1-15-2010

Testing of Great Bay Oysters for Two Protozoan Pathogens

Douglas E. Grout
New Hampshire Fish and Game Department

TESTING OF GREAT BAY OYSTERS FOR TWO PROTOZOAN PATHOGENS

A Final Report to

Piscataqua Region Estuaries Partnership

Submitted by

Douglas E. Grout
New Hampshire Fish and Game Department
Region 3, Marine Division
225 Main Street
Durham, NH 03824

Date of Report

January 15, 2010

This project was funded in part by a grant from the Piscataqua Region Estuaries Partnership as authorized by the U.S. Environmental Protection Agency's National Estuary Program.

Table of Contents

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 New Hampshire Fish and Game Department, (NHF&G) continues to assess the presence and intensity of both disease conditions in oysters from the major beds within the Great Bay estuarine system. Histological examination of Great Bay oysters has also revealed other endoparasites.

Introduction

The American oyster, *Crassostrea virginica*, may be invaded by a variety of parasites. Two particularly damaging protozoan parasites, *Haplosporidium nelsoni* (MSX) and *Perkinsus marinus* (Dermo), have caused widespread high mortalities along the Southern and Middle Atlantic Coast 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). It has since spread so that it now is reported from Florida to Maine. The presence of MSX in New England was first noted in 1960 from oysters taken at Milford, Connecticut (Sindermann and Rosenfield, 1967). In 1967, oysters from Wellfleet, Massachusetts were found to contain MSX ((Krantz et al, 1972). The presence of MSX in the Piscataqua River oysters was first documented in 1983 although unspeciated haplosporidian plasmodia were seen by Maine Department of Marine Resource scientists in 1979 (S. Sherburne, Maine Department of Marine Resources, per com.). Following this, MSX is not recorded again until 1994 when a Maine based aquaculture operation, Spinney Creek Shellfish, Inc., found Piscataqua River specimens contained MSX. Oysters from these same beds were examined a year later (1995) and again MSX was found, this time in higher prevalence than the previous year (Ken LaValley, Spinney Creek Shellfish, Inc., per com.).

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

Dermo has spread from South and Middle Atlantic sources up the coast and into the Gulf of Maine during the past three decades. North of Chesapeake Bay, cold waters are believed to act as a controlling factor that prevents year-round persistence of Dermo, probably making its virulence to oysters in New England waters minor compared to MSX. However, the recent warming of the Gulf of Maine may be responsible for increases in Dermo prevalence and now appears to be an increasing threat to Great Bay oysters. Dermo was first demonstrated to be present in the Great Bay system in 1996. Oysters from Spinney Creek, a small tidal pond off the Piscataqua River, were seen to harbor Dermo when examined by University of Maryland scientists. Following this, samples were taken from Great Bay and the Piscataqua River, and these showed Dermo-like particles also. Dermo tests from Great Bay system specimens will be reviewed in greater detail below.

Project Goals and Objectives

Based on the results of oyster monitoring by New Hampshire Fish and Game Department and from information gained by survey of oyster harvesters, the years 1995 to 2005 were a period of reduced oyster abundance and harvest decline. It is highly likely the presence of both MSX and Dermo contributed significantly to this decline in Great Bay oyster stock. However, recent spatfalls (2006 to present) have been good and this provides some optimism for recovery of the stock. It is important to maintain some surveillance of these disease conditions as the presence or absence of such potentially damaging pathogens may help explain future oyster abundance variability. The objective of this study is to monitor the presence of MSX and Dermo in Great Bay oysters**.**

Methods

In the fall of 2009, oysters were collected from five locations (Fig. 1): Piscataqua River, Woodman Point, Nannie Island, Oyster River, and Adams Point.

Oysters sampled were of variable sizes, generally ranging from 60 to 80mm shell height. Site samples consisted of 20 individuals for all sites. Collected oysters were cleaned of attached epifauna and shipped to Rutgers University, Haskin Shellfish Research Laboratory, for testing.

MSX determinations were accomplished by tissue section histology. They were processed using standard techniques and examined microscopically for pathological conditions or 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 present, are shown in Table 1. Dermo RFTM results for all years of testing are shown in Table 2.

