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TESTING OF GREAT BAY OYSTERS FOR TWO PROTOZOAN PATHOGENS

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 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 unspiciated 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 possibly it is 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. Dermo tests from Great Bay system specimens will be reviewed in greater detail below.

Project Goals and Objectives

It appears, based on recent oyster population monitoring and from the information gained by survey of oyster harvesters, that the last decade and a half has been a period of reduced oyster abundance and harvest decline. It is highly likely the presence of both MSX and Dermo has contributed significantly to recent

declines in the Great Bay oyster 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 2008, oysters were collected from six locations (Fig. 1):

Piscataqua River, Woodman Point, Nannie Island, Oyster River, Adams Point and Squamscott River.

Oysters sampled were of variable sizes, generally ranging from 40 to 100mm shell height. Site samples consisted of 20 individuals for all sites except the Piscataqua and Squamscott Rivers, which were 10 individual samples. 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 fourteen 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 2008 tests showed MSX to be present at all sites. Prevalence varied by site, but few advanced infections were noted. 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 a 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 2008, 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. Over the last decade, except for one year (2002), the percentage of total prevalence has been below 33%. Advanced prevalence for all sampled oysters has always been below 8%.

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 few years, DERMO appears to be increasing in prevalence (Figure 3). Dermo results for 2008 show the continued presence of this

pathogen at all sampled sites with advanced infections at all sites except Piscataqua River (Table 2).

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 earlier 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 show some presence of ciliates. Xenomas, produced as a result of ciliate presence, were seen at all sites except the Squamscott River. For the year 2008, percentages of xenoma prevalence varies with a high of 60% at Piscataqua River. Low prevalence of 25% was seen at Oyster River and Adams Point.

The 2008 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 over the past sixteen years (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 is quickly considered as a possible cause. 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.

A comparison of MSX infection prevalence for the Piscataqua River and all other sites does not reveal any alarming difference either for total infected or systemic infection (Table 1). A similar comparison for the Dermo results also yield nothing to suggest the Piscataqua River oysters are notably more infected than other sites. Only the xenoma comparison shows the Piscataqua River to have the highest prevalence of all sites and this only marginally. In fact, the levels of infection at two other sites, Squamscott River and Woodman Point, closely approach the 60% prevalence found at the Piscataqua.

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 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.

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, 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 2008 show continued presence of MSX in Great Bay with infection prevalence levels similar to those recorded over the past eight years. Dermo was seen for the seventh successive year after a period 1997 to 2002 when it was found in oysters only at very low prevalence. 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, the Nature Conservancy, the 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. A special thank you is due to Executive Secretary Ralph Johnston, New Hampshire Fish and Game, Marine Fisheries Division, who has typed and prepared the graphics on this report for the past ten years. To all others on the team we extend our gratitude for their cooperation.

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Table 1. MSX Test Results

<u>Date</u>	<u>Location</u>	<u>No. Tested</u>	<u>No. Infected</u> ¹⁾	<u>No. Systemic Infection</u> ¹⁾
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
5/27/96	Adams Pt.	10	0	0
5/27/96	Nannie Island	10	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
10/20/02	Salmon Falls - disease resistant	20	2 (10%)	0
10/20/02	Salmon Falls - natives	18	5 (28%)	0
10/31/02	Nannie Island	24	9 (37%)	4 (17%)
10/28/03	Nannie Island	26	2(7.7%)	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
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%)
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
11/28/06	Berrys Brook	16	6(38%)	0
12/7/06	Nannie Island	20	4(20%)	0
		6		
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
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
10/27/08	Squamscott River	10	3(30%)	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

