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Testing of Great Bay Oysters for Two Protozoan Pathogens

New Hampshire Fish and Game Department

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A Final Report to

Piscataqua Region Estuaries Partnership

Submitted by

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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 (NHF&G) continues to assess the presence and intensity of both of these disease conditions in oysters from the major 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 are now found in New Hampshire waters.

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 from Florida all the way to Nova Scotia. The presence of MSX in New England was initially detected from 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 unspeciated 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 specimens 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. In later years, other such 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 prevents Dermo from persisting year-round, which likely renders its virulence to oysters in New England as minor compared to MSX. Recent warming of the Gulf of Maine (GoMOOS, 2010), 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, as well as information obtained via surveys of oyster harvesters, both abundance and harvest of oysters declined from 1995 through 2005. It is highly likely that the presence of MSX and Dermo contributed significantly to these declines in the Great Bay oyster stock. Recent spatfalls (2006 to present), however, have been promising, with spat abundance at levels greater than those of the late 1990s through the mid 2000s. This provides some optimism for the recovery of the stock. It is imperative to maintain surveillance of these disease conditions, given that the presence (and 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 2010, oysters were collected from six locations (Figure 1): the Piscataqua, Oyster, and Squamscott Rivers, as well as Woodman Point, Adams Point, and Nannie Island.

The oysters sampled varied in size, generally ranging from 60mm to 80mm shell height. Site samples consisted of twenty individuals for all sites except the Piscataqua River, where seventeen individuals were sampled. 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.

The MSX results show a widespread distribution of infection throughout the Great Bay system during the sixteen 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 2008, but a consistent pattern cannot be inferred. At all locations in 2009, there was a general increase in both the total numbers infected and the numbers of more advanced, and potentially lethal, systemic infections. This uptick in MSX infection frequency was seen following a seven-year period (2002 through 2008) of relatively reduced infections. Systemic infections are those in which MSX is present in tissue other than the epithelial cells of the gills and palps of the oyster. 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 2010 MSX testing results show that all sites contained infected oysters. Three of the six sample sites show no systemic infections. It is very interesting to see that the two sites that had the highest incidence of systemic infections in 2009 (the Oyster River at 35%; Nannie Island at 25%) had no systemic infections observed in 2010. Woodman Point (the other site free of systemic infection in 2010) had the same infection level of 15% in 2009. Adams Point and the Piscataqua River sites had systemic infections in 2010, with levels about the same as the previous year. The Squamscott River site is yet to have regular annual testing for protozoan pathogens; therefore, trends there cannot be inferred. It is important to note that widespread mortality of oysters in Great Bay was not observed in 2010.

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 2010. 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. These increases are not carried over to 2010. Levels of infection from 2010, in fact, have dropped from 2009, and are now similar to those from 2004 through 2008 (Table 1 and Figure 2).

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). 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 has 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 for 2010 show a continuation of high levels of Dermo infections at all sample sites. Unlike the variable results for locations and years recorded for MSX samples, those of Dermo are more spatially and temporally consistent. While the infection levels are high, without reported mortality amongst oysters in Great Bay during 2010, the Dermo infections, for now at least, should be considered subpatent.

The tissue examination of Great Bay oysters has produced interesting findings that are both incidental and unforeseen. 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). All sampled locations in 2010 show some presence of ciliates. These percentages of xenoma prevalence vary, with a high of 80% at Adams Point, while other sites showed prevalence of about 50% or slightly greater except for the Piscataqua River, which was 35%.

Because of the continuing low abundance of oysters in the sampled bed in the Piscataqua River (B. Smith memoranda, 2008 and 2010), the information regarding the prevalence of MSX and Dermo at this sample site is especially important. The levels of infection in the Piscataqua are not markedly different from those at other such sites; therefore, factors other than protozoan pathogens must be considered in the Piscataqua River beds to better understand this oyster decline.

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). These highest mortalities were found in the Piscataqua and Salmon Falls Rivers. 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 did not begin until 1996, although it 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 been received to support 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 2007 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 the time, it is not reasonable to conclude that protozoan pathogens were the cause of that drop in oyster abundance.

