

**Final Project Report: Sustainable Fisheries Goal Implementation Team Project 7: Development of a Striped Bass Health Indicator**

Prepared by Rebecca Scott of EcoAnalytics LLC

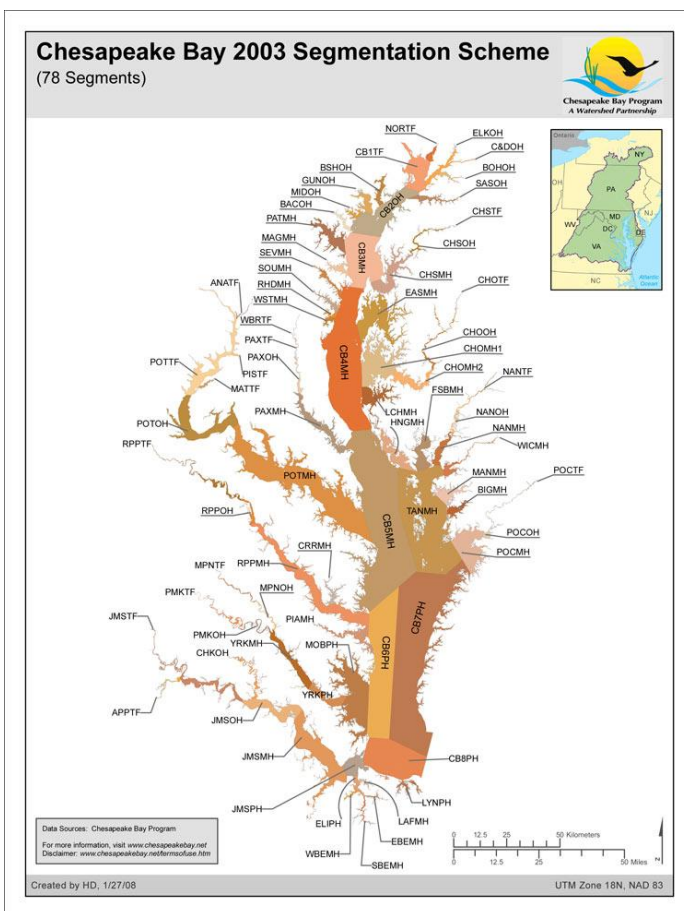
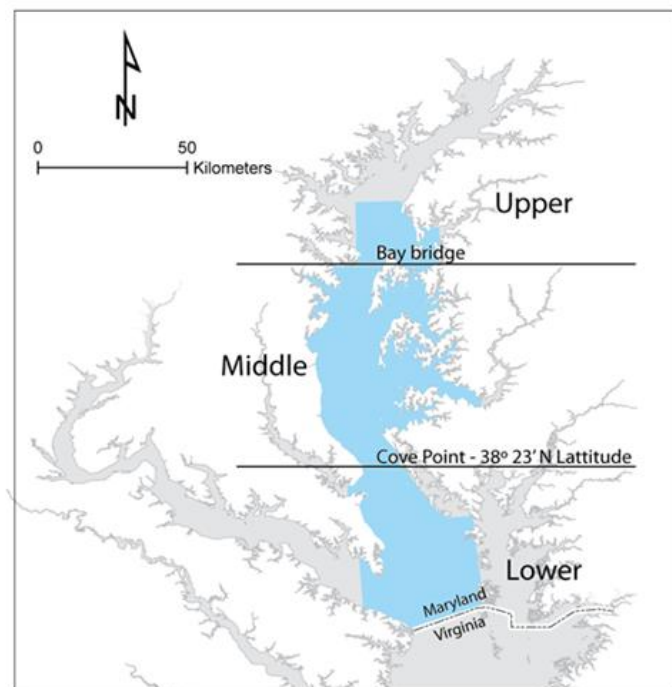
## **Project Background**

The Sustainable Fisheries Goal Implementation Team tasked EcoAnalytics LLC with the development of a health indicator for Chesapeake Bay striped bass that would help inform coastwide management, as the Bay serves as the spawning grounds for a majority of the coastwide stock. As of the current date, the only striped bass indicators used by the Chesapeake Bay Program are an assessment of the coastwide stock conducted by the Atlantic States Marine Fisheries Commission, as well as state-specific juvenile surveys, which may fail to capture specific trends in the overall Chesapeake Bay stock (<http://www.chesapeakebay.net/track/health/bayhealth>).

The Fish and Wildlife Health Program of the Maryland Department of Natural Resources conducts a fish health survey that began in 1998, in a response to the discovery of mycobacteriosis in Chesapeake Bay fish in 1997 (Heckert et al. 2001, Rhodes et al. 2001). Mycobacteriosis is a chronic, progressive disease currently affecting a large proportion of Bay striped bass, and is in most cases visceral, with the spleen, liver and kidneys being primarily affected with granulomatous inflammation containing acid-fast bacteria. Dermal symptoms of mycobacteriosis include scale loss, ulceration, and changes in pigmentation. Mycobacteriosis in wildlife is generally considered irreversible (Hines, M., J. Kreegar, et al. (1995), and there has been no evidence of disease regression and clearance in long term laboratory studies of mycobacteriosis in fish (Bruno, D. W., J. Griffiths, et al. (1998), Gauthier, D. T., M. W. Rhodes, et al. (2003)).

Sampling for striped bass continues to occur annually, giving a working data set of 17 years (through 2014). While data from 2014 sampling is now available, updated age-at-length keys for 2014 have not yet been provided. Fish are collected from Maryland Bay Waters, from the Chester River down to the Virginia border, with sampling generally occurring in the fall. Figure 1 shows the general sampling area of the survey. Extensive laboratory processing of sampled fish yields information on infection status, body condition, body fat, and lesion or granuloma severity, among many others. Because of the availability of this comprehensive data set, it was decided that an evaluation of mycobacteriosis in Chesapeake Bay striped bass would be an appropriate product in response to the call for a fish health indicator.

▼ **Figure 1** Approximate sampling area of Maryland Department of Natural Resources Fish and Wildlife Health Program's striped bass survey.



▲ **Figure 2** The Chesapeake Bay Program's Bay Segmentation Scheme (2003). Water quality data was only utilized from segments present in the sampling area (Figure 1).

## Methods

### **Apparent Prevalence as an Indicator**

In order to provide a visualization of the mycobacteriosis epidemic in Chesapeake Bay striped bass, we decided to focus efforts on the use of apparent prevalence as an indicator. Apparent prevalence can be defined as the proportion of organisms that test positive for infection by the focal disease, or the number of infected individuals in the population divided by the total population size. By capturing trends in prevalence over time and determining the factors

influencing prevalence, we are able to begin to piece together the story of how the fish come in to contact with the disease, and how the disease spreads throughout the population over time. Given the ongoing concerns surrounding Chesapeake Bay water quality, we put emphasis on analyses comparing apparent prevalence with external water quality factors. Using binomial logistic regressions in R programming software, we were able to demonstrate correlations between overall disease prevalence and important water quality factors associated with nutrient loading, such as nitrogen and phosphorus components and total suspended solids (Figures 17 through 26). In addition, we were able to connect these water quality factors to the amount of *Mycobacterium* spp. in the water column (Jacobs et al 2009) and the prevalence of mycobacteriosis in age 1 fish (Figures 29 and 30)

### **Estimating Disease-Associated Mortality**

At the start of the project we intended to use a force-of-infection modeling approach to produce a time series of values for the additional mortality as a result of mycobacterial infection, or the “disease-associated mortality”. The disease-associated mortality includes any mortality associated with infection, including increased susceptibility to fishing or predation. With the current data from MD DNR Fisheries Service, data on older age classes is sparse, which, in turn, prohibited model convergence such that force-of-infection modeling approaches for estimating disease-associated mortality were not possible. However, for a few cohorts, adequate samples of older age classes were available for simpler approaches to estimate disease-associated mortality. We derived an approach for estimating diseased-associated mortality using cohort data and a simple theory of mortality..

