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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

Submitted by

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Date of Report

March 2020

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.



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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 (NH) 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 unspiciated 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. MSX test results for natural oyster beds in NH from 1995 to 2019 are shown in Table 1.

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, 2017), 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. Dermo test results for natural oyster beds in NH from 1996 to 2019 are shown in Table 2.

## **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 2008 and 2014 to 2017), 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 2019, oysters were collected and tested from five natural beds (Figure 1): Squamscott River, Oyster River, Adams Point, Woodman Point, and Piscataqua River. No live adult oysters were found in the Nannie Island sample for use in testing in 2019.

Oysters sampled varied in size, generally ranging from about 74mm to 98mm shell height. Site samples consisted of 10 individuals per site. 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.

Aquacultured oysters were also sampled from four farms located in NH tidal waters (Table 3). Thirty oysters, generally ranging in size from 67mm to 108mm, were donated each from four farms and tested by the Haskin Shellfish Research Laboratory in the same manner as those from the natural oyster beds.

## **Results and Discussion**

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-five 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 present, but a consistent pattern cannot be inferred.

In 2019, the Oyster River, Piscataqua River, and Squamscott River sites showed MSX presence. No oysters showed evidence of the more advanced systemic infection. Samples from the other two locations tested were free of MSX.

A graph of MSX prevalence, both total and advanced, from all sites (Figure 2) has been developed to track the overall presence of MSX in the Great Bay estuary for the period of 1995 through 2019. The first year of monitoring showed an initial high percentage of total prevalence in Great Bay, which was quickly followed by a general downward trend of total prevalence (1998 through 2008). In 2009, the overall percentage of both MSX prevalence and systemic infection increased markedly (NHFG, 2009). The overall percentage of MSX prevalence decreased dramatically in 2010 then gradually increased to another spike in 2014. After 2014, the overall prevalence decreased to its lowest percentages since sampling began. The percentage of advanced prevalence of MSX in the Great Bay oysters has trended downward since the peak in 2009 to their lowest rates in the time series with the exception of a moderate spike in 2013. Results from 2019 indicate a continued decline in MSX presence with two of the five sites sampled being free of the pathogen (Table 1).

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 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 1996 through 2002 (except for the Salmon Falls River). A general increasing trend in both total and advanced prevalence of Dermo started in 2004 and peaked in 2014 (Figure 3). Although some years show a remission, the infections are re-occurring in subsequent years on the natural beds. The 2019 test results show an increase in Dermo total prevalence and advanced prevalence approaching the levels seen in 2014.

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 2019, (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 in natural oyster beds (Figure 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 showed a downward trend in advanced stage presence of Dermo, though the total prevalence remained high. It is progressive and nearly always fatal, and the results from 2019 exhibited its progressive nature with an increase in advanced stages in Great Bay oysters. 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. 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 2019, ciliate prevalence was highest at the Oyster River and Piscataqua River sites, both at 50% of oysters sampled. Xenomas were seen at all sites except the Squamscott River.

With funds from the US Department of Agriculture, additional oysters were tested in 2019 from aquaculture farms located in Hampton Harbor, Upper Little Bay, and Lower Little Bay. All aquacultured oysters were free of both the MSX and Dermo pathogens, and between 27% and 37% of the oysters tested showed the presence of gill ciliates.

## **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; annual testing began in 1996.

In the spring of 1996, testing at the major recreational oyster 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, funds 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 for MSX and 1996 for Dermo, the presence of these two protozoan parasites are widely distributed within the Great Bay oyster

stock. 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, however little is known of the pathogenicity of this condition. Despite the presence of these protozoan parasites, no large-scale mortality of oysters since the 1995 epizootic has been observed. In 2008, however, a sharp decline in oyster abundance at one site (Piscataqua River) was noted. Because the prevalence of MSX and Dermo at this site was not greater than other sites at that time, it is not reasonable to conclude that protozoan pathogens were the cause of that decrease in oyster abundance. Observations by NHFG that year reported an oily film over the oysters that occurred following dredging of the lower Cocheco River.

Oyster tests in 2019 show continued presence of MSX in Great Bay with total infection prevalence at levels down from the previous five years and among the lowest of the time series. There was no indication of advanced infections in 2019.