The MSX results, over the fifteen years of testing, show a widespread distribution of infection throughout the Great Bay system. Levels of prevalence vary site to site and within sites over time. It appears, based on early test results, that the Piscataqua River area was most severely impacted by the 1995 epizootic (Barber et al 1997). Systemic infections in the upper reaches of the Piscataqua River and Salmon Falls River ranged from 25% to 50% compared to generally lower values in Great Bay proper (Table 1.). An exception to this general pattern is shown in the 1997 Nannie Island data that show relatively high values for both numbers infected and number of systemic infections.

The year 2009 tests show MSX to be present at all sites. When compared to the past decade-plus results these data show a generally increased level of both the overall prevalence of infection and the numbers with advanced infection. Advanced infections are those that show the

MSX to be present systemically in tissue other than the epithelial cells of the gills and palps of the oyster. It is important to recognize that MSX infection can be progressive therefore a spreading of the pathogens throughout an individual is possible with time.

To track the overall estuary presence of MSX for the period of 1997 to 2009, a combined sites prevalence graphic (Figure 2) has been developed. From this, one can see an initial high spike of total prevalence in the early years of monitoring (1997/1998) followed by a reduced total prevalence. Now, with the addition of the 2009 results, the combined sites MSX prevalence jumps upward markedly. The latest total prevalence value is surpassed only by the earliest (1997 and 1998) years. More troubling is the sharp rise in systemic (advanced) infections which, at 17%, are the highest ever recorded. While the rise in MSX prevalence is of concern, it is important to note that no widescale mortality of Great Bay oysters was observed in 2009.

Early Dermo results show the presence of Perkinsus-like particles at all locations sampled except for Seal Rock, Fox Point and Bellamy River. All except the Sturgeon Bed and Piscataqua River sites were light infections that appeared to show low frequency within the sample lot (i.e., prevalence). Over the past decade Dermo prevalence has generally increased except for a drop in 2008. Dermo results for 2009 show a rise in the presence of this pathogen at all sampled sites with advanced infections at all sites except Woodman Point and Piscataqua River (Table 2). However, without reported mortality among oysters in Great Bay during 2009, these infections should be considered subpatent.

The tissue examination of Great Bay oysters has produced interesting incidental findings. 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 earler tissue samples for Great Bay shows that they have been seen since the examinations in the late 1990s, but their numbers have increased since 2000 (Scarpa, et al, 2006). All sampled locations in 2009 show some presence of ciliates. For the year 2009, percentages of xenoma prevalence varies with a high of 75% at Nannie Island. Other sites showed prevalences over 50% except for the Piscataqua River which was 25%.

The 2009 analysis of Great Bay system protozoan disease pathogens is especially important because of an apparent recent die-off of oysters in the Piscataqua River. Annual monitoring of oyster abundance has been accomplished since 1991 (NHF&G memorandum, B. Smith to D. Grout, Nov. 12, 2008) and the results of the October 2008 sampling showed a dramatic Piscataqua River drop in density of all size classes. With this sort of decline, oyster disease assessment is very important. This is especially true due to the earliest (mid-90's) outbreak of disease that was first noted there and resulted in the initiation of an ongoing program of oyster disease monitoring.

The 2008 oyster disease report (New Hampshire Fish and Game Department, 2009) discusses the possibility of MSX and/or Dermo as a contributing cause of the oyster density decline in the Piscataqua. The conclusion reached, based on comparative review of Piscataqua disease prevalence with other sampled sites, did not support the idea of sporozoan pathogens as causative agents for the drop in oyster abundance there.

Now, with 2009 data, another comparative assessment for the Piscataqua site is possible. It is important to note that the exact location of sampling, 2008 to 2009, differed slightly. The 2009 sample site was about one quarter mile down river from that of 2008. The Piscataqua 2009 MSX levels are among the lowest of all sites tested and no advanced infections are seen there. Dermo levels are also low in comparison to the other four tested sites. Only the Piscataqua and Nannie sites were free of advanced infections. Based on this and previous data it seems unlikely pathogenic protozoans can be considered to be the cause of low oyster densities here.