Oyster tests in 2010 show continued presence of MSX in Great Bay, with total infection prevalence at levels below all other years tested. The prevalence of advanced infections in 2010 is at levels less than the early test years (1997 through 2000) and 2009, but roughly the same as both 2004 and 2008. Dermo was either nonexistent or existed in only low prevalence for an eight-year period (1997 through 2002), except at the Salmon Falls River site. The marked increase in Dermo prevalence since 2004 is noteworthy. Also present, but of unknown pathogenicity, are ciliate produced xenomas in gill tissue. A sharp drop in oyster abundance in 2008 at the Piscataqua River cannot be attributed to MSX or Dermo infections.

Recommendations

- This testing program should continue with samples 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 ciliate 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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Smith, B. memo to Grout, D., November 25, 2010 (p.3) *Review of Oyster Data - 2010*

Table 1. MSX Test Results - 1995 - 2010

Date	Location	No. Tested	No. Infected [1]	% of No. Tested	No. Systemic Infection [1]	% of No. Tested
9/05/95 [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
4/12/96	Nannie Island	30	3	10	0	0
5/27/96	Adams Pt.	10	0	0	0	0
5/27/96	Nannie Island	10	0	0	0	0
3/17/97	Fox Pt.	30	5	16.6	1	3.3
9/08/97	Bellamy River	25	10	40	2	8
9/08/97	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
12/9/98	Adams Point	25	7	28	2	8
12/9/98	Nannie Island	25	11	44	2	8
12/9/98	Squamscott River	25	17	68	7	28
12/9/98	Piscataqua River	18	7	39	3	11
10/21/99	Nannie Island	20	7	35	6	30
11/4/00	Piscataqua River	20	6	30	3	15
11/4/00	Adams Point	20	7	35	5	25
11/4/00	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
11/4/01	Oyster River	20	5	25	4	20
11/4/01	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
11/6/05	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
11/1/06	Oyster River	20	8	40	1	5

Table 1. MSX Test Results - 1995 - 2010 (continued)

Date	Location	No. Tested	No. Infected [1]	% of No. Tested	No. Systemic Infection [1]	% of No. Tested
11/2/06	Woodman Point	20	6	30	1	5
11/7/06	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
12/7/06	Nannie Island	20	4	20	0	0
11/7/06	Nannie Island experimental reef	20	6	30	2	10
11/7/06	Adams Point experimental reef	20	4	20	1	5
1128/06	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
11/4/09	Oyster River	20	10	50	7	35
11/6/09	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
12/8/09	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

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 - 2010

Date	Location	No. Tested	No. Oysters in each infection category[1]						%
			0.5	1	2	3	4	5	Prevalence
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%
3/17/97	Fox Pt.	30	0	0	0	0	0	0	0%
8/14/97	Piscataqua River	25	2	2	0	0	1	0	20%
8/17/97	Adams Pt.	25	4	0	0	0	0	0	16%
8/14/97	Oyster River	25	1	0	0	0	0	0	4%
8/14/97	Nannie Island	25	1	0	0	0	0	0	4%
9/08/97	BellamyRiver	25	0	0	0	0	0	0	0%
9/08/97	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%
12/9/98	Adams Pt.	25	0	0	0	0	0	0	0%
12/9/98	Nannie Island	25	0	0	0	0	0	0	0%
12/9/98	Squamscott River	25	0	0	0	0	0	0	0%
12/9/98	Piscataqua River	18	0	0	0	0	0	0	0%
10/21/99	Nannie Island	20	0	0	0	0	0	0	0%
11/4/00	Piscataqua River	20	0	0	0	0	0	0	0%
11/4/00	Adams Pt.	20	0	0	0	0	0	0	0%
11/4/00	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%
11/4/01	Oyster River	20	0	0	0	0	0	0	0%
11/4/01	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%
11/6/05	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%
11/1/06	Oyster River	20	3	3	4	6	0	0	80%
11/2/06	Woodman Point	20	3	8	8	1	0	0	100%
11/7/06	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 - 2010 (continued)

Date	Location	No. Tested	No. Oysters in each infection category[1]						% Prevalence
			0.5	1	2	3	4	5	
12/7/06	Nannie Island	20	2	5	4	0	1	0	60%
11/7/06	Nannie experimental reef	20	2	7	6	3	0	0	90%
11/7/06	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%

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.