Dermo was either nonexistent or found in only low prevalence for an eight-year period (1996 through 2003), except at the Salmon Falls River site in 2001 and 2002 when a total prevalence of 60% and 50%, respectively, was recorded. The marked increase in Dermo since 2004 is noteworthy. Total levels, as well as advanced prevalence, increased in 2019, approaching those seen in 2014.

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 Squamscott River site.

While both MSX and Dermo have been seen throughout the Great Bay oyster beds, and previously 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 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, US Department of Agriculture, 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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**Table 1. MSX test results for natural oyster beds in NH, 1995 – 2019.**

| <b>Date</b>           | <b>Location</b>                  | <b>No. Tested</b> | <b>No. Infected <sup>1</sup></b> | <b>% of No. Tested</b> | <b>No. Systemic Infection <sup>1</sup></b> | <b>% of No. Tested</b> |
|-----------------------|----------------------------------|-------------------|----------------------------------|------------------------|--|------------------------|
| 09/05/95 <sup>2</sup> | Piscataqua River (Summer Bed)    | 25                | 18                               | 72                     | 10   | 40                     |
| 10/27/95 <sup>2</sup> | Salmon Falls                     | 16                | 13                               | 81                     | 8  | 50                     |
| 10/27/95 <sup>2</sup> | Piscataqua River (Summer Bed)    | 20                | 14                               | 70                     | 5  | 25                     |
| 10/27/95 <sup>2</sup> | Sturgeon Bed                     | 20                | 13                               | 65                     | 8  | 40                     |
| 10/27/95 <sup>2</sup> | 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                                | 17                     | 1  | 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                                | 8                      | 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. (continued)**

| <b>Date</b> | <b>Location</b>                 | <b>No. Tested</b> | <b>No. Infected <sup>1</sup></b> | <b>% of No. Tested</b> | <b>No. Systemic Infection <sup>1</sup></b> | <b>% of No. Tested</b> |
|-------------|---------------------------------|-------------------|----------------------------------|------------------------|--|------------------------|
| 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    | Berry 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. (continued)**

| <b>Date</b> | <b>Location</b>  | <b>No. Tested</b> | <b>No. Infected <sup>1</sup></b> | <b>% of No. Tested</b> | <b>No. Systemic Infection <sup>1</sup></b> | <b>% of No. Tested</b> |
|-------------|------------------|-------------------|----------------------------------|------------------------|--|------------------------|
| 10/16/14    | Adams Point      | 10                | 5                                | 50                     | 0  | 0                      |
| 10/17/14    | Nannie Island    | 9                 | 3                                | 33                     | 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                                | 17                     | 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  | 13                     |
| 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                                | 13                     | 0  | 0                      |
| 10/07/16    | Adams Point      | 8                 | 0                                | 0                      | 0  | 0                      |
| 10/07/16    | Nannie Island    | 8                 | 1                                | 13                     | 0  | 0                      |
| 10/07/16    | Oyster River     | 8                 | 0                                | 0                      | 0  | 0                      |
| 11/01/16    | Piscataqua River | 8                 | 0                                | 0                      | 0  | 0                      |
| 10/17/17    | Adams Point      | 8                 | 0                                | 0                      | 0  | 0                      |
| 10/18/17    | Oyster River     | 8                 | 2                                | 25                     | 0  | 0                      |
| 10/19/17    | Woodman Point    | 8                 | 0                                | 0                      | 0  | 0                      |
| 10/20/17    | Piscataqua River | 8                 | 2                                | 25                     | 0  | 0                      |
| 10/23/18    | Squamscott River | 8                 | 0                                | 0                      | 0  | 0                      |
| 10/24/18    | Woodman Point    | 8                 | 1                                | 13                     | 0  | 0                      |
| 10/29/18    | Oyster River     | 8                 | 4                                | 50                     | 1  | 13                     |
| 10/30/18    | Piscataqua River | 8                 | 2                                | 25                     | 0  | 0                      |
| 11/02/18    | Adams Point      | 8                 | 1                                | 13                     | 0  | 0                      |
| 11/02/18    | Nannie Island    | 8                 | 0                                | 0                      | 0  | 0                      |
| 10/15/19    | Oyster River     | 10                | 1                                | 10                     | 0  | 0                      |
| 10/16/19    | Woodman Point    | 10                | 0                                | 0                      | 0  | 0                      |
| 10/16/19    | Adams Point      | 10                | 0                                | 0                      | 0  | 0                      |
| 10/18/19    | Squamscott River | 10                | 2                                | 20                     | 0  | 0                      |
| 10/22/19    | Piscataqua River | 10                | 2                                | 20                     | 0  | 0                      |