The comparison of MSX, Dermo and xenomas prevalence levels between the Piscataqua River and the other five sampled sites does not provide a reasonable answer that explains the sharp drop in oyster abundance at that site. Other environmental factors must be further examined there to better understand this oyster decline.

Conclusions

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

During this same time, the Piscataqua and Salmon Falls River beds in Maine waters were the sites of similar oyster MSX mortality (Ken LaValley, Spinney Creek Shellfish, Inc., per. com.). The 1995 Great Bay Estuary MSX epizootic caused over 80% mortality in the areas most affected (Barber et al 1997). Highest mortalities were found in the Piscataqua and Salmon Falls Rivers. Other areas in the estuary did not, at this time, appear to be as heavily infected. It is important to note that no testing specific for Dermo was done immediately following the reported fall 1995 oyster mortality. Dermo testing began in 1996 and has been done annually since then.

In 1996 spring testing at the major New Hampshire recreational oystering beds, Nannie Island and Adams Point, showed no systemic infections of MSX. The 1996 season did not result in oyster mortalities of the type observed in the previous year. In recent years, monies from PREP have been received to support a more expansive testing program for both MSX and Dermo.

Based on tests performed annually since 1995, there are two protozoan parasites (ie, MSX and Dermo) now widely distributed within the Great Bay oyster stock. Severity of infection and prevalence vary from site to site and over time at a specific site. We also know 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. Despite the presence of these protozoan parasites, there has been no observable large scale mortality of oysters from the 1995 event to 2007. In 2008, however, a sharp decline in oyster abundance at one site (Piscataqua River) was noted. Because the prevalence of MSX and Dermo at Piscataqua River is not clearly greater than other sites, it is not reasonable to claim these protozoan pathogens are the cause of the oyster abundance drop.

Oyster tests in 2009 show continued presence of MSX in Great Bay with total infection prevalence showing an increase to levels exceeded only by the years 1997 and 1998. The prevalence of advanced infections in 2009 is the highest ever seen over the entire test time series. Dermo was seen for the eighth successive year after a period, 1997 to 2002, when it was found in oysters only at very low prevalence at one 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 effect of ciliate xenomas should be further studied.

Acknowledgment

Testing of Great Bay system oysters is a team effort. Others involved besides NHF&G, include UNH, Jackson Estuarine laboratory personnel, Piscataqua Region Estuaries Partnership and Rutgers-Haskin Shellfish Research Laboratory. This report has been prepared by the New Hampshire Fish and Game Department and we assume all responsibility for its accuracy. To all others on the team we extend our gratitude for their cooperation**.**

References

Barber, B. J., R. Langan and T.C. Howell, 1997 *Haplosporidium nelsoni (MSX) Epizootic in the Piscataqua River Estuary*. Jour. Parasitol. Vol 83 No. 1, Feb. 1997.

Haskin, H.H. and J. D. Andrews, 1988 *Uncertainties and speculations about the life cycle of the eastern oyster pathogen Haplosporidium nelsoni (MSX) In: W. S. Fisher (ed) Disease Processes in Marine Bivalve Mollusca*, American Fisheries Society, Bethesda, MD. pp. 5-22.

Krantz, G. E., L. R. Buchanan, C. A. Farley, and H.A. Carr, 1972 *Minchinia Nelsoni in Oysters from Massachusetts Waters*, Proceedings of the National Shell Fisheries Association. Vol. 62, June 1972.

New Hampshire Fish and Game Department, 2009, *Testing of Great Bay Oysters for Two Protozoan Pathogens,* Piscataqua Region Estuarine Partnership Report.

Scarpa E, S. Ford, B. Smith and D. Bushek *An Investigation of Ciliate Xenomas in Crassostrea Virginica,* Proceedings National Shellfish Assoc. 2006

Sindermann, C.J. and A Rosenfield, 1967. *Principal Diseases of Commercially Important Marine Bivalve Mollusca and Crustacea*, U. S. Fish and Wildlife Service. Fish Bulletin 66:335-385

Smith, B. memo to Grout, D., November 11, 2008 (p.3) *Review of Oyster Data - 2008*

Table 1. MSX Test Results - 1995 - 2009

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 - 2009 (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

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 - 2009

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%
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 - 2009 (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%

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.