From the simple theory of population ecology, mortality can be treated as negative population growth using the differential equation for year class size:

**Equation 1** 
$$\frac{dN}{dt} = -ZN$$

..with N being the number of individuals in the population and Z being a constant instantaneous mortality.

When  $N(0)=N_0$ , the solution to equation 1 is

**Equation 2** 
$$N(t) = N_0 e^{-Zt}$$

This signifies that year-class abundance decreases at each age (t) at a constant instantaneous mortality. After a one-year period, the year class size can be calculated as:

**Equation 3** 
$$N(1) = N_0 e^{-Z}$$

From this the annual survival fraction (rate), S, for a year class over a one-year period can be estimated from the ratio of year-class abundances.

**Equation 4** 
$$S = \frac{N_1}{N_0} = e^{-Z}$$

The annual mortality fraction (rate), A, is calculated as 1-S.

For this study we had age-class prevalence data available rather than numbers. Prevalence is estimated as:

**Equation 5** 
$$\hat{P}_t = \frac{I_t}{N_t}$$

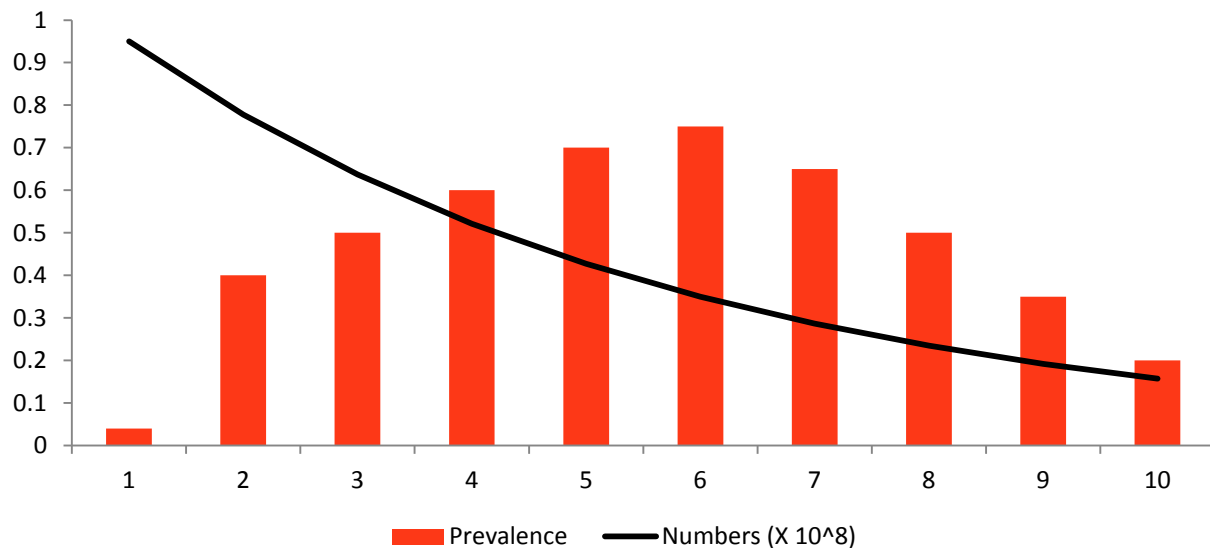
..where  $\hat{P}_t$  is the estimated prevalence for an age class and  $I_t$  is the number infected for an age class.

Replacing N with P in Equation 4, we derive an estimate for the annual survival fraction of infected individuals,  $S_I$ , over a one-period

**Equation 6** 
$$S_I = \frac{P_1}{P_0} = e^{-Z_I}$$

..where  $Z_1$  is the annual mortality associated with infection. From this the annual mortality fraction for infected individuals ( $A_1$ ) can be estimated as  $1-S_1$ .

In the case of mycobacteriosis, simply substituting  $\hat{P}_t$  for  $N_t$  in Equation 2 would not be appropriate for estimating disease-associated mortality as the prevalence by age class increases in younger age classes while the numbers by age classes decreases. This phenomenon is illustrated in Figure 3. For younger age classes,  $dI/Dt > 0$ , whereas  $dN/Dt < 0$ , so applying the exponential decay of equation 1 to  $P$  is not appropriate; however, for older age classes the exponential decay equation is reasonable.



**Figure 3.** Typified age-at size and age-specific mycobacteriosis prevalence patterns for striped bass.

For older age classes,  $dI/Dt < 0$  and  $dN/Dt < 0$  such that  $dP/dt < 0$ . In this case, the numbers-infected-at-age decline faster than numbers-at-age, and mycobacteriosis is a chronic condition with no recovery (for infection diagnosis based on splenic pathology), thus suggesting that decrease in prevalence by age class ( $dP/dt$ ) is an indicator of disease-associated mortality. Substituting Equation 5 into Equation 6,

**Equation 7**

$$S_I = \frac{\frac{I_1}{N_1}}{\frac{I_0}{N_0}} = \frac{I_1}{I_0} \times \frac{N_0}{N_1} = \frac{I_1}{I_0} \times \frac{1}{S}$$

and using  $S_I$  to estimate  $A_I$ ,

**Equation 8**

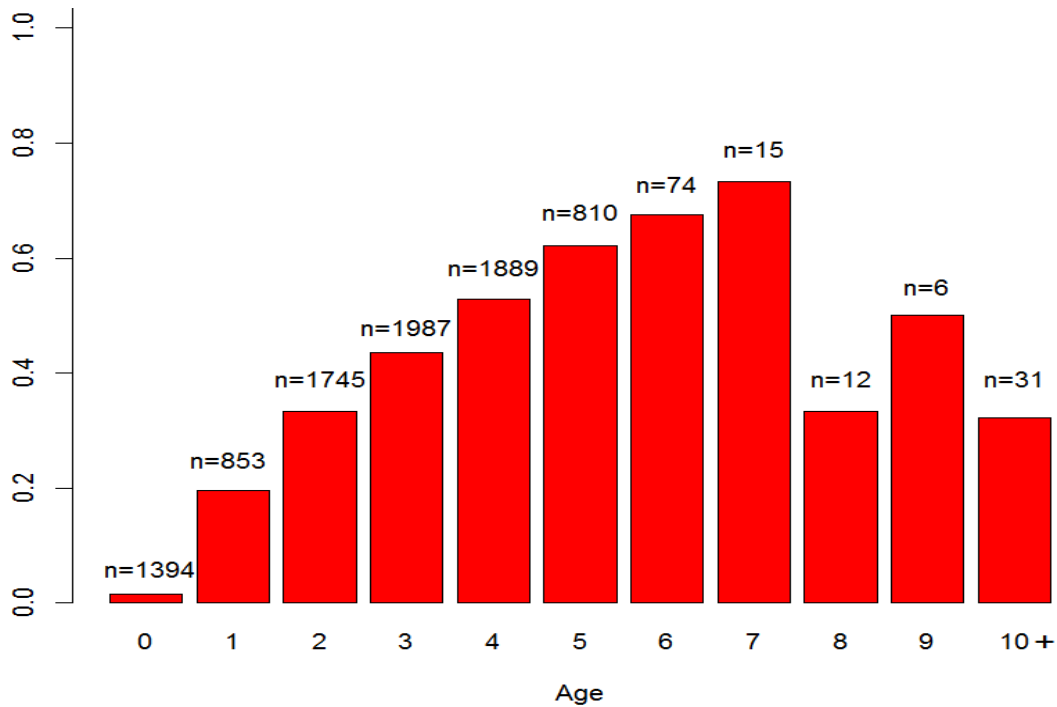
$$A_I = 1 - \frac{I_1}{I_0} \times \frac{1}{S}$$