1- Presence of MSX plasmodia when found in palps and gills only are recorded as infections. 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 for natural oyster beds in NH, 1996 – 2019.**

| Date     | Location                         | No. Tested | No. Oysters in each infection category <sup>1</sup> |   |   |   |   |   | % 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 | Berry Brook                      | 16         | 0   | 0 | 0 | 0 | 0 | 0 | 0            |

**Table 2. (continued)**

| Date     | Location                 | No. Tested | No. Oysters in each infection category <sup>1</sup> |   |   |   |   | % 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   |



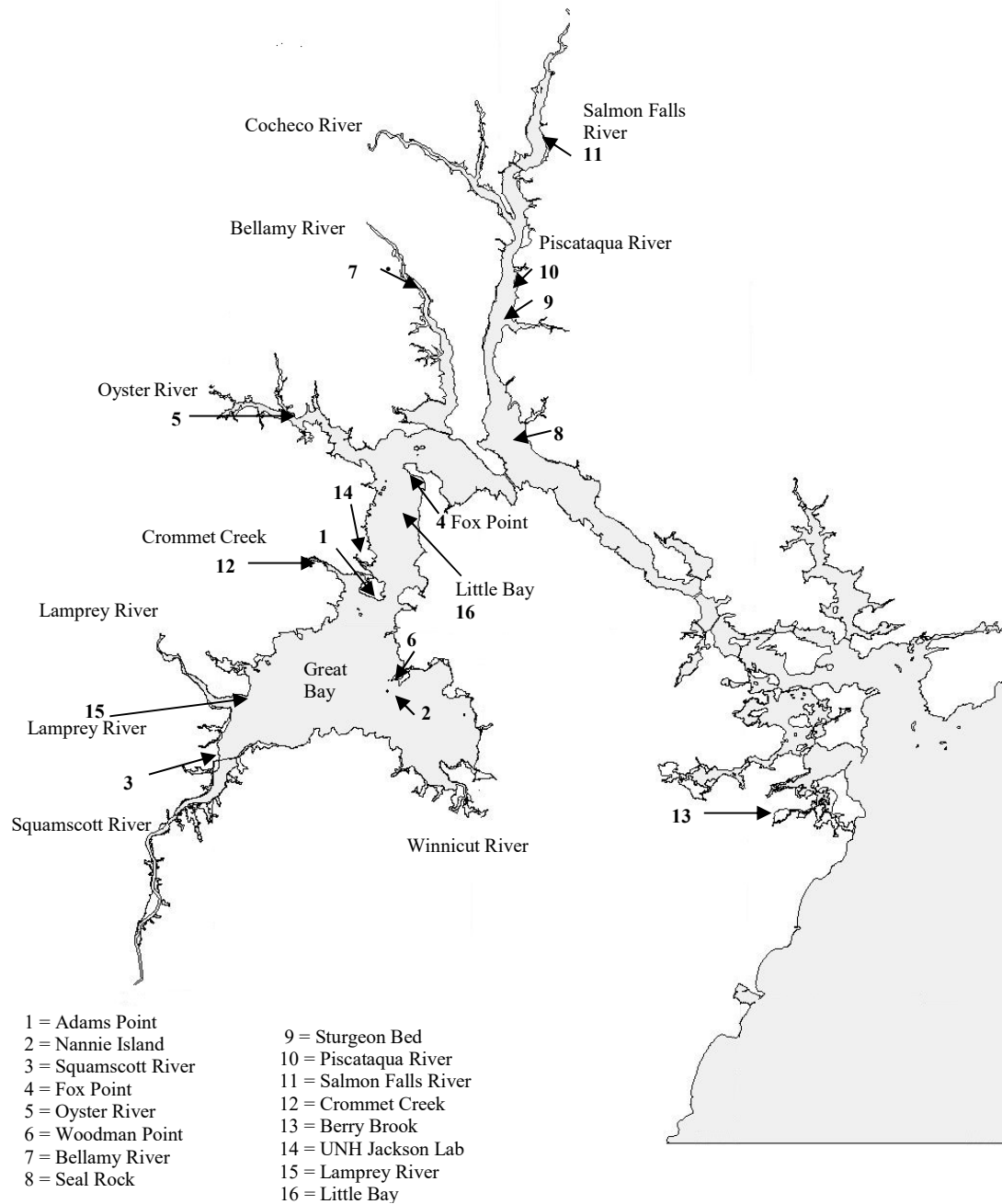
**Table 2. (continued)**

| Date     | Location         | No. Tested | No. Oysters in each infection category <sup>1</sup> |   |   |   |   |   | % 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 | 89           |
| 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           |
| 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 | 88           |
| 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 | 88           |
| 11/01/16 | Piscataqua River | 8          | 2   | 0 | 1 | 0 | 0 | 0 | 38           |
