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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

The New Hampshire Estuaries Project

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 New Hampshire Estuaries Project (NHEP), 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, some open for harvest, 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 to the degree 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 established 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 Haskins 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 abundance monitoring and from the information gleaned 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 2007, oysters were collected from five locations (Fig. 1):

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

Native oysters sampled were a combination of sizes: adults are those 60mm shell height and greater and those smaller are termed spat and represent the 2006 year class. Site samples consisted of 20 individuals per location. Collected oysters were cleaned of attached epifauna and shipped to Rutgers University, Haskins 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 the past twelve years of testing are shown in Table 2.

The MSX results, over the thirteen 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 2007 tests were analyzed different from those of earlier years. To look at the possibility of disease resistance in the large 2006 year class, gained by natural selection, samples were sorted into two groups; adults (those 60mm shell height {SH}and greater) and spat (those less than 60mm SH). The term "spat" is hereafter applied to the oysters in this year's study that are presumably the 2006 year class.

In fact, these would be more properly termed juveniles. The results of the adult/spat comparison of MSX total prevalence is shown below:

Location	Adult (a)	(b)	Spat (a)	(b)
Piscataqua River	N-18	- 38.9%	N-2	- 0%
Oyster River	N-15	- 33.3%	N-5	- 40%
Woodman Point	N-10	- 30%	N-10	- 20%
Nannie Island	N-13	- 23.1%	N-7	- 28.6%
Adams Point	N-14	- 21.4%	N-6	- 33.3%

(a) = number tested (b) = percent prevalence of MSX

Grouping all samples shows the total spat infection prevalence at 26% and that for adults at 30%.

From this, one would conclude there is no demonstrable difference between the presence of MSX for the two size classes.

Figure 2 compares the 2007 total prevalence and advanced infection prevalence with previous years. This shows that MSX, after the initially high epizootic of the mid-1990s, has remained present at all sample locations at prevalence levels similar to previous years. Though advanced infections are uncommon among total infected oysters, the progression from light to heavy infections are likely if the host is stressed by adverse environmental conditions (e.g., temperature or salinity extremes).

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 2007 show the continued presence of this pathogen at all sampled sites with very high prevalence at Oyster River, Piscataqua River and Woodman Point.

The same type of adult/spat infection prevalence comparison done for MSX was applied to Dermo. The results are shown below:

Location	Adult (a)	(b)	Spat (a)	(b)
Piscataqua River	N-18	- 88.9%	N-2	- 100%
Oyster River	N-15	- 100%	N-5	- 100%
Woodman Point	N-10	- 90%	N-10	- 90%
Nannie Island	N-13	- 38.5%	N-7	- 28.6%
Adams Point	N-14	- 71.4%	N-6	- 16.7%

(a) = number tested (b) = percent prevalence of Dermo

Grouping all samples shows the total spat infection prevalence at 63% and that for adults at 78%. As with the similar adult/spat MSX comparison, there is no reason to believe the recently produced 2006 year class has any Dermo resistance to infection.

The tissue examination of Great Bay oysters has produced one very interesting incidental finding. 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 show some presence of ciliates. For the year 2007, percentages of xenoma prevalence varies from a high of 100% to less than 46% for Oyster River and Nannies Island, respectively.

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 oyster grounds as a cause of 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 NHEP have been received to support a more expansive testing program for both MSX and Dermo.

Based on tests performed annually since 1995, we find two protozoan parasites (ie, MSX and Dermo) are 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 since the 1995 event.

Oyster tests in 2007 show continued presence of MSX in Great Bay with infection prevalence levels similar to those recorded over the past seven years. Dermo was seen for the sixth successive

year after a near six year absence in oysters. The marked increase in Dermo prevalence since 2004 is noteworthy. Also present but of unknown pathogenicity are ciliate produced xenomas in gill tissue.