..shows that our estimate of  $A_I$  is a measure of the proportion of annual mortality associated with infection. In practice, this is a conservative estimate as  $P$  for any given age-class and year is likely to be an underestimate of actual prevalence because of disease-associated mortality. More details regarding the cohorts used in these calculations are shown in Figure 31, and the results are shown in Table 2.

## **Results**

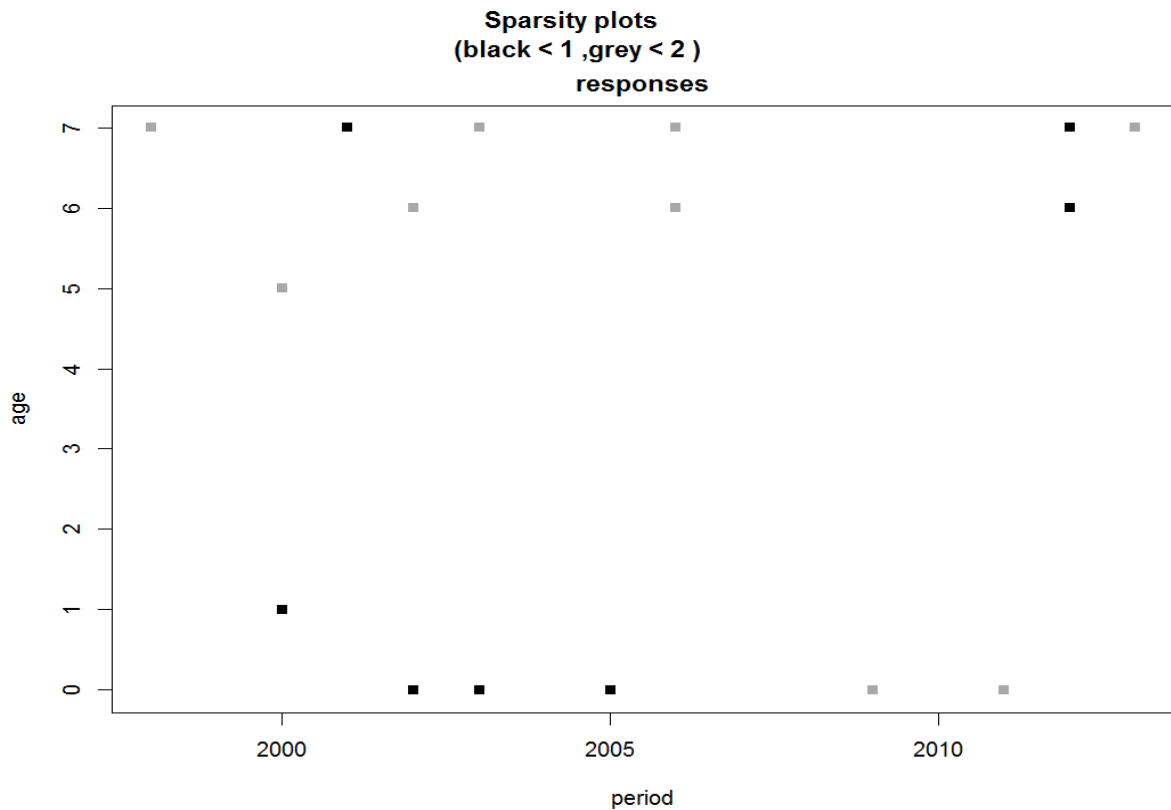
### **Initial data exploration**

Before possible indicators were considered, the data set underwent initial exploration to examine possible sources of bias and to evaluate disease prevalence in the population. Figure 4 shows the apparent prevalence of mycobacteria in sampled fish, as broken down by age class (determined by annual Maryland Department of Natural Resources Age-at-Length keys).



**Figure 4** Apparent prevalence of mycobacteriosis in sampled fish, by age. Sample sizes are shown.

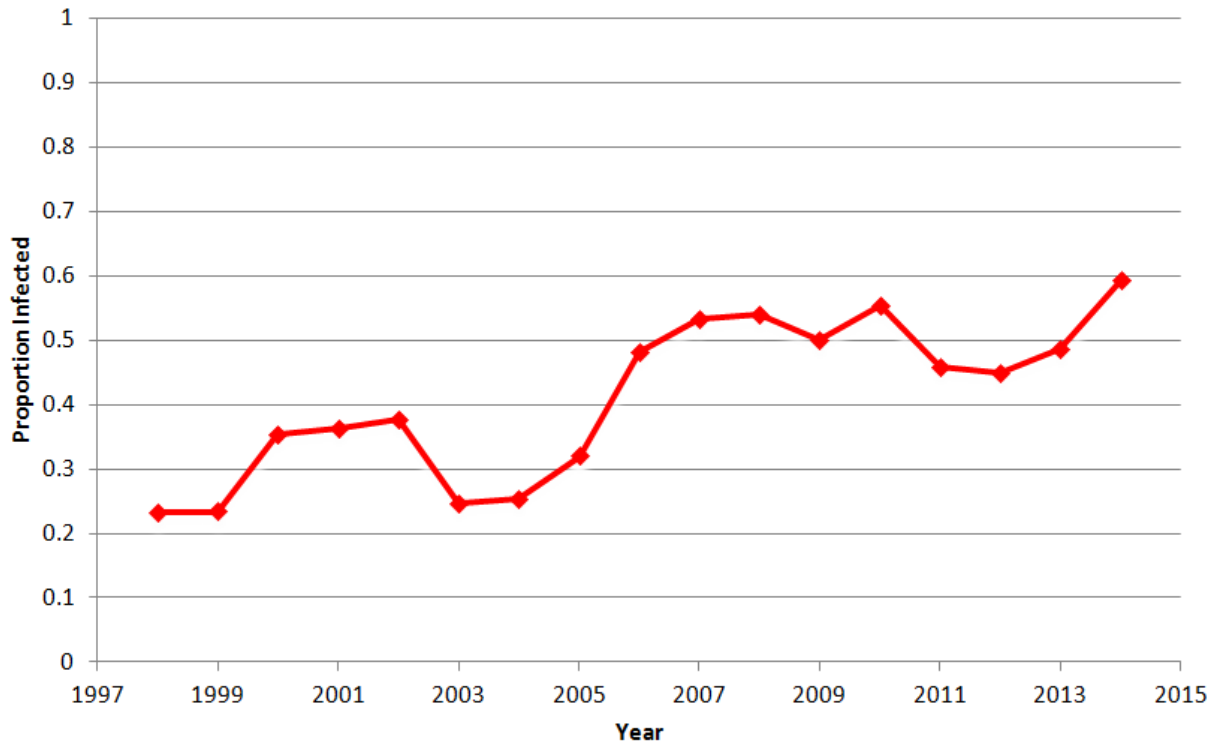
The data show an increase in prevalence with age until a drop in prevalence is reached at age 8, which may be due to mortality of older age class fish as a result of infection. This mortality is often referred to as “disease-associated mortality”, described above. The increase of prevalence with age is a visualization of the “force-of-infection” of mycobacteriosis, or the rate at which disease-free animals become infected. The greatest force-of-infection appears to be occurring between ages 0 and 1, as prevalence is increasing from less than 1 percent to almost 20 percent in the first year of life. Also shown by Figure 4 is the sample size of each age class; because older, larger fish were relatively rare in the sampling, the decision was made to use only data from age 1-6 fish in many analyses, with age 0 fish also being excluded due to low prevalence. This is demonstrated by a sparsity plot (Figure 5), which shows inadequate data in older age classes as well as sparsity signals for age 0 fish, due to the high population of “0” data points (code for disease-negative).