| 10/17/17 | Adams Point      | 8          | 2   | 3 | 2 | 0 | 0 | 0 | 88           |
| 10/18/17 | Oyster River     | 8          | 1   | 1 | 2 | 0 | 0 | 0 | 50           |
| 10/19/17 | Woodman Point    | 8          | 1   | 3 | 1 | 0 | 0 | 0 | 63           |
| 10/20/17 | Piscataqua River | 8          | 1   | 1 | 1 | 4 | 0 | 1 | 100          |
| 10/23/18 | Squamscott River | 8          | 2   | 0 | 2 | 0 | 0 | 0 | 50           |
| 10/24/18 | Woodman Point    | 8          | 2   | 2 | 1 | 2 | 0 | 0 | 25           |
| 10/29/18 | Oyster River     | 8          | 1   | 1 | 4 | 0 | 0 | 0 | 75           |
| 10/30/18 | Piscataqua River | 8          | 2   | 1 | 2 | 0 | 0 | 0 | 63           |
| 11/02/18 | Adams Point      | 8          | 3   | 3 | 1 | 0 | 0 | 0 | 88           |
| 11/02/18 | Nannie Island    | 8          | 0   | 0 | 1 | 0 | 0 | 0 | 13           |
| 10/15/19 | Oyster River     | 10         | 1   | 1 | 2 | 1 | 4 | 0 | 90           |
| 10/16/19 | Woodman Point    | 10         | 0   | 1 | 3 | 2 | 3 | 0 | 90           |
| 10/16/19 | Adams Point      | 10         | 0   | 2 | 2 | 2 | 1 | 1 | 80           |
| 10/18/19 | Squamscott River | 10         | 1   | 1 | 0 | 0 | 2 | 0 | 40           |
| 10/22/19 | Piscataqua River | 10         | 0   | 2 | 2 | 3 | 2 | 1 | 100          |