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, 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 New Hampshire Estuaries Project 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**.**

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Table 1. MSX Test Results

Date	Location	No. Tested	No. Infected [1]	No. Systemic Infection [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

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%)**

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

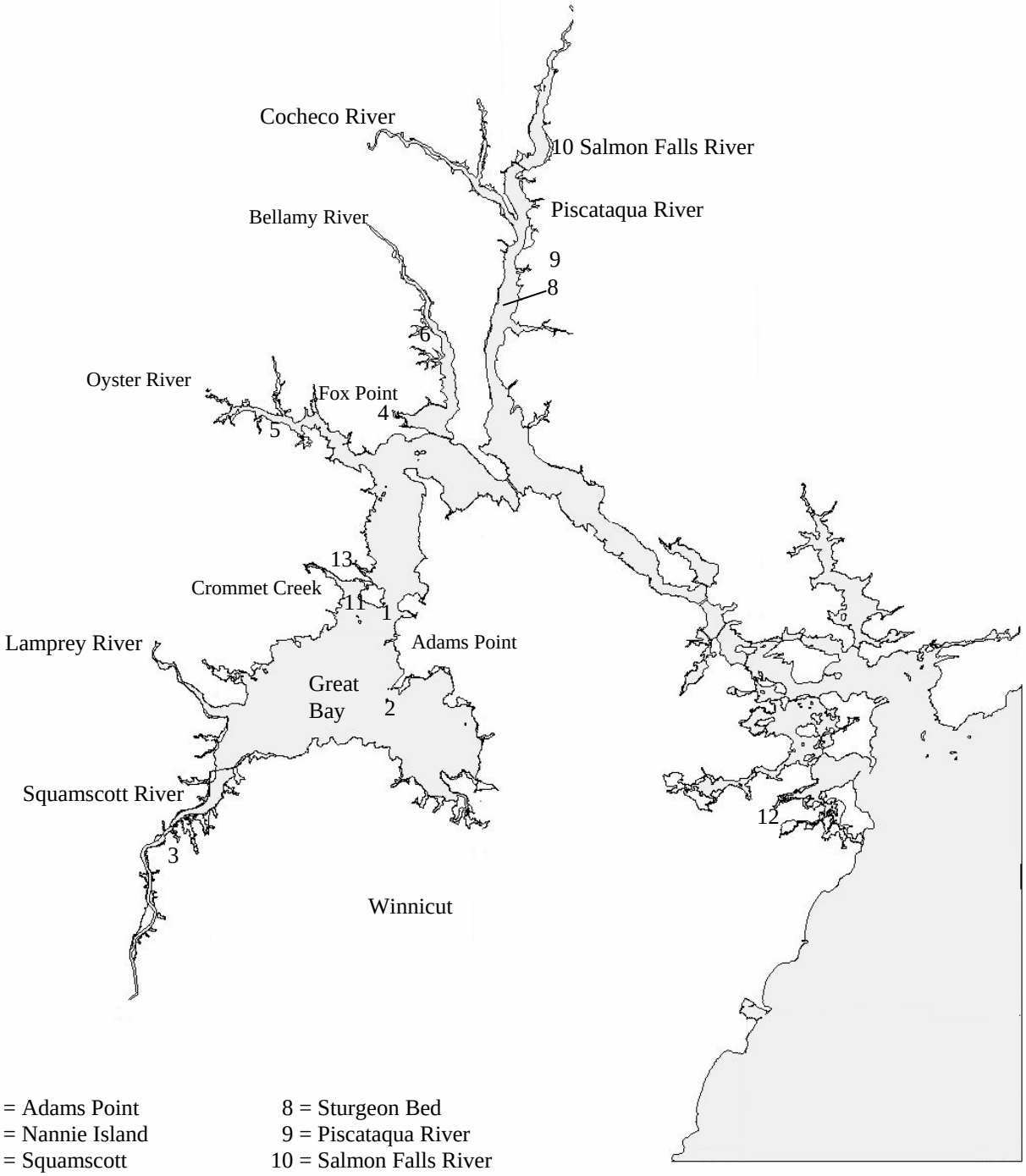
Date	Location	No. Tested	No. Oysters in each infection category[1)] 0.5	1	2	3	4	5	Prevalence
12/16/96	**Nannie Island**	**25**	**1**						**4%**
12/16/96	**Seal Rock**	**25**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
12/16/96	**Sturgeon Bed**	**25**	**2**				**1**		**12%**
3/17/97	**Fox Pt.**	**30**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
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	**BellamyRiver**	**25**	**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**
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**
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%**

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. Oyster Sampling Stations



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