sampled and tested in 1995. This initial histological examination was conducted by Dr. Bruce Barber, University of Maine (Barber et al., 1997). In later years, these tests have been performed by the Haskin Shellfish Research Laboratory, Rutgers University. (Results of all MSX tests are covered below.)

Dermo (*Perkinsus marinus*) has spread up the coast from South and Middle Atlantic sources into the Gulf of Maine. During the past three decades, cold waters north of Chesapeake Bay were believed to act as a controlling factor that prevented Dermo from persisting year-round, which may have rendered its virulence to oysters in New England as minor compared to MSX. Recent warming of the Gulf of Maine (NERACOOS, 2015), however, may be responsible for increases in the prevalence of Dermo, and it now appears to be an increasing threat to oysters in Great Bay. This protozoan pathogen was first demonstrated to be present in the Great Bay system in 1996, when scientists from the University of Maryland found oysters in Spinney Creek (a small tidal pond off the Piscataqua River) contained Dermo. Following this, other samples taken from Great Bay and the Piscataqua River showed Dermo-like particles as well. (Tests for Dermo on specimens from the Great Bay system will be reviewed in greater detail below.)

Project Goals and Objectives

Based on the results of oyster monitoring by the New Hampshire Fish and Game Department (Smith, B., NHFG annual memos), as well as information obtained via surveys of oyster harvesters, both abundance and harvest of oysters declined from 1995 to 1996 (NHFG, 1997). It is highly likely that the presence of MSX and Dermo contributed significantly to these declines in the Great Bay oyster stock. More recent spatfalls (2006 to 2009 and 2014), however, are promising, with spat abundance at levels greater than those of the late 1990s through the early 2000s. This provided some optimism for the recovery of the stock. However, the most recent surveys of larger oysters show the stock once more slipping downward. It is imperative to maintain surveillance of these disease conditions, given that the presence (or absence) of such potentially damaging pathogens could indeed help explain the variability of oyster abundance in the future. The objective of this study is to monitor the presence of MSX and Dermo in Great Bay oysters.

Methods

During the fall of 2016, oysters were collected and tested from four natural beds (Figure 1): the Oyster River, Adams Point, Nannie Island, and Piscataqua River. Testing of the Squamscott River and Woodman Pt. sites were not accomplished in 2016 because of budget constraints.

The oysters sampled varied in size, generally ranging from about 58mm to 91mm shell height. Site samples consisted of 8 individuals per sites.. The oysters were cleaned of attached epifauna and then shipped to Haskin Shellfish Research Laboratory (Rutgers University) for testing.

MSX determinations were made by tissue section histology. Using standard techniques, the tissue sections were examined microscopically for pathological conditions and parasites, particularly MSX. Dermo testing involved the standard Ray's fluid thioglycollate medium (RFTM) incubation of rectal and mantle tissues.

Results and Discussion

The results of all recent histological tests for MSX (1995 to the present) are shown in Table 1. Dermo RFTM results for all years of testing are shown in Table 2.

MSX infection frequencies can be categorized according to the presence of the MSX protozoan in various locations within the host oyster. Light infections are those that involve only the gills and adjacent palps epithelium. More advanced systemic infections are those in which MSX is present in tissue other than gills and palps of the oyster (i.e. digestive organs and blood). It is important to recognize that an MSX infection can be progressive; therefore, the spreading of the pathogen throughout an individual is possible over time.

The MSX results show a widespread distribution of infection throughout the Great Bay system during the twenty years of testing. Prevalence varies both site to site and within each site over time. Based on early test results, it appears that the Piscataqua River was the area most severely impacted by the 1995 epizootic (Barber et al., 1997). Systemic infections in the upper reaches of the Piscataqua and Salmon Falls Rivers ranged from 25% to 50%, compared to generally lower values in Great Bay proper (Table 1). Some seemingly isolated, higher frequencies of infection were found at various locations from 1996 through 2016, but a consistent pattern cannot be inferred.

In 2016 only Nannie Island showed MSX presence in a single oyster, which did not show evidence of the more advanced, systemic infection. Samples from the other three locations tested were free of MSX.

A graphic of combined sites prevalence (Figure 2) has been developed to track the overall presence of MSX in the Great Bay estuary for the period of 1997 through 2016. From this, one can see an initial high spike of total prevalence in the early years of monitoring (1997 through 2002), followed by a reduced total prevalence. In 2009, the combined sites MSX prevalence increased markedly and the number of systemic infections also rose (NHFG, 2009). These increases were not repeated in 2010, 2011 or 2012. In 2013, however, there was an increase in both total and advanced prevalence. In 2014 MSX prevalence was down again. The 2015 results show another drop in both total and advanced infection prevalence with total prevalence being the lowest of the time series (Table 1 and Figure 2). Results from 2016 indicate a continued decline in MSX presence with three of the four sites sampled being free of the pathogen.