**Figure 5** A sparsity plot of the overall sample data set demonstrating inadequate prevalence data for older age classes and age 0 fish.

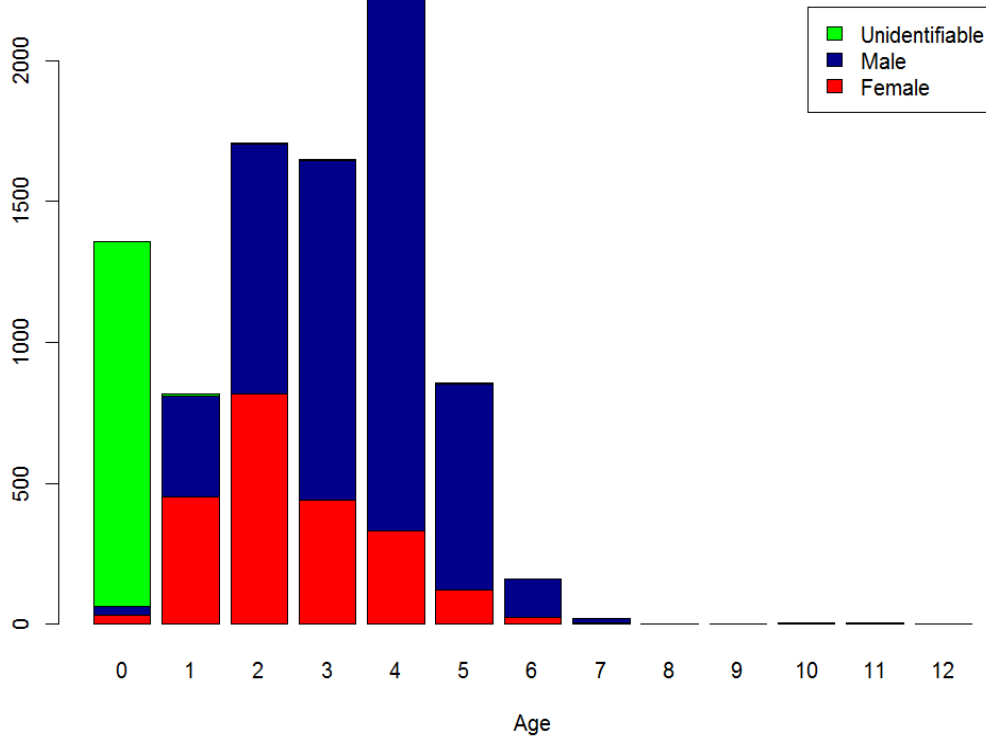
Figure 6 demonstrates the overall trend in infection over time for the entire sample population, excluding age 0 fish. The prevalence of mycobacteriosis in Maryland striped bass appears to have reached a peak of ~59% in 2014. These numbers are consistent with results from similar studies conducted in Virginia (Gauthier et al 2008). Note that 2014 data is not used in any age-specific analyses from this point forward as final 2014 age-at-length keys have not been published by Maryland DNR at time of writing.



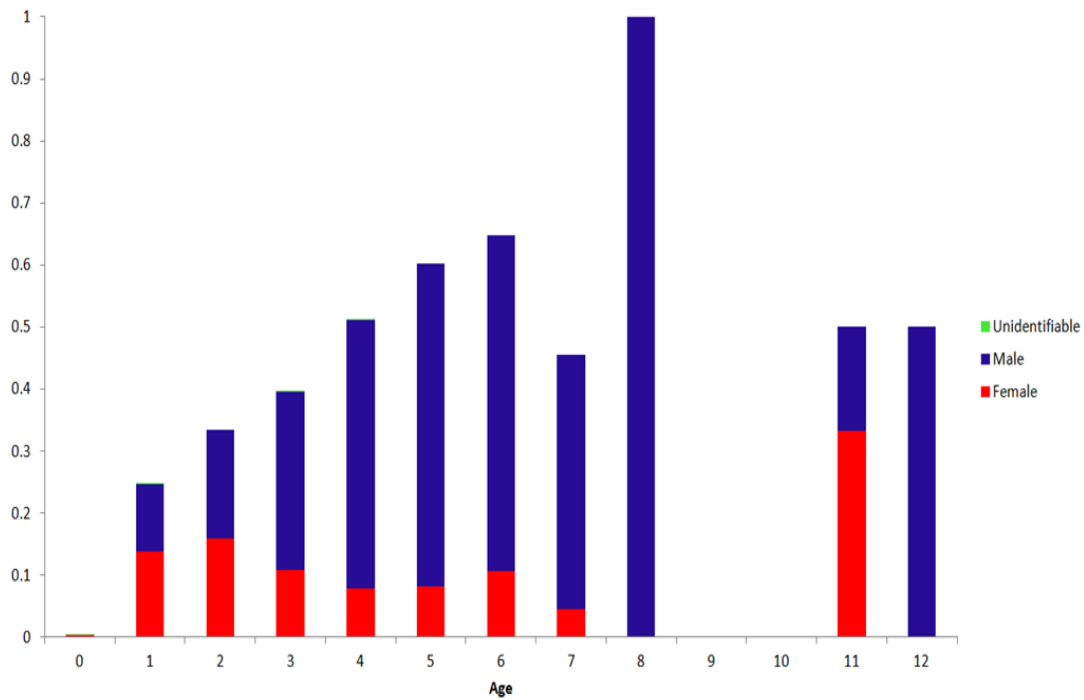


**Figure 6** Overall trend in mycobacteriosis prevalence in Maryland striped bass over time (1998-2014). Age 0 fish were excluded from this comparison.

Gender structure of sampled fish was analyzed for potential bias, as prevalence differed between genders in a similar Virginia study (Gauthier et al 2008). The results are shown in Figures 7 and 8. The number of ages 1 and 2 fish sampled was approximately divided in half between males and females, but males began to dominate in numbers in ages 3 and up. The majority of age 0 fish were unidentifiable by gender. These numbers were reflected in Figure 8, which examined gender in relation to disease prevalence. Figure 8 can be interpreted as the gender structure of disease positive fish; similarly to the patterns in Figure 7, approximately half of diseased fish were male for ages 1 and 2, with the proportion of males increasing with age (likely due to the larger number of males in the sample in relation to females shown by Figure 7). The comparison of Figures 7 and 8 suggest that the data did not indicate a gender bias associated with infection.