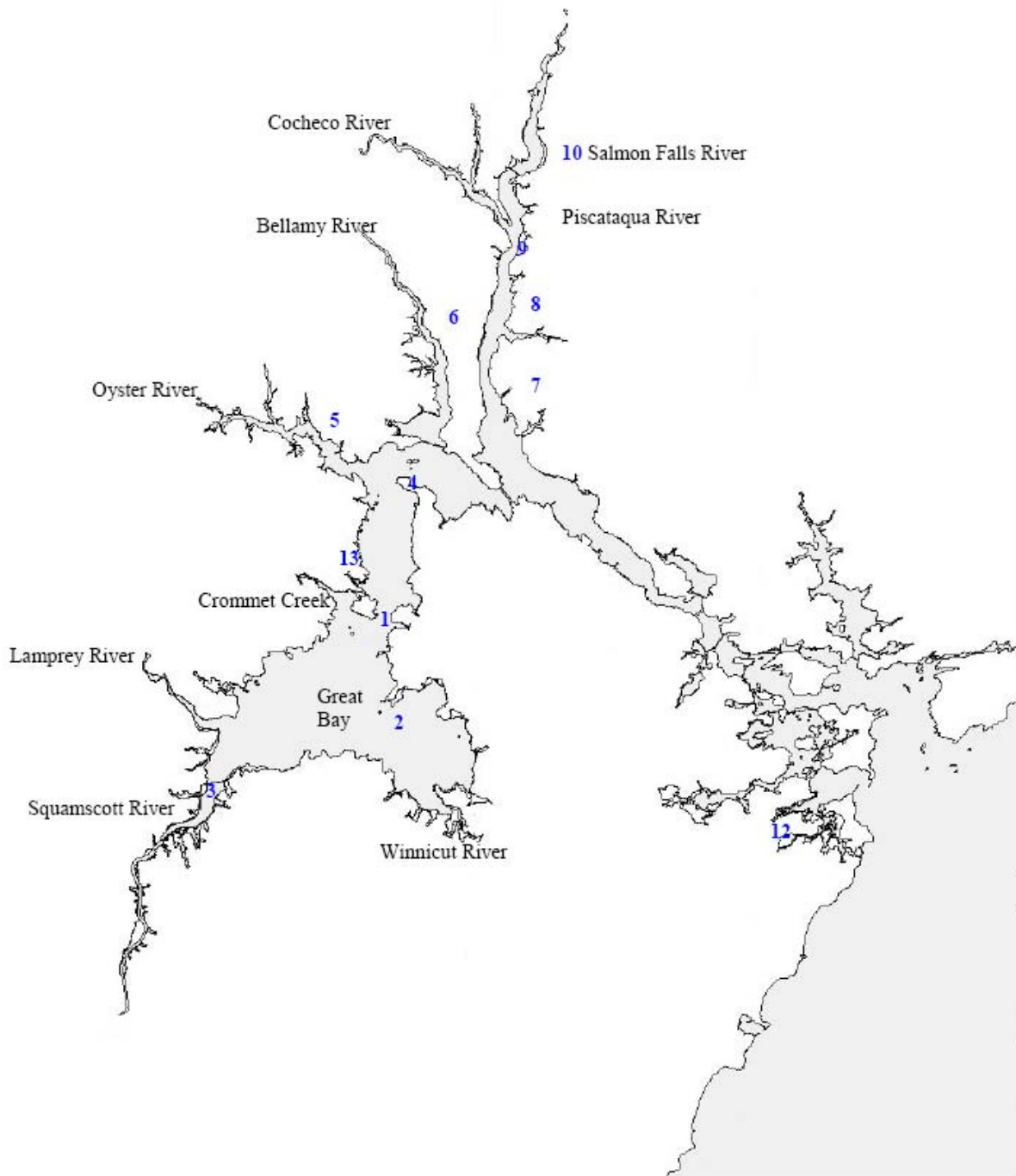
Date	Location	No. Tested	<u>No. Oysters in each infection category¹⁾</u>					Prevalence
			0.5	1	2	3	4	