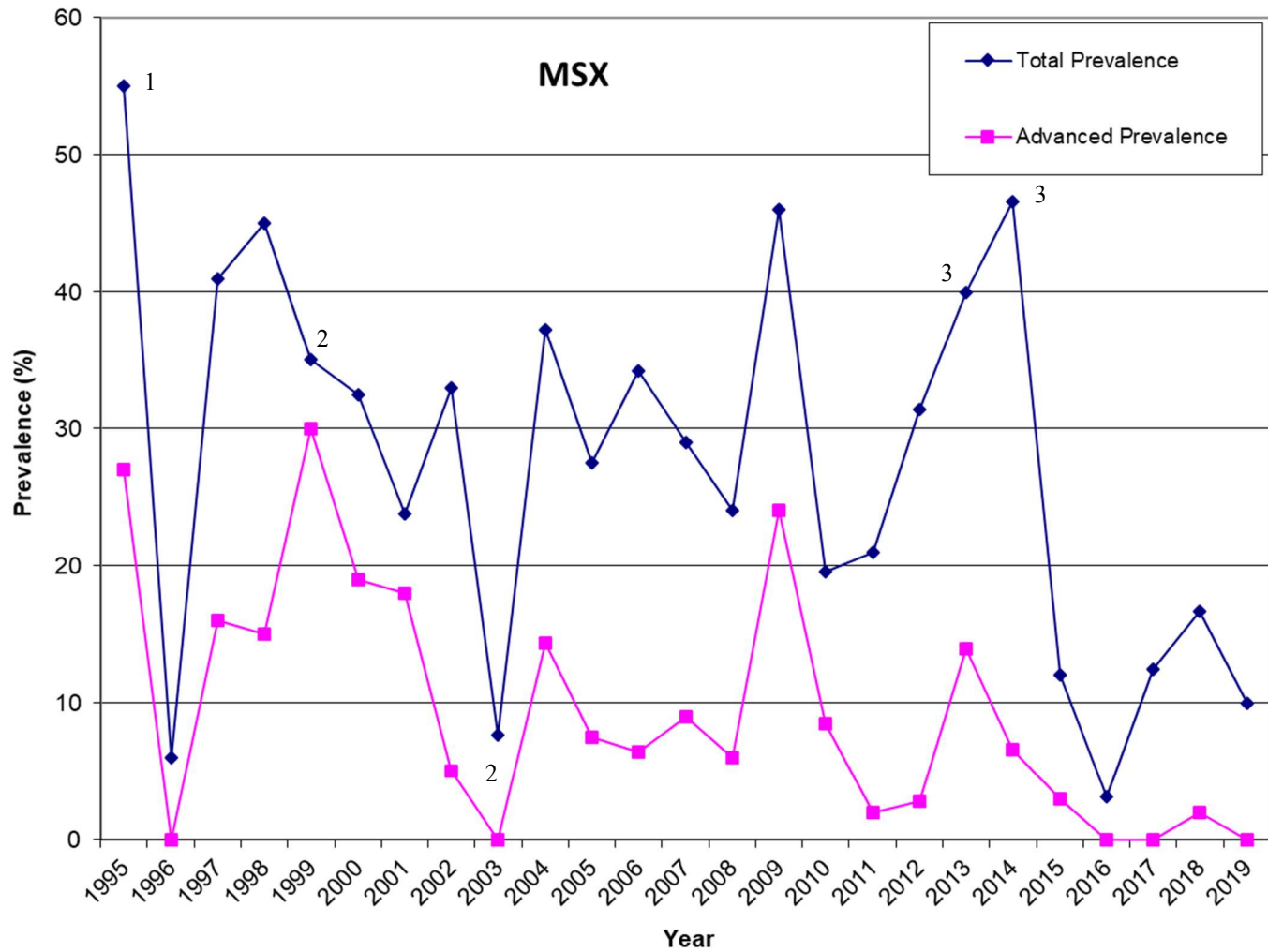
<sup>1</sup>-Infection categories are based on the severity of infection. Categories 0.5 to 2 are generally thought of as light or minor, whereas categories 3 to 5 are moderate to heavy and may pose an infection threat to Dermo-free oysters.

**Table 3. MSX and Dermo test results from aquacultured oysters in NH, 2019.**

| <b>Date fixed/<br/>cultured</b> | <b>Location</b>  | <b>Farm</b>          | <b>Mean Shell Length (mm)</b> | <b>StDev</b> | <b>No. Tested</b> | <b>No. Infected MSX</b> | <b>No. Infected Dermo</b> | <b>Gill Ciliate Prevalence (%)</b> |
|---------------------------------|------------------|----------------------|-------------------------------|--------------|-------------------|-------------------------|---------------------------|------------------------------------|
| 8/28/2019                       | Hampton Harbor   | Swell Oyster Co.     | 67.81                         | 5.01         | 30                | 0                       | 0                         | 27                                 |
| 8/28/2019                       | Lower Little Bay | Rising Tide Oysters  | 90.34                         | 6.36         | 30                | 0                       | 0                         | 33                                 |
| 8/28/2019                       | Upper Little Bay | Bay Point Oyster Co. | 107.39                        | 7.71         | 30                | 0                       | 0                         | 33                                 |
| 9/11/2019                       | Upper Little Bay | Fat Dog Oyster Co.   | 92.77                         | 7.36         | 30                | 0                       | 0                         | 37                                 |