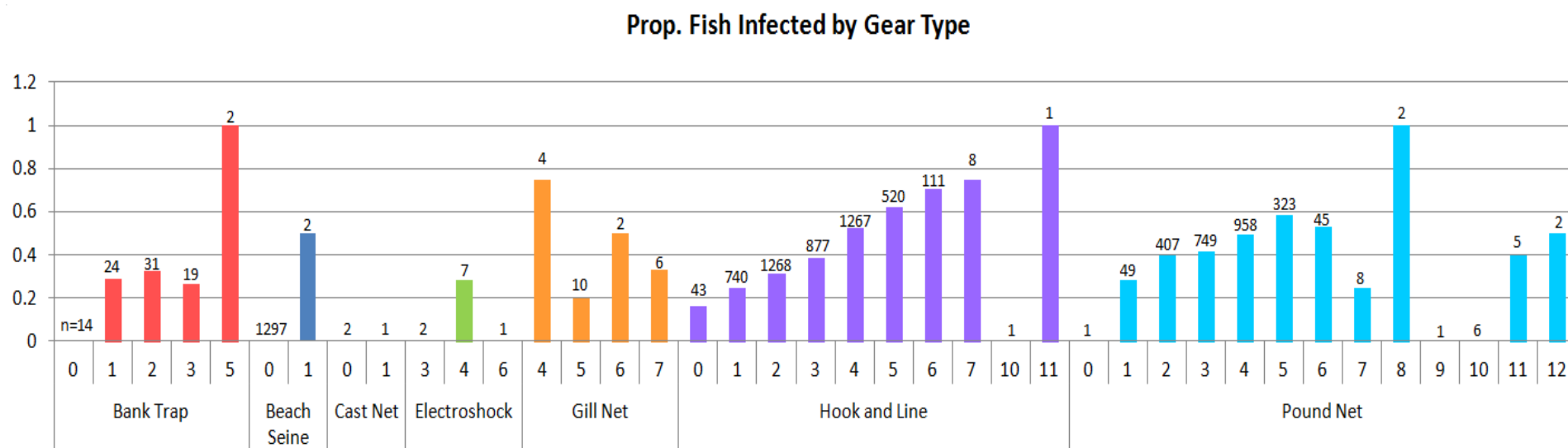


**Figure 7** Gender structure of sampled fish, by age.



**Figure 8** Gender structure by age class for disease-positive fish.

The gear type used in sampling was also examined as a possible source of bias, shown in Figure 9. Sample sizes are shown above each bar in the figure, demonstrating that most fish were collected using hook and line or pound net. The low sample sizes for other gear types make gear-associated bias unlikely. While over twelve hundred fish were collected by beach seine, all of these fish were age 0 with the exception of two age 1 fish, and so were excluded from analyses.