Early Dermo results from 1996 and 1997 show the presence of Perkinsus-like particles at every location sampled except for Seal Rock, Fox Point, and the Bellamy River (Table 2). Other than the Sturgeon Creek bed, as well as the Piscataqua River sites, these were light infections that appeared to show low frequency within the total sample lot. Dermo prevalence was comparatively low for the years 1997 through 2002 (except for the Salmon Falls River). From 2004 through 2009, Dermo increased both in overall prevalence and in the frequency of the more serious, advanced stages, which pose a direct threat of infection to Dermo-free oysters. Results between 2010 and 2015 indicated an increasing prevalence on natural beds. The 2016 test results show an overall increase in Dermo prevalence from 2015, and remained within the range observed since the increased prevalence in 2006. The combined sites Dermo prevalence is shown by Figure 3.

Unlike the variable results for locations and years recorded for MSX samples, those of Dermo are more spatially and temporally consistent. While there has been no convincing evidence of oyster mortality due to Dermo prevalence, there are some irrefutable facts that would seem to implicate it as an agent in oyster stock decline in the Great Bay system. Upon review of the annual oyster survey record, 2008 to 2016, (Smith, B. and Sullivan, K., NHFG annual memos) it is clear the standing stock of adult oysters has trended downward. This has occurred even with historically high recruitment years in 2006 and 2007. This has been seen at both natural oyster beds where recreational harvest is ongoing (i.e. Nannie Is, Woodman Pt. and Adams Pt.) and at beds that are not harvested (i.e. Oyster River and Squamscott River).

During the period of time in which Dermo infections have trended upward (Fig. 3), it is especially noteworthy to mention the prevalence of advanced infections, a condition well recognized as a cause of oyster mortality (Paynter, 1996, Sindermann and Rosenfield, 1967; Burreson et al, 1994 and Craig et al 1989). With the drop in adult oyster stocks in Great Bay, along with the concomitant rise in Dermo prevalence it is reasonable to believe Dermo is implicated.

Recent years show a downward trend in advanced stage presence of Dermo. Because it is progressive and nearly always fatal one should not suppose this is an overall reduction of the threat to Great Bay oysters. The prevalence is high and the non-advanced individuals will most likely progress to advanced infection and death over time. Direct mortality is one of several types of adverse impact on oysters. Sublethal effects including reduced reproductive functions may also be possible (Paynter, 1996).

The tissue examination of Great Bay oysters has produced interesting findings that are incidental to the principal objective studied. Large ciliate-produced xenomas are now being observed in the gills of the tissue cross sections. Over the past few years, the presence of xenomas has received increased attention. A review of earlier tissue samples for Great Bay shows that these xenomas have been present since the examinations in the late 1990s, but their numbers have increased since 2000 (Scarpa et al., 2006). In 2016, ciliate prevalence was highest at the Piscataqua River (50%). Xenomas were seen at all sample locations except for Oyster River. The highest xenoma presence was found at the Piscataqua River (25%).

Conclusions

Evidence of large-scale oyster mortality within the Great Bay estuary first gained regional attention in the fall of 1995. This prompted examinations of oysters from several beds in New Hampshire. Results of these examinations focused on the presence of *Haplosporidium nelsoni* (MSX), an oyster pathogen well-known as a cause of oyster epizootics throughout the middle Atlantic coast.

During this same time, oyster beds in the Piscataqua and Salmon Falls Rivers (Maine) incurred similar, MSX-related mortality (Ken LaValley, University of New Hampshire Cooperative Extension, per. com.). The 1995 Great Bay Estuary MSX epizootic caused more than 80% mortality in the areas most affected (Barber et al., 1997). The highest mortalities were found in the Piscataqua and Salmon Falls Rivers while other areas in the estuary did not appear to be as heavily infected. It is important to note that testing specifically for Dermo was not performed immediately after the reported oyster mortality in the fall of 1995. Dermo testing began in 1996, and has continued annually since then.

In the spring of 1996, testing at the major recreational oystering beds in New Hampshire (Nannie Island and Adams Point) showed no systemic infections of MSX. The entire 1996 season did not result in oyster mortalities of the type observed in the previous year. In recent years, monies from the Piscataqua Region Estuaries Partnership have supported a more expansive testing program for both MSX and Dermo.

Based on the tests performed annually since 1995, there are two protozoan parasites now widely distributed within the Great Bay oyster stock: MSX and Dermo. Severity of infection and prevalence vary greatly from site to site, as well as over time at a specific site. It is also known that a ciliated protozoan is forming intracellular xenomas of a size previously unseen in Atlantic Coast oysters. Little is known of the pathogenicity of this condition, however. Despite the presence of these protozoan parasites, no large-scale mortality of oysters from the 1995 event through to 2016 has been observed. In 2008, however, a sharp decline in oyster abundance at one site (the Piscataqua River) was noted. Because the prevalence of MSX and Dermo at this site was not clearly greater than other sites at that time, it is not reasonable to conclude that protozoan pathogens were the cause of that drop in oyster abundance. Observations by NHFG that year reported an oily film over the oysters.

Oyster tests in 2016 show continued presence of MSX in Great Bay with total infection prevalence at levels down from the previous five years and lowest of the time series. There was no prevalence of advanced infections in 2016.