12/16/96	Nannie Island	25	1							4%
12/16/96	Seal Rock	25	0	0	0	0	0	0	0	0
12/16/96	Sturgeon Bed	25	2					1		12%
3/17/97	Fox Pt.	30	0	0	0	0	0	0	0	0
8/14/97	Piscataqua River	25	2	2				1		20%
8/17/97	Adams Pt.	25	4							16%
8/14/97	Oyster River	25	1							4%
8/14/97	Nannie Island	25	1							4%
9/08/97	Bellamy River	25	0	0	0	0	0	0	0	0
9/08/97	Squamscott River	25	1							4%
11/17/97	Adams Pt.	25	1							4%
11/17/97	Nannie Island	25	0	0	0	0	0	0	0	0
11/17/97	Oyster River	25	0	0	0	0	0	0	0	0
11/17/97	Piscataqua River	25	0	0	0	0	0	0	0	0
12/9/98	Adams Pt.	25	0	0	0	0	0	0	0	0
12/9/98	Nannie Island	25	0	0	0	0	0	0	0	0
12/9/98	Squamscott River	25	0	0	0	0	0	0	0	0
12/9/98	Piscataqua River	18	0	0	0	0	0	0	0	0
10/21/99	Nannie Island	20	0	0	0	0	0	0	0	0
11/4/00	Piscataqua River	20	0	0	0	0	0	0	0	0
11/4/00	Adams Pt.	20	0	0	0	0	0	0	0	0
11/4/00	Nannie Island	20	0	0	0	0	0	0	0	0
11/15/00	Oyster River	20	0	0	0	0	0	0	0	0
10/10/01	Nannie Island	25	0	0	0	0	0	0	0	0
10/18/01	Salmon Falls (disease resistant)	25	3	0	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	0
11/4/01	Adams Point	20	0	0	0	0	0	0	0	0
10/14/02	Adams Point	20	1	2	0	0	0	0	0	15%
10/14/02	Oyster River	20	0	0	0	0	0	0	0	0
10/31/02	Nannie Island	24	2	0	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	0	5%
10/28/03	Nannie Island	25	2	1	0	2	0	0	0	20%
10/27/04	Oyster River	25	2	0	2	0	0	0	0	16%
11/18/04	Nannie Island	17	5	2	2	1	0	0	0	65%
11/19/04	Adams Point	20	3	4	2	4	0	0	0	65%
11/19/04	Crommet Creek	23	0	1	0	1	0	0	0	8%
11/6/05	Oyster River	20	3	3	5	0	2	0	0	65%
11/14/05	Adams Point	20	6	7	3	1	1	0	0	90%
11/16/05	Woodman Point	20	4	4	8	2	0	0	0	90%
11/17/05	Squamscott River	20	0	1	0	0	0	0	0	5%
10/31/06	Piscataqua River	20	0	9	2	3	1	0	0	75%
11/1/06	Oyster River	20	3	3	4	6	0	0	0	80%
11/2/06	Woodman Point	20	3	8	8	1	0	0	0	100%
11/7/06	Squamscott River	39	3	1	1	0	0	0	0	13%
11/22/06	Adams Point	20	2	8	4	5	1	0	0	100%
11/28/06	Berrys Brook	16	0	0	0	0	0	0	0	0
12/7/06	Nannie Island	20	2	5	4	0	1	0	0	60%
11/7/06	Nannie experimental reef	20	2	7	6	3	0	0	0	90%
11/7/06	Adams experimental reef	20	3	6	7	3	0	0	0	95%
11/28/06	UNH - Jackson (spat)	20	0	0	0	0	0	0	0	0
			8							
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	Nannie Island	20	1	5	0	1	0	1	40%
10/10/08	Oyster River	20	6	7	1	2	1	0	85%
10/22/08	Piscataqua River	20	1	1	1	0	0	0	30%
10/23/08	Squamscott River	10	1	1	2	0	1	0	50%
10/27/08	Woodman Point	10	3	5	4	3	2	2	95%

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



- | | |
|----------------------|-------------------------|
| 1 = Adams Point | 8 = Sturgeon Bed |
| 2 = Nannie Island | 9 = Piscataqua River |
| 3 = Squamscott River | 10 = Salmon Falls River |
| 4 = Fox Point | 11 = Crommet Creek |
| 5 = Oyster River | 12 = Berrys Brook |
| 6 = Bellamy River | 13 = UNH Jackson Lab |
| 7 = Seal Rock | |

Figure 2 Combined Sites MSX Prevalence 1997 to 2008



Figure 3 Combined Sites DERMO Prevalence 1997 to 2008