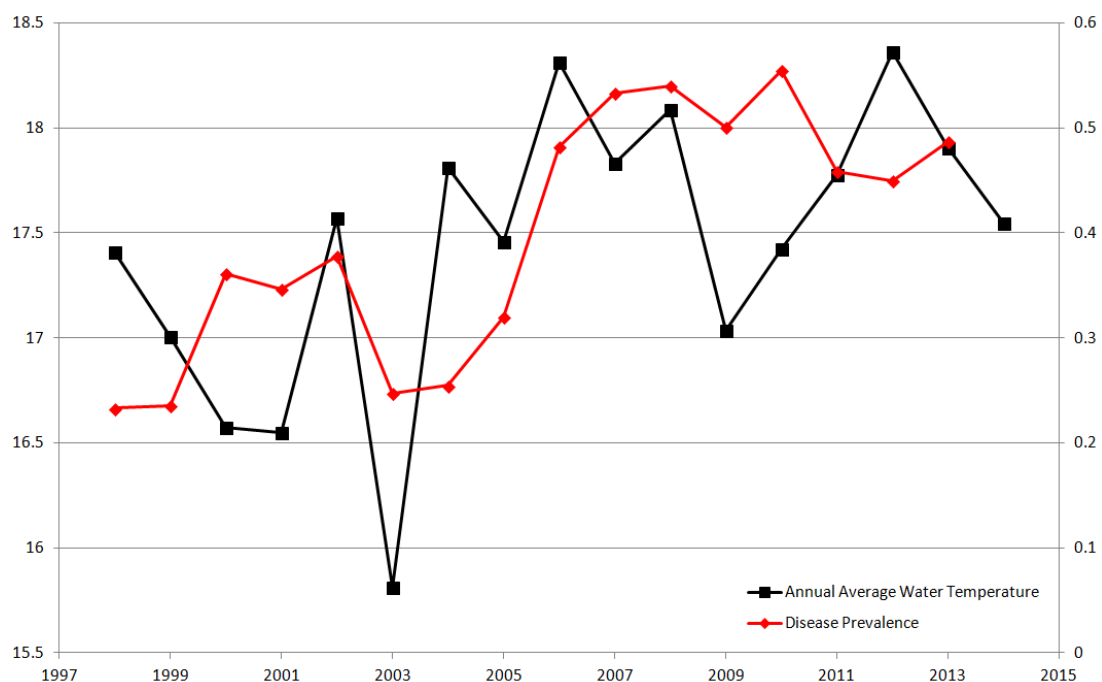


**Figure 9** Examination of disease prevalence by age, by collection gear type. Sample sizes are shown.

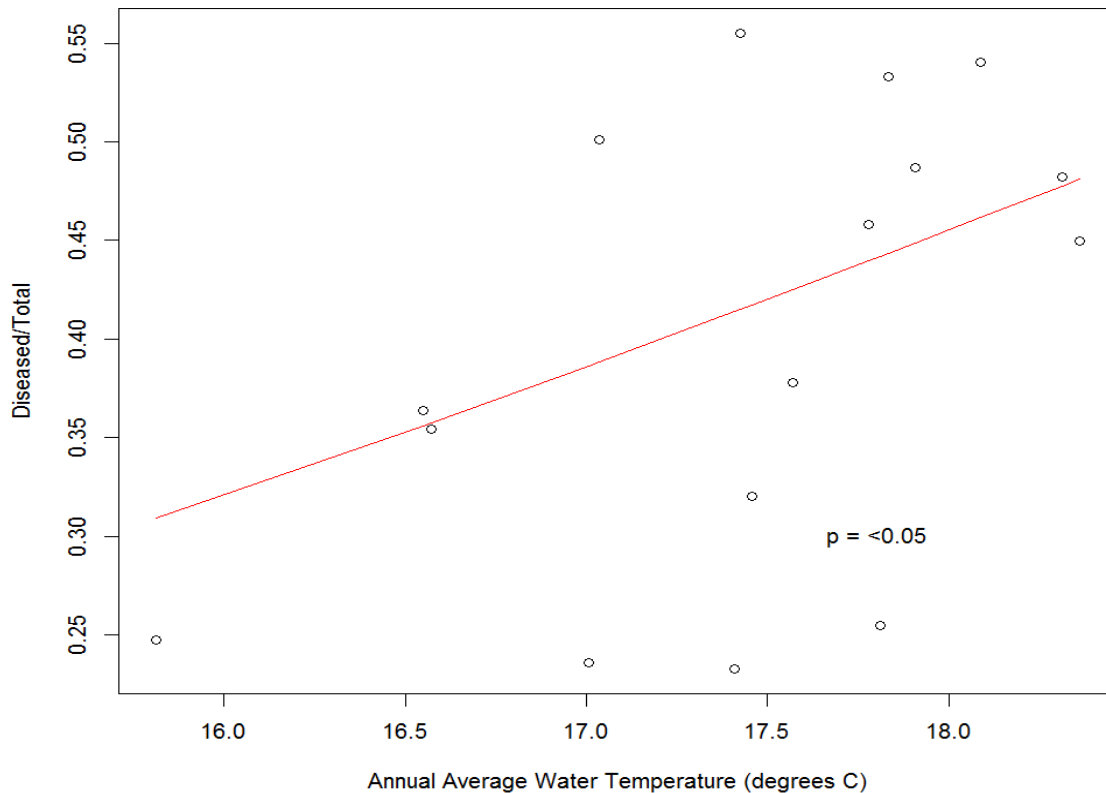
## Water Quality Covariates

### Water Temperature

Water temperature data was taken from the Chesapeake Bay Program's Water Quality data hub, and only data from the Bay Segments in which sampling occurred were used (Figure 2). Figure 10 compares average annual water temperatures ( $^{\circ}\text{C}$ ) with the prevalence of mycobacteriosis in the sample population. A positive correlation is demonstrated, with higher water temperatures correlating with a higher prevalence of mycobacteriosis. This relationship was found to be statistically significant, with a  $p$  value  $< 0.05$ , demonstrated in the logistic regression plot (Figure 11). The regression output suggests a 1.33x increase in the odds of a fish becoming disease positive for every  $1^{\circ}\text{C}$  increase in water temperature. To put this in perspective, the water temperature in the sampled area has fluctuated  $\sim 2.5^{\circ}\text{C}$  since the beginning of the survey in 1998.

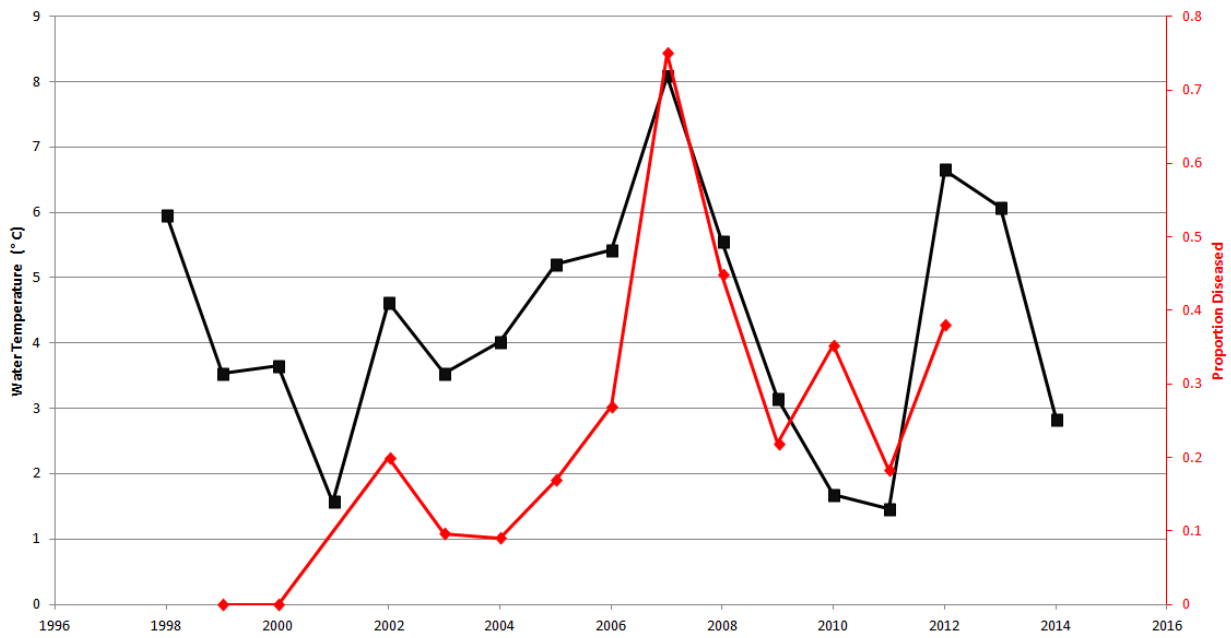


**Figure 10** A comparison between average annual water temperatures and disease prevalence (age 1-6 fish).

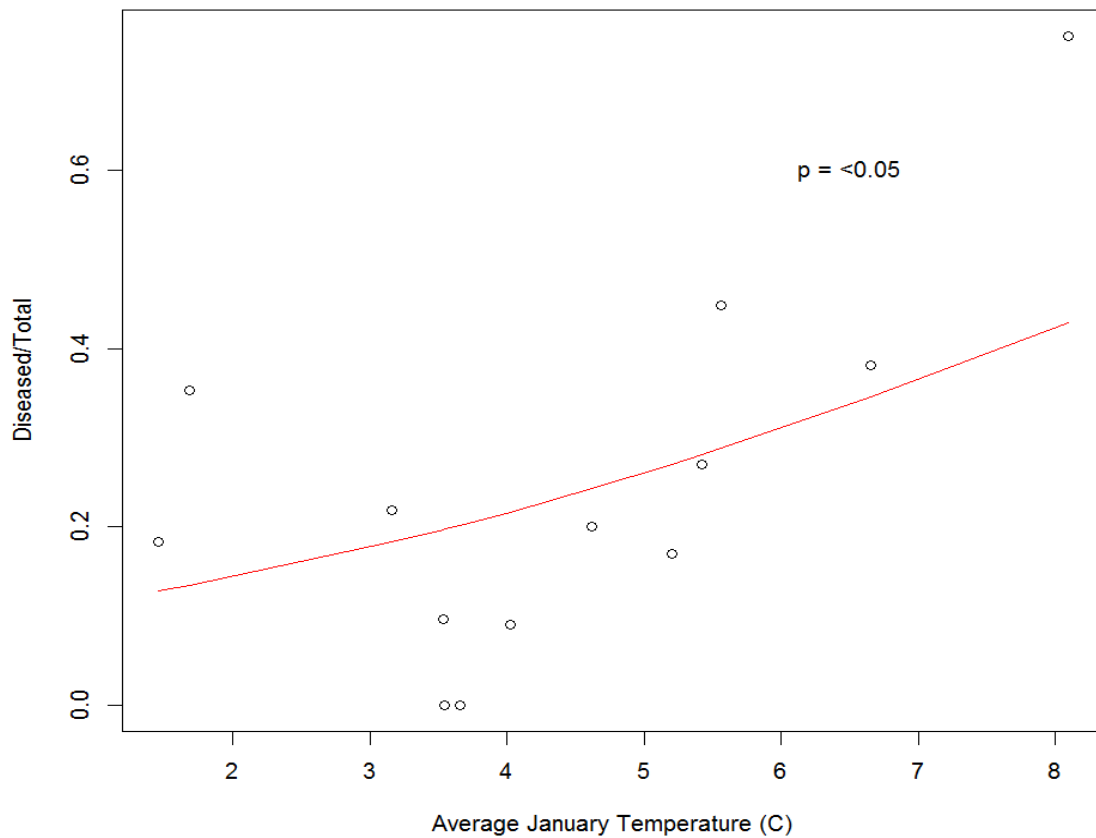


**Figure 11** A logistic regression comparing average annual water temperatures with disease prevalence in age 1-6 fish. The regression yielded statistical significance with  $p < 0.05$ .