Dermo was either nonexistent or found in only low prevalences for an eight-year period (1996 through 2003), except at the Salmon Falls River site in 2001 when a 60% prevalence was seen. The next year, another high (50%) was found there also. The marked increase in Dermo since 2004 is noteworthy with the 2016 levels remaining within the high range observed over the past decade.

Also present, but of unknown pathogenicity, are ciliate produced xenomas in gill tissue. The ciliate protozoans were found in all sites. Xenomas resulting from ciliate presence were seen at all but the Oyster River site.

While both MSX and Dermo have been seen throughout the Great Bay oyster beds and at some aquaculture sites, the observation of disease-related mortalities since that reported in 1995, has not occurred at a scale observable by oyster harvesters or NHFG. However, the presence of these pathogens poses a potential threat of epizootic and may be adversely affecting spawning and recruitment.

Recommendations

- This testing program should continue with samples taken from major oyster beds within the Great Bay system.
- Movement of oysters from bed to bed within the Great Bay system should be carefully controlled as it may lead to distribution of infective stages of protozoan pathogens. MSX is not yet known to be transmitted oyster to oyster, but lacking clear evidence of the exact means of transmission, it is still prudent to control movement throughout the area.
- The presence of ciliates and the resulting xenomas should be studied further.

Acknowledgment

This testing of oysters in the Great Bay system has been a team effort led by the New Hampshire Fish and Game Department's Marine Fisheries Division. Necessary support has been provided by the University of New Hampshire, Jackson Estuarine Laboratory personnel, the Piscataqua Region Estuaries Partnership, and the Haskin Shellfish Research Laboratory, Rutgers University. This report has been prepared by the New Hampshire Fish and Game Department, which assumes all responsibility for its accuracy. To all others on the team, we extend our gratitude for their cooperation.

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Sullivan, K. memo to Grout D., January 26, 2017 (p.12) *Review of Oyster Data - 2016*

Table 1. MSX Test Results - 1995 - 2016

Date	Location	No. Tested	No. Infected ¹	% of No. Tested	No. Systemic Infection ¹	% of No. Tested
09/05/95 ²	Piscataqua River (Summer Bed)	25	18	72	10	40
10/27/95 ²	Salmon Falls	16	13	81	8	50
10/27/95 ²	Piscataqua River (Summer Bed)	20	14	70	5	25
10/27/95 ²	Sturgeon Bed	20	13	65	8	40
10/27/95 ²	Stacy Bed (Seal Rock)	20	9	45	2	10
11/06/95	Adams Point	20	8	40	3	15
11/06/95	Nannie Island	20	3	15	1	5
12/18/95	Oyster River	20	10	50	6	30
04/12/96	Nannie Island	30	3	10	0	0
05/27/96	Adams Pt.	10	0	0	0	0
05/27/96	Nannie Island	10	0	0	0	0
03/17/97	Fox Pt.	30	5	16.6	1	3.3
09/08/97	Bellamy River	25	10	40	2	8
09/08/97	Squamscott River	25	11	44	5	20
11/17/97	Adams Point	25	10	40	5	20
11/17/97	Nannie Island	25	13	52	7	28
11/17/97	Oyster River	25	9	36	2	8
11/17/97	Piscataqua River	25	15	60	5	20
12/09/98	Adams Point	25	7	28	2	8
12/09/98	Nannie Island	25	11	44	2	8
12/09/98	Squamscott River	25	17	68	7	28
12/09/98	Piscataqua River	18	7	39	3	11
10/21/99	Nannie Island	20	7	35	6	30
11/04/00	Piscataqua River	20	6	30	3	15
11/04/00	Adams Point	20	7	35	5	25
11/04/00	Nannie Island	20	6	30	5	25
11/15/00	Oyster River	20	7	35	2	10
10/10/01	Nannie Island	24	5	21	4	17
10/18/01	Salmon Falls - disease resistant	20	1	5	1	5
01/18/01	Salmon Falls - native	21	9	43	6	29
11/04/01	Oyster River	20	5	25	4	20
11/04/01	Adams Point	20	5	25	4	20
10/14/02	Oyster River	20	9	45	1	5
10/14/02	Adams Point	20	9	45	0	0
10/20/02	Salmon Falls - disease resistant	20	2	10	0	0
10/20/02	Salmon Falls - natives	18	5	28	0	0
10/31/02	Nannie Island	24	9	37	4	17
10/28/03	Nannie Island	26	2	7.7	0	0
10/27/04	Oyster River	24	6	25	1	4
11/18/04	Nannie Island	17	5	29	1	6
11/19/04	Adams Point	19	2	11	1	5
11/19/04	Crommet Creek	23	18	78	9	39
11/06/05	Oyster River	20	7	35	1	5
11/14/05	Adams Point	20	7	35	2	10
11/16/05	Woodman Point	20	2	10	0	0
11/17/05	Squamscott River	20	6	30	3	15
10/31/06	Piscataqua River	20	11	55	2	10
11/01/06	Oyster River	20	8	40	1	5

Table 1. MSX Test Results - 1995 - 2016 (continued)