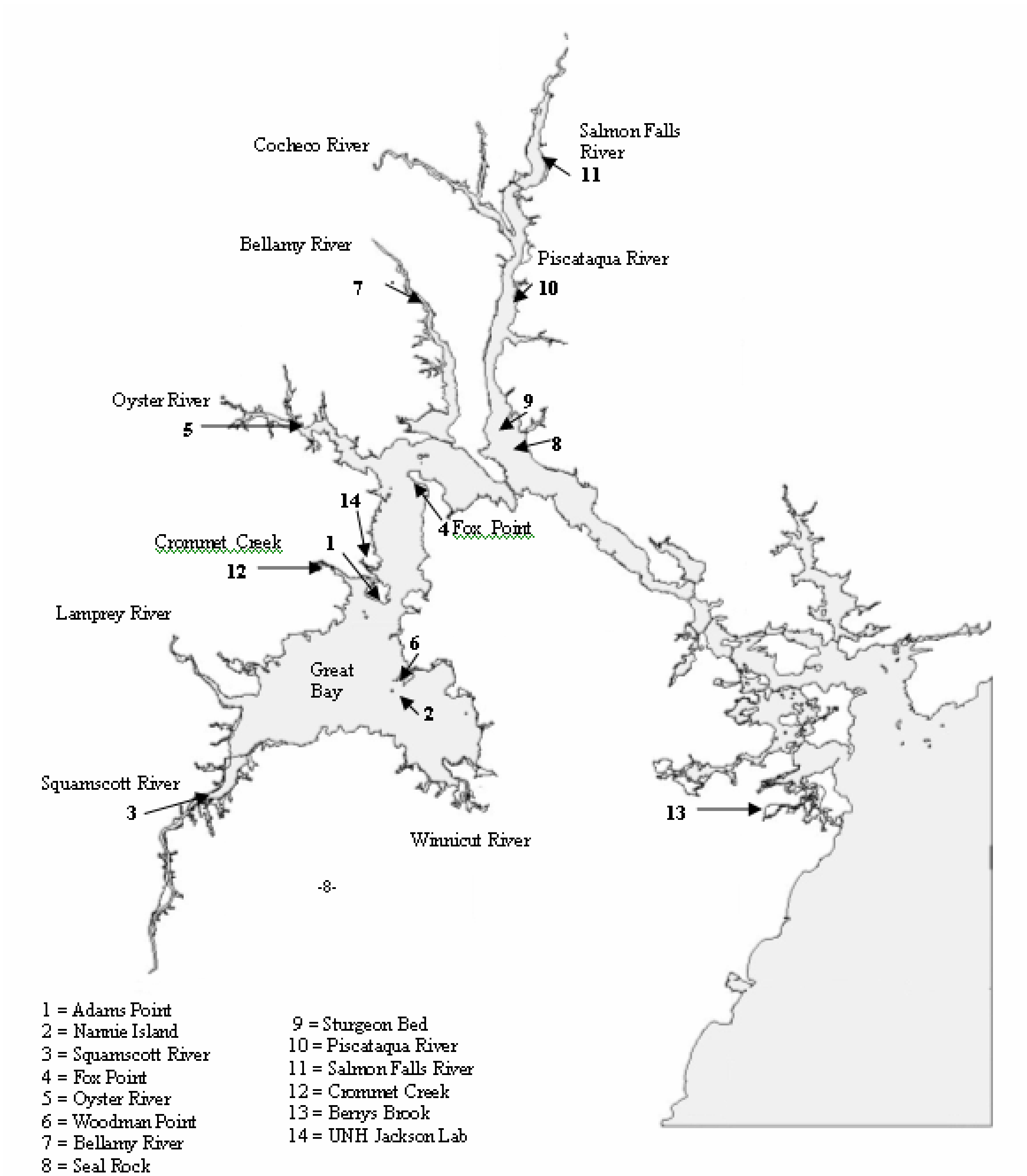

Figure 1. Study Area and Sample Locations

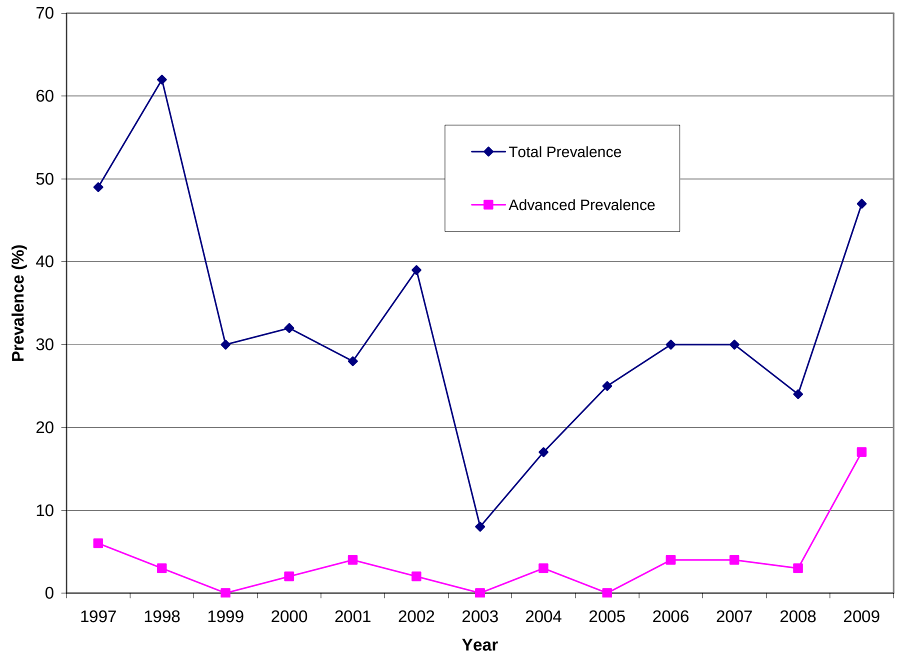