Within the analyses of water temperature as an explanatory factor, an interesting correlation was found between average January water temperatures and the prevalence of disease within age 1 fish (Figure 12). While this may seem an oddly specific comparison, it may provide a piece of the narrative regarding the initiation of infection in young age classes. The results of the logistic regression for this comparison are shown in Figure 13.



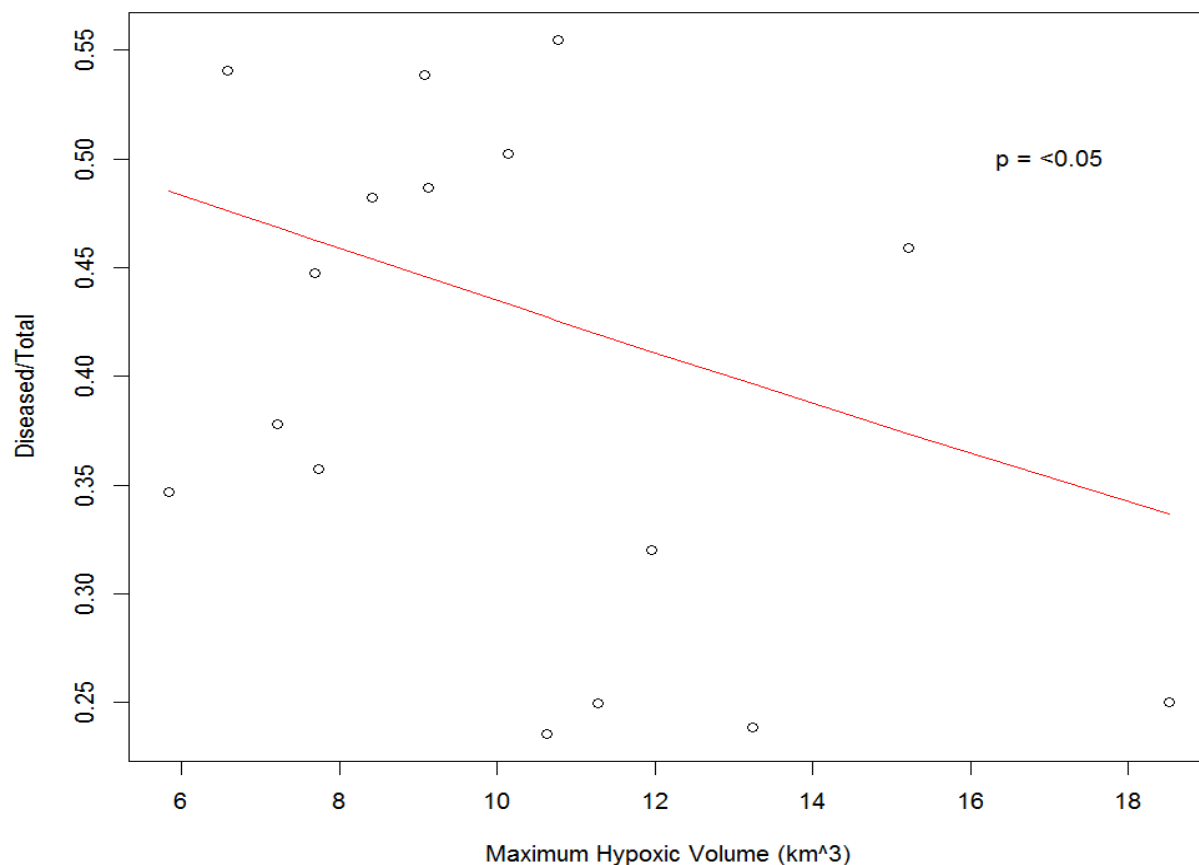
**Figure 12** Average January water temperatures over time plotted with disease prevalence in age 1 fish.



**Figure 13** A logistic regression comparing average January water temperatures with disease prevalence in age 1 fish. The regression yielded statistical significance with  $p < 0.05$ .

### Dissolved Oxygen/Hypoxic Volume

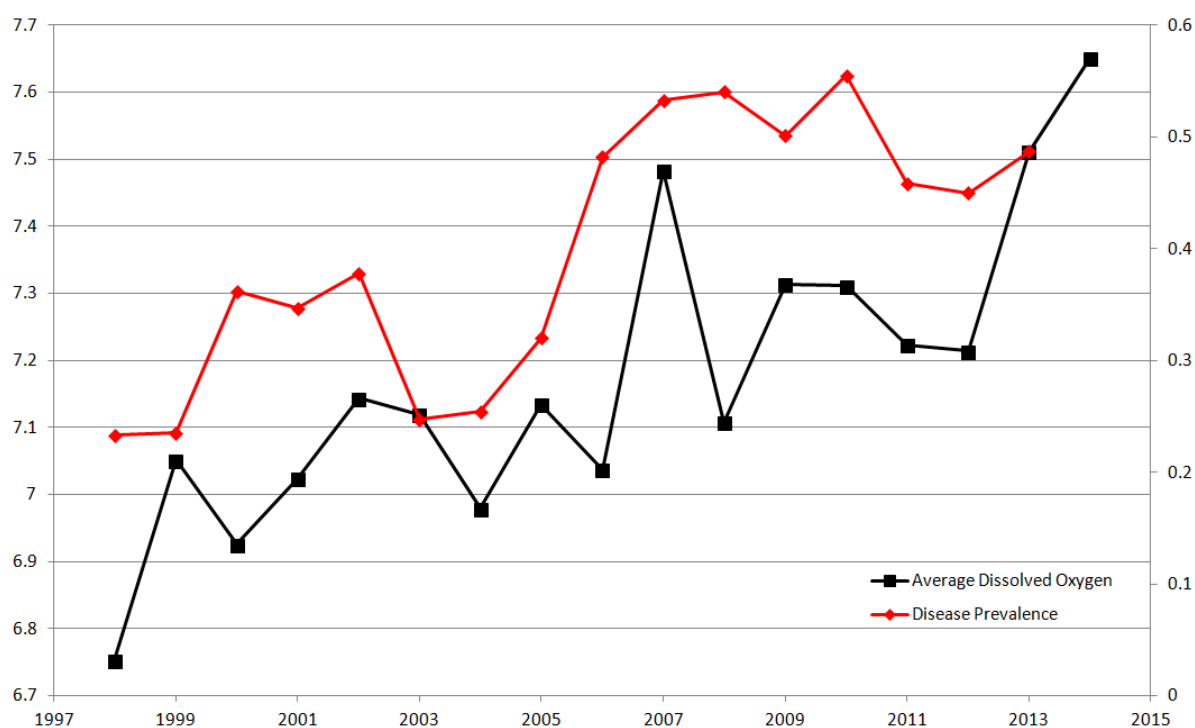
Data for Bay hypoxic volume ( $\text{DO} < 0.2 \text{ mg/L}$ ) was acquired from Jeremy Testa of the University of Maryland's Chesapeake Biological Laboratory. Hypoxic volume was examined along with water temperature because of their combined effects on habitat squeeze, which would likely cause an increase in transmission of mycobacteriosis as fish are packed more densely into less area of suitable habitat. A logistic regression for the relationship between maximum annual hypoxic volume and disease prevalence yielded statistical significance, although inversely (Figure 14). The inverse relationship shown in Figure 14 may be a result of mortality from the combination of mycobacteriosis and high hypoxic volume.