Date	Location	No. Tested	No. Infected ¹	% of No. Tested	No. Systemic Infection ¹	% of No. Tested
11/02/06	Woodman Point	20	6	30	1	5
11/07/06	Squamscott River	40	24	60	6	15
11/22/06	Adams Point	20	1	5	0	0
11/28/06	Berrys Brook	16	6	38	0	0
12/07/06	Nannie Island	20	4	20	0	0
11/07/06	Nannie Island experimental reef	20	6	30	2	10
11/07/06	Adams Point experimental reef	20	4	20	1	5
11/28/06	UNH Jackson Lab	20	4	20	1	5
10/16/07	Piscataqua River	20	7	35	1	5
10/23/07	Oyster River	20	7	35	3	15
10/24/07	Woodman Point	20	5	25	3	15
11/21/07	Nannie Island	20	5	25	1	5
12/07/07	Adams Point	20	5	25	1	5
10/08/08	Adams Point	20	1	5	0	0
10/09/08	Woodman Point	20	4	20	3	15
10/10/08	Oyster River	20	8	40	2	10
10/22/08	Nannie Island	20	3	15	1	5
10/23/08	Piscataqua River	10	5	50	0	0
10/27/08	Squamscott River	10	3	30	0	0
11/04/09	Oyster River	20	10	50	7	35
11/06/09	Adams Point	20	9	45	5	25
11/12/09	Nannie Island	20	11	55	5	25
11/13/09	Woodman Point	20	7	40	3	15
12/08/09	Piscataqua River	20	9	45	4	20
10/21/10	Oyster River	20	2	10	0	0
10/19/10	Adams Point	20	5	25	4	20
10/20/10	Nannie Island	20	2	10	0	0
10/18/10	Woodman Point	20	3	15	0	0
10/26/10	Piscataqua River	17	7	41	3	18
11/16/10	Squamscott River	20	4	20	3	15
10/21/11	Adams Point	20	6	30	1	5
10/26/11	Oyster River	20	4	20	0	0
10/28/11	Woodman Point	20	3	15	0	0
11/04/11	Nannie Island	20	4	20	0	0
11/07/11	Squamscott River	20	4	20	1	5
10/19/12	Nannie Island	10	5	50	0	0
10/25/12	Woodman Point	10	3	30	0	0
11/02/12	Oyster River	10	4	40	1	10
11/05/12	Lamprey River	10	5	50	0	0
11/09/12	Adams Point	10	0	0	0	0
12/04/12	Squamscott River	10	2	20	0	0
12/06/12	Adams Point EXP	10	3	30	1	10
10/29/13	Woodman Point	10	5	50	2	20
10/30/13	Adams Point	10	3	30	1	10
10/31/13	Oyster River	10	3	30	1	10
11/04/13	Nannie Island	10	2	20	1	10
11/15/13	Piscataqua River	10	7	70	2	20
12/03/13	Little Bay A	5	1	20	0	0
12/03/13	Little Bay B	5	2	40	0	0

Table 1. MSX Test Results - 1995 - 2016 (continued)

Date	Location	No. Tested	No. Infected ¹	% of No. Tested	No. Systemic Infection ¹	% of No. Tested
10/16/14	Adams Point	10	5	50	0	0
10/17/14	Nannie Island	9	3	33.3	0	0
10/28/14	Oyster River	10	6	60	1	10
10/30/14	Woodman Point	10	6	60	1	10
11/19/14	Piscataqua River	6	1	16.7	1	11
12/15/14	Little Bay A	10	1	10	0	0
12/15/14	Little Bay B	10	1	10	0	0
12/15/14	Little Bay C	10	1	10	0	0
10/20/15	Adams Point	9	0	0	0	0
10/21/15	Oyster River	8	2	25	1	12.5
10/22/15	Nannie Island	7	1	14	0	0
10/26/15	Woodman Point	1	0	0	0	0
11/02/15	Piscataqua River	8	1	12.5	0	0
10/07/16	Adams Point	8	0	0	0	0
10/07/16	Nannie Island	8	1	12.5	0	0
10/07/16	Oyster River	8	0	0	0	0
11/01/16	Piscataqua River	8	0	0	0	0

1-Presence of MSX plasmodia when found in palps and gills only are recorded as infections only. When plasmodia are found in tissue other than palps and gills (i.e. digestive gland, haemolymph, gonads) the infection is considered systemic.