Figure 2. Combined Sites MSX Prevalence 1997 to 2009.

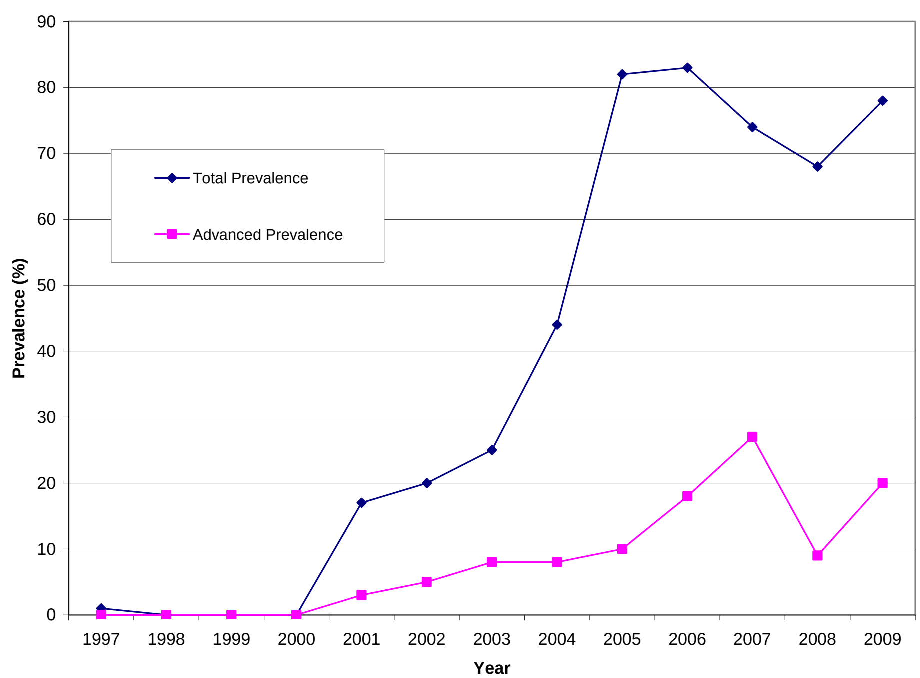

Figure 3. Combined Sites Dermo Prevalence 1997 to 2009.