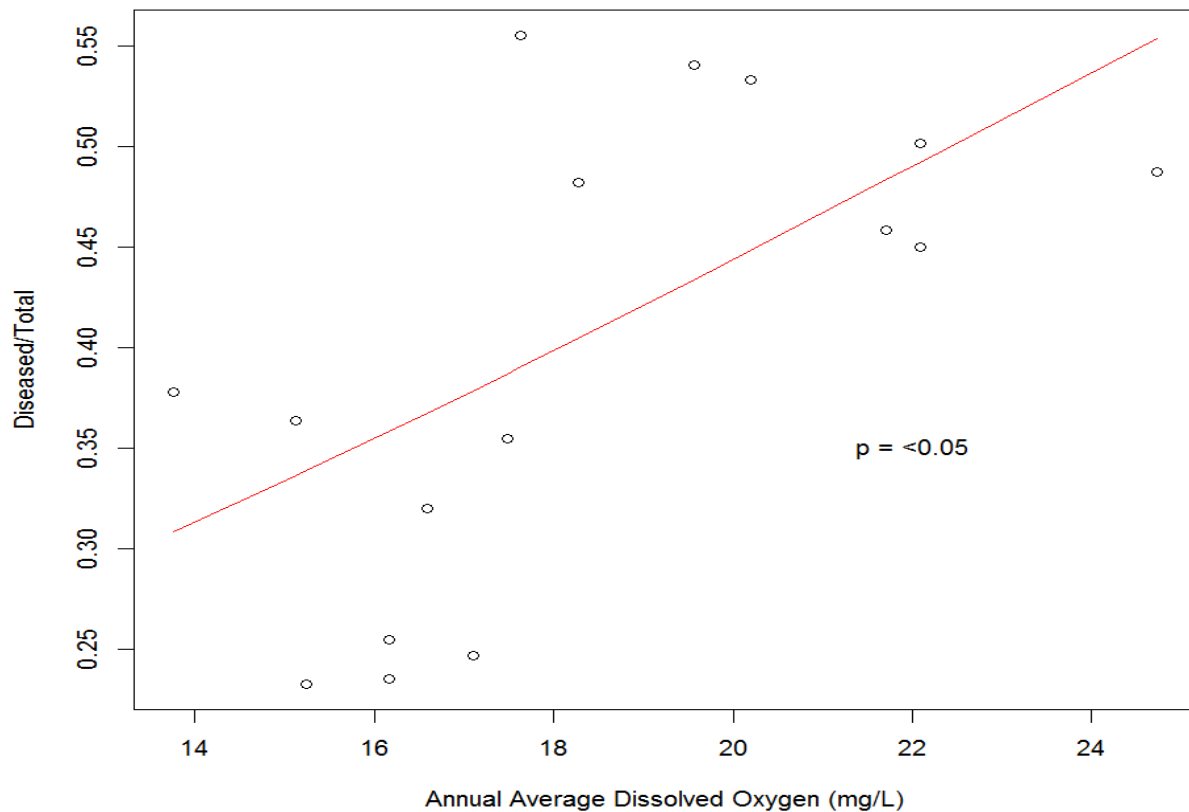
**Figure 14** A logistic regression comparing maximum hypoxic volume with disease prevalence. The regression yielded statistical significance with  $p < 0.05$ .



Dissolved oxygen in Bay water was examined along with hypoxic volume, using data from the Chesapeake Bay Program's Water Quality data hub from the Bay Segments in which sampling occurred (Figure 2). Figure 15 shows the comparison of average annual dissolved oxygen levels (mg/L) with the prevalence of mycobacteriosis in the sample population. A positive correlation is demonstrated, with higher levels of dissolved oxygen correlating with a higher prevalence of mycobacteriosis. This was to be expected given the inverse relationship between prevalence and hypoxic volume, and we believe it can be explained using the same reasoning of mortality at extreme lows of dissolved oxygen. This relationship was found to be statistically significant, with a  $p$  value  $< 0.05$ , demonstrated in the logistic regression plot (Figure 16).



**Figure 15** A comparison between average annual dissolved oxygen levels (mg/L) and disease prevalence (age 1-6 fish).



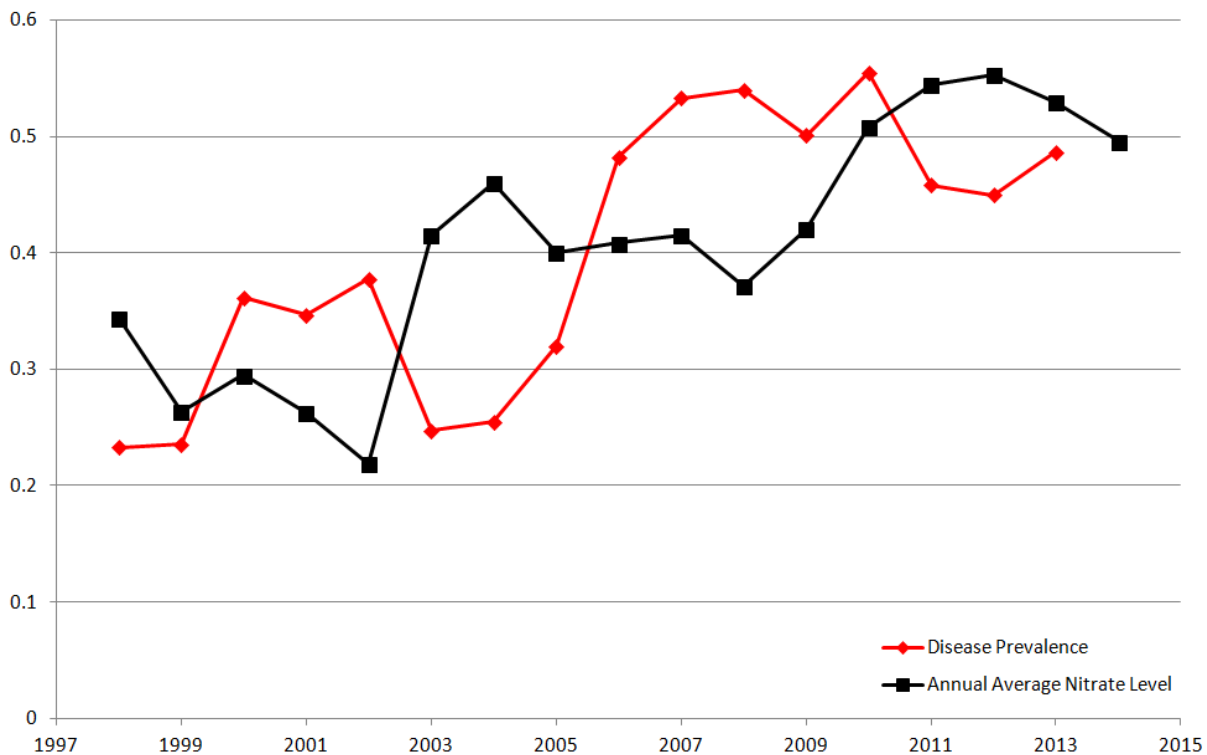
**Figure 16** A logistic regression comparing average annual dissolved oxygen levels with disease prevalence in age 1-6 fish. The regression yielded statistical significance with  $p < 0.05$ .

### Nitrates/Total Nitrogen