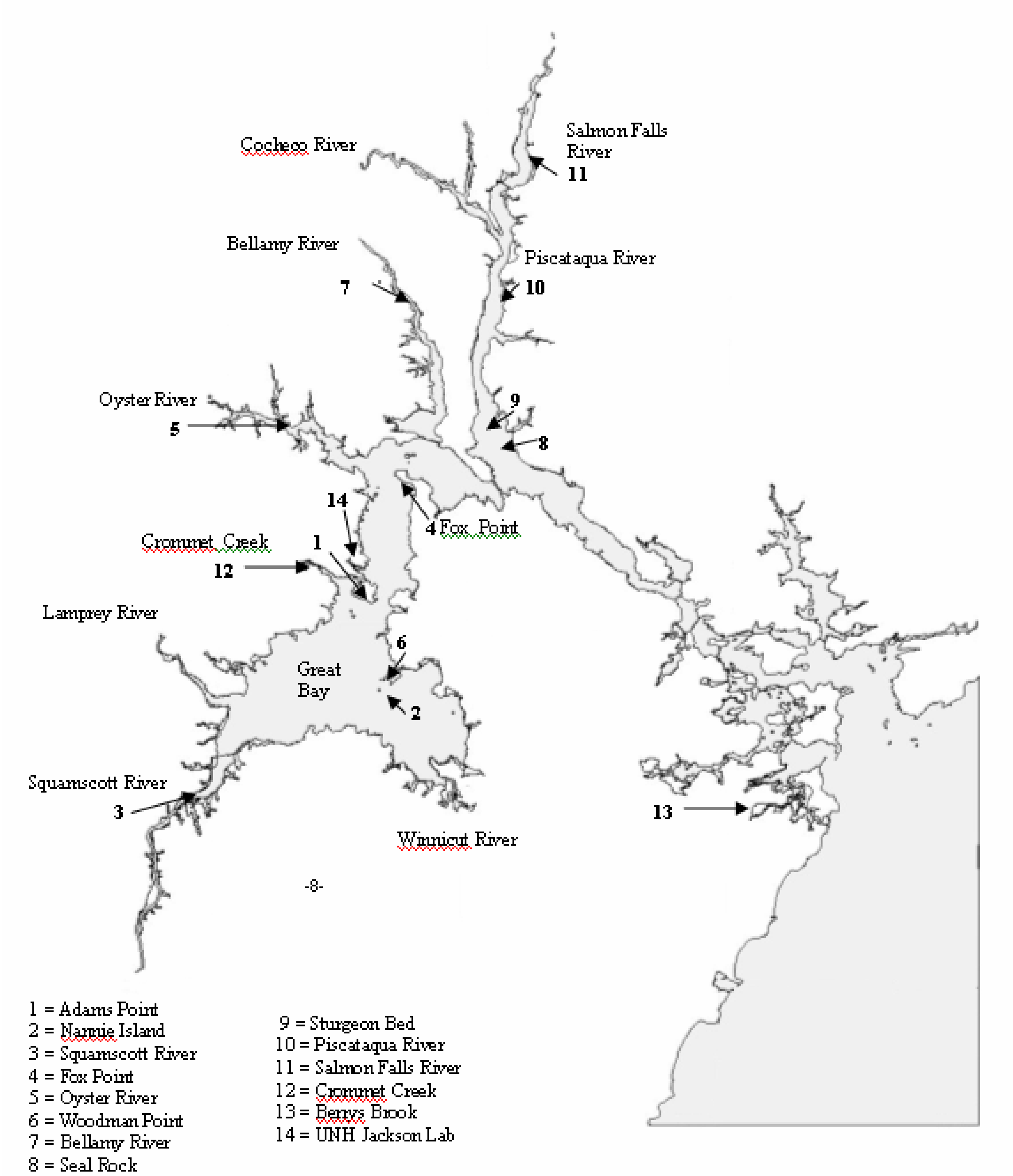

Figure 1. Study Area and Sample Locations

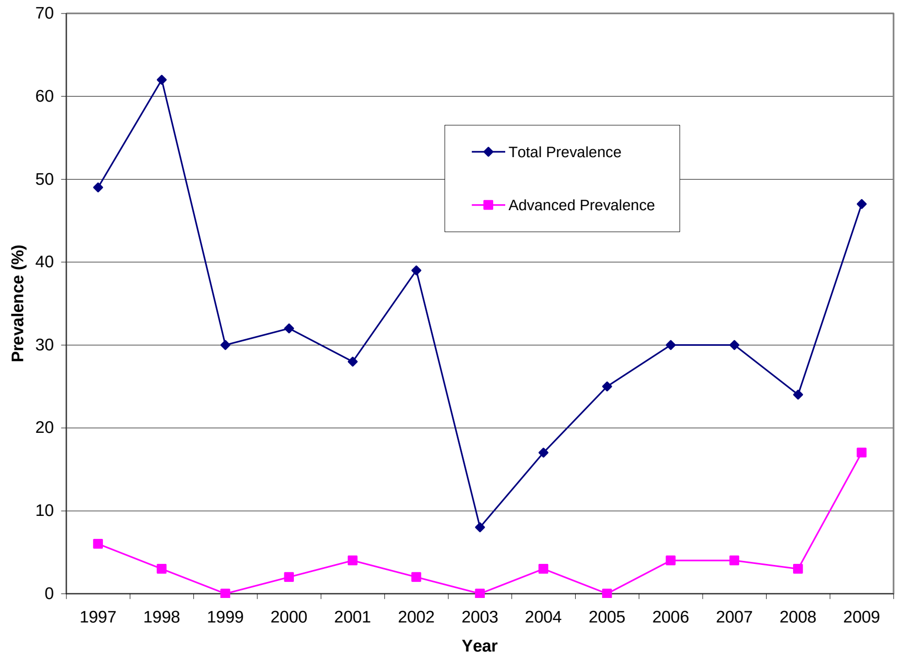