**Figure 1. Study area and sample locations.**

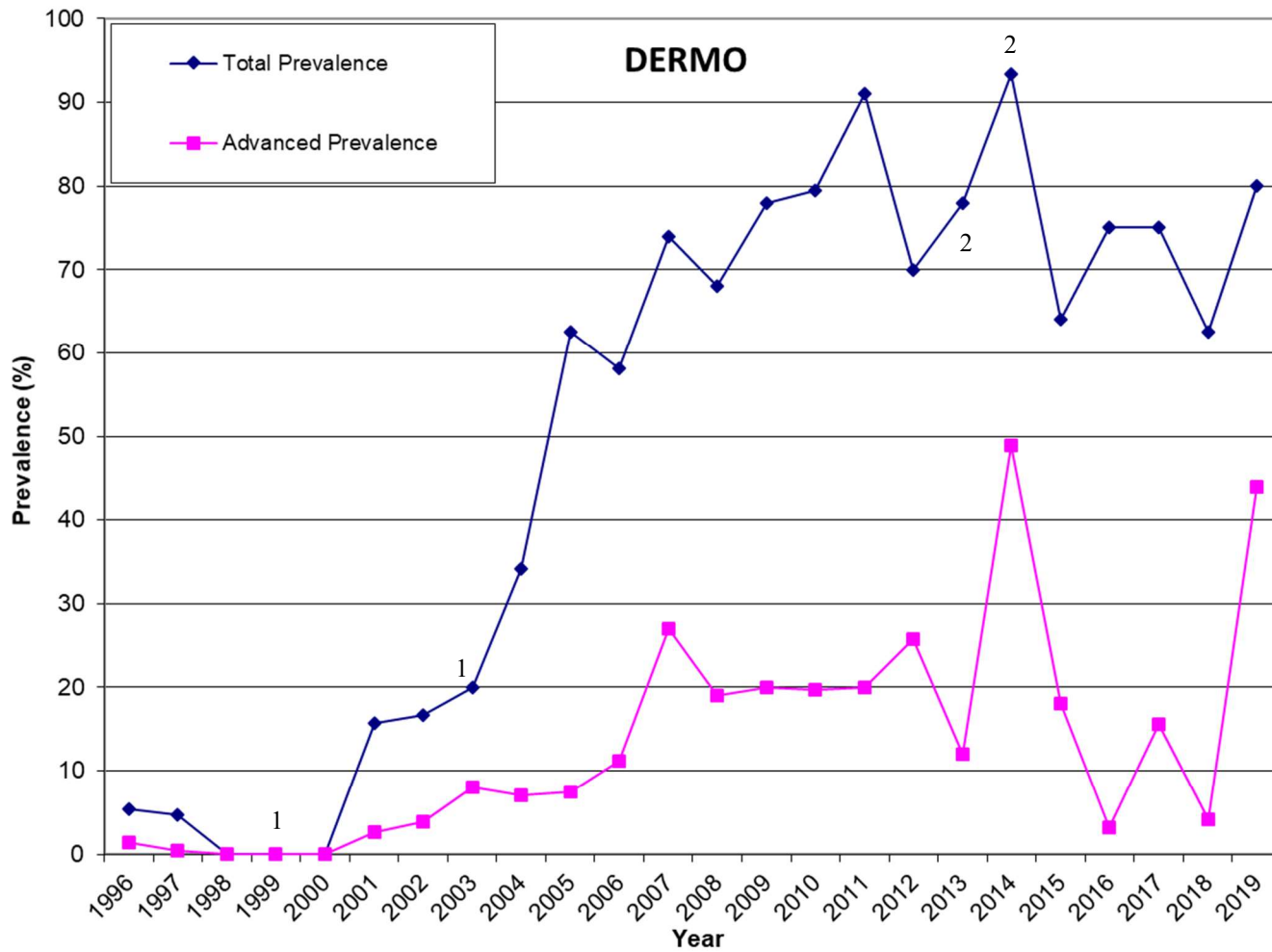


<sup>1</sup>Data for September and October sampling in 1995 from Barber et al., 1997 (see Table 1).

<sup>2</sup>Only the Nannie Island bed was sampled in 1999 and 2003.

<sup>3</sup>Does not include aquaculture sampling in 2013 and 2014.

**Figure 2. Total and advanced MSX prevalence in the Great Bay Estuary, 1995 -2019.**



<sup>1</sup>Only the Nannie Island bed was sampled in 1999 and 2003.

<sup>2</sup>Does not include aquaculture sampling in 2013 and 2014.

**Figure 3. Total and advanced Dermo prevalence in the Great Bay Estuary, 1996 -2019.**