2-Data from Barber et al 1997

Table 2. Dermo Test Results - 1996 - 2016

Date	Location	No. Tested	No. Oysters in each infection category ¹					% Prevalence	
			0.5	1	2	3	4		5
12/16/96	Nannie Island	25	1	0	0	0	0	0	4%
12/16/96	Seal Rock	25	0	0	0	0	0	0	0%
12/16/96	Sturgeon Bed	25	2	0	0	0	1	0	12%
03/17/97	Fox Pt.	30	0	0	0	0	0	0	0%
08/14/97	Piscataqua River	25	2	2	0	0	1	0	20%
08/17/97	Adams Pt.	25	4	0	0	0	0	0	16%
08/14/97	Oyster River	25	1	0	0	0	0	0	4%
08/14/97	Nannie Island	25	1	0	0	0	0	0	4%
09/08/97	Bellamy River	25	0	0	0	0	0	0	0%
09/08/97	Squamscott River	25	1	0	0	0	0	0	4%
11/17/97	Adams Pt.	25	1	0	0	0	0	0	4%
11/17/97	Nannie Island	25	0	0	0	0	0	0	0%
11/17/97	Oyster River	25	0	0	0	0	0	0	0%
11/17/97	Piscataqua River	25	0	0	0	0	0	0	0%
12/09/98	Adams Pt.	25	0	0	0	0	0	0	0%
12/09/98	Nannie Island	25	0	0	0	0	0	0	0%
12/09/98	Squamscott River	25	0	0	0	0	0	0	0%
12/09/98	Piscataqua River	18	0	0	0	0	0	0	0%
10/21/99	Nannie Island	20	0	0	0	0	0	0	0%
11/04/00	Piscataqua River	20	0	0	0	0	0	0	0%
11/04/00	Adams Pt.	20	0	0	0	0	0	0	0%
11/04/00	Nannie Island	20	0	0	0	0	0	0	0%
11/15/00	Oyster River	20	0	0	0	0	0	0	0%
10/10/01	Nannie Island	25	0	0	0	0	0	0	0%
10/18/01	Salmon Falls (disease resistant)	25	3	0	0	0	0	0	12%
10/18/01	Salmon Falls (native)	25	6	5	1	1	1	1	60%
11/04/01	Oyster River	20	0	0	0	0	0	0	0%
11/04/01	Adams Point	20	0	0	0	0	0	0	0%
10/14/02	Adams Point	20	1	2	0	0	0	0	15%
10/14/02	Oyster River	20	0	0	0	0	0	0	0%
10/31/02	Nannie Island	24	2	0	0	0	0	0	8%
11/20/02	Salmon Falls (native)	18	4	2	1	1	1	2	50%
11/20/02	Salmon Falls (crossbreeds)	20	1	0	0	0	0	0	5%
10/28/03	Nannie Island	25	2	1	0	2	0	0	20%
10/27/04	Oyster River	25	2	0	2	0	0	0	16%
11/18/04	Nannie Island	17	5	2	2	1	0	0	65%
11/19/04	Adams Point	20	3	4	2	4	0	0	65%
11/19/04	Crommet Creek	23	0	1	0	1	0	0	8%
11/06/05	Oyster River	20	3	3	5	0	2	0	65%
11/14/05	Adams Point	20	6	7	3	1	1	0	90%
11/16/05	Woodman Point	20	4	4	8	2	0	0	90%
11/17/05	Squamscott River	20	0	1	0	0	0	0	5%
10/31/06	Piscataqua River	20	0	9	2	3	1	0	75%
11/01/06	Oyster River	20	3	3	4	6	0	0	80%
11/02/06	Woodman Point	20	3	8	8	1	0	0	100%
11/07/06	Squamscott River	39	3	1	1	0	0	0	13%
11/22/06	Adams Point	20	2	8	4	5	1	0	100%
11/28/06	Berrys Brook	16	0	0	0	0	0	0	0%

Table 2. Dermo Test Results - 1996 - 2016 (continued)

Date	Location	No. Tested	No. Oysters in each infection category ¹						% Prevalence
			0.5	1	2	3	4	5	
12/07/06	Nannie Island	20	2	5	4	0	1	0	60%
11/07/06	Nannie experimental reef	20	2	7	6	3	0	0	90%
11/07/06	Adams experimental reef	20	3	6	7	3	0	0	95%
11/28/06	UNH - Jackson (spat)	20	0	0	0	0	0	0	0%
10/16/07	Piscataqua River	20	4	2	6	4	1	1	90%
10/23/07	Oyster River	20	7	1	5	4	2	1	100%
10/24/07	Woodman Point	20	3	6	1	4	3	1	90%
11/21/07	Nannie Island	20	2	0	3	0	2	0	35%
12/07/07	Adams Point	20	1	1	5	2	1	1	55%
10/08/08	Adams Point	20	3	3	4	4	1	1	80%
10/09/08	Woodman Point	20	1	5	0	1	0	1	40%
10/10/08	Oyster River	20	6	7	1	2	1	0	85%
10/22/08	Nannie Island	20	1	1	1	0	0	0	30%
10/23/08	Piscataqua River	10	1	1	2	0	1	0	50%
10/27/08	Squamscott River	10	3	5	4	3	2	2	95%
11/04/09	Oyster River	20	3	4	5	2	3	3	100%
11/06/09	Adams Point	20	3	2	6	3	1	3	90%
11/12/09	Nannie Island	20	3	9	4	0	0	0	80%
11/13/09	Woodman Point	20	0	6	4	2	1	2	75%
12/08/09	Piscataqua River	20	2	6	1	0	0	0	45%
10/21/10	Oyster River	20	3	6	6	2	2	0	95%
10/19/10	Adams Point	20	2	7	3	1	3	2	90%
10/20/10	Nannie Island	20	1	2	8	3	1	0	75%
10/18/10	Woodman Point	20	2	4	5	3	3	2	95%
10/26/10	Piscataqua River	17	5	4	1	1	0	0	64%
11/16/10	Squamscott River	20	8	3	0	0	0	0	55%
10/21/11	Adams Point	20	2	4	9	1	0	1	85%
10/26/11	Oyster River	20	3	8	2	3	2	2	100%
10/28/11	Woodman Point	20	4	5	4	6	1	0	100%
11/04/11	Nannie Island	20	6	7	4	0	1	0	90%
11/07/11	Squamscott River	20	9	1	3	2	1	0	80%
10/19/12	Nannie Island	10	0	1	3	3	1	0	80%
10/25/12	Woodman Point	10	0	1	2	4	1	2	100%
11/02/12	Oyster River	10	1	3	1	2	1	1	90%
11/05/12	Lamprey River	10	2	0	3	0	0	0	50%
11/19/12	Adams Point	10	4	1	1	0	2	0	80%
12/04/12	Squamscott River	10	3	0	1	1	0	0	50%
12/06/12	Adams Point EXP	10	2	2	0	0	0	0	40%
10/29/13	Woodman Point	10	3	1	3	2	0	0	90%
10/30/13	Adams Point	10	3	4	1	0	0	0	80%
10/31/13	Oyster River	10	2	5	1	0	0	0	80%
11/04/13	Nannie Island	10	3	1	4	1	0	1	100%
11/15/13	Piscataqua River	10	0	2	0	0	1	1	40%
12/03/13	Little Bay A	5	0	0	0	0	0	0	0
12/03/13	Little Bay B	5	0	0	0	0	0	0	0

Table 2. Dermo Test Results - 1996 - 2016 (continued)

Date	Location	No. Tested	No. Oysters in each infection category ¹					% Prevalence	
			0.5	1	2	3	4		5
10/16/14	Adams Point	10	0	2	2	3	1	1	90%
10/17/14	Nannie Island	9	0	0	0	3	4	1	88.9%
10/28/14	Oyster River	10	0	1	5	2	1	1	100%
10/30/14	Woodman Point	10	1	3	3	1	1	1	100%
11/19/14	Piscataqua River	6	1	0	2	1	1	0	83.3%
12/15/14	Little Bay A	10	0	0	0	0	0	0	0%
12/15/14	Little Bay B	10	1	0	0	0	0	0	10%
12/15/14	Little Bay C	10	0	0	0	0	0	0	0%
10/20/15	Adams Point	12	2	1	4	1	1	0	75%
10/21/15	Oyster River	13	0	1	1	1	3	1	54%
10/22/15	Nannie Island	7	0	4	1	0	1	0	86%
10/26/15	Woodman Point	6	0	1	0	1	0	0	33%
11/02/15	Piscataqua River	12	4	2	2	0	0	0	67%
10/07/16	Adams Point	8	4	1	1	1	0	0	87.5%
10/07/16	Nannie Island	8	1	5	2	0	0	0	100%
10/07/16	Oyster River	8	1	3	2	0	0	0	87.5%
11/01/16	Piscataqua River	8	2	0	1	0	0	0	37.5%

1- Infection categories are based on the severity of infection. Categories 0.5 to 2 are generally thought of as light or minor, whereas categories 3 to 5 are moderate to heavy and may pose an infection threat to Dermo-free oysters.

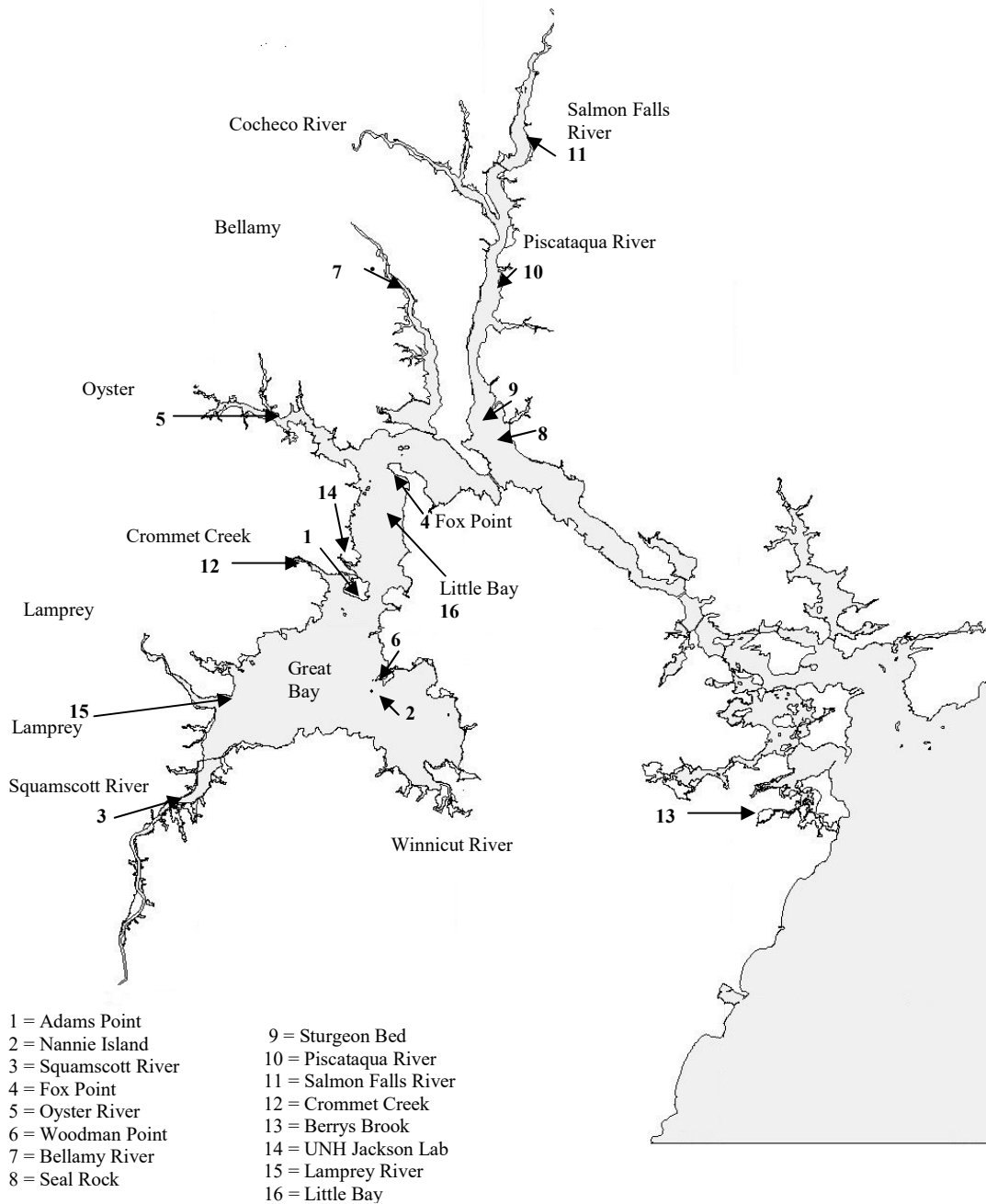


Figure 1. Study Area and Sample Locations

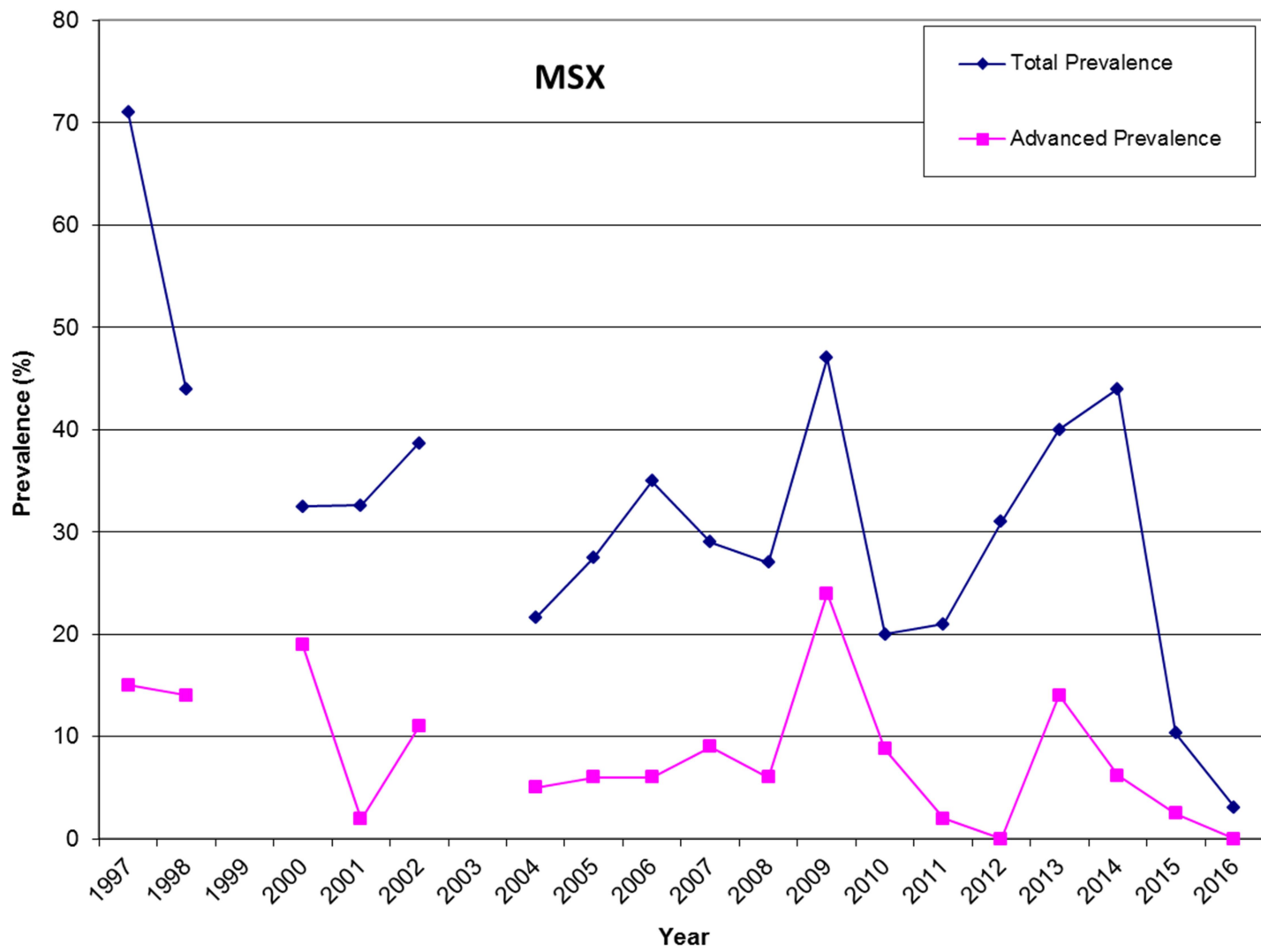


Figure 2. MSX

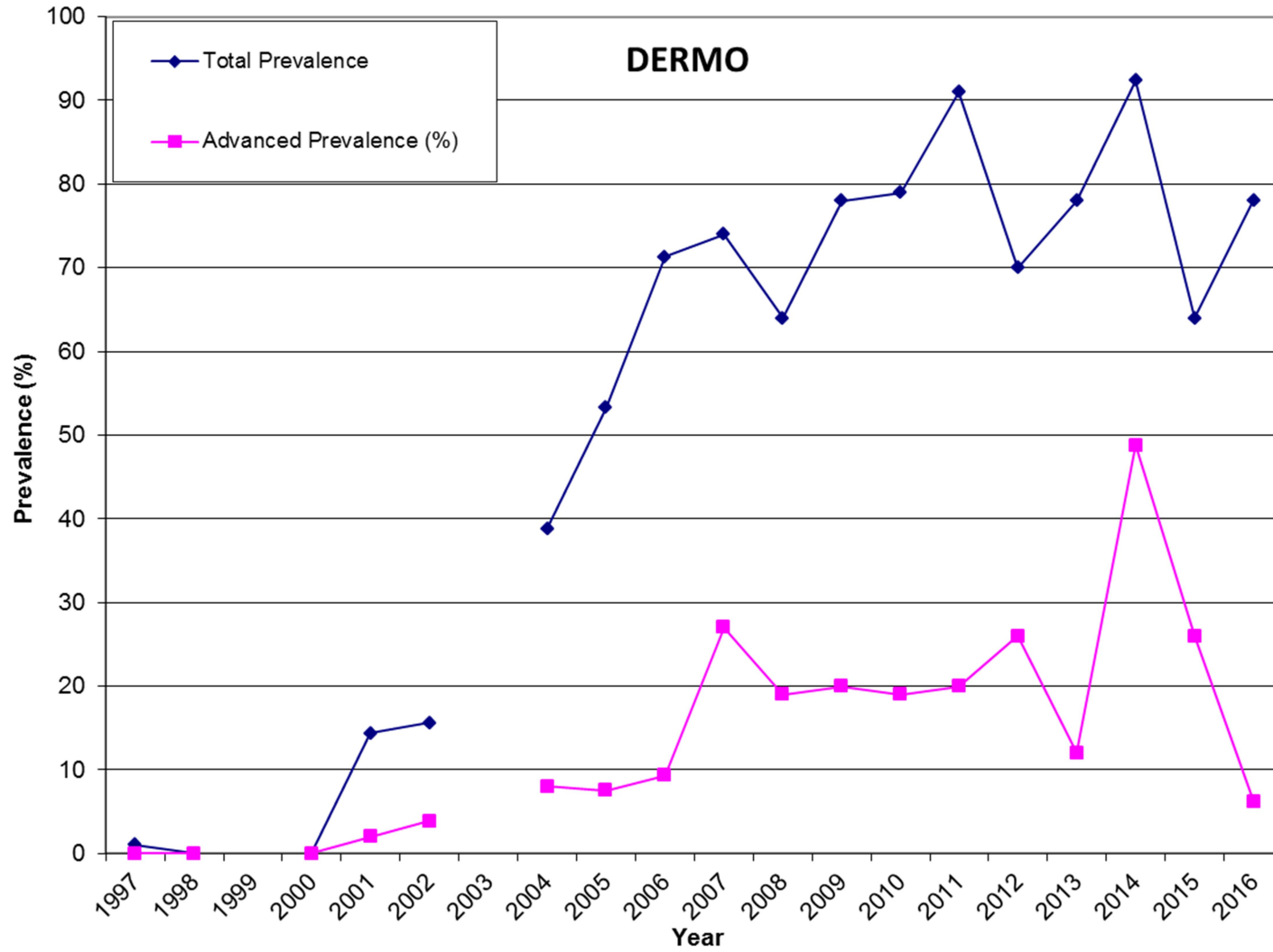


Figure 3. DERMO