Figure 2. Combined Sites MSX Prevalence 1997 to 2010.

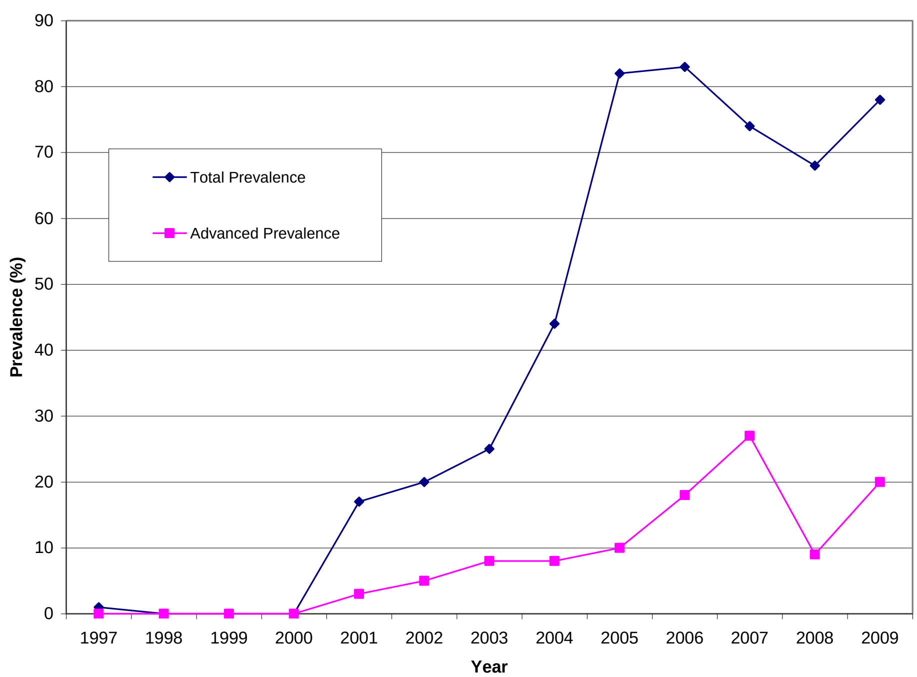

Figure 3. Combined Sites Dermo Prevalence 1997 to 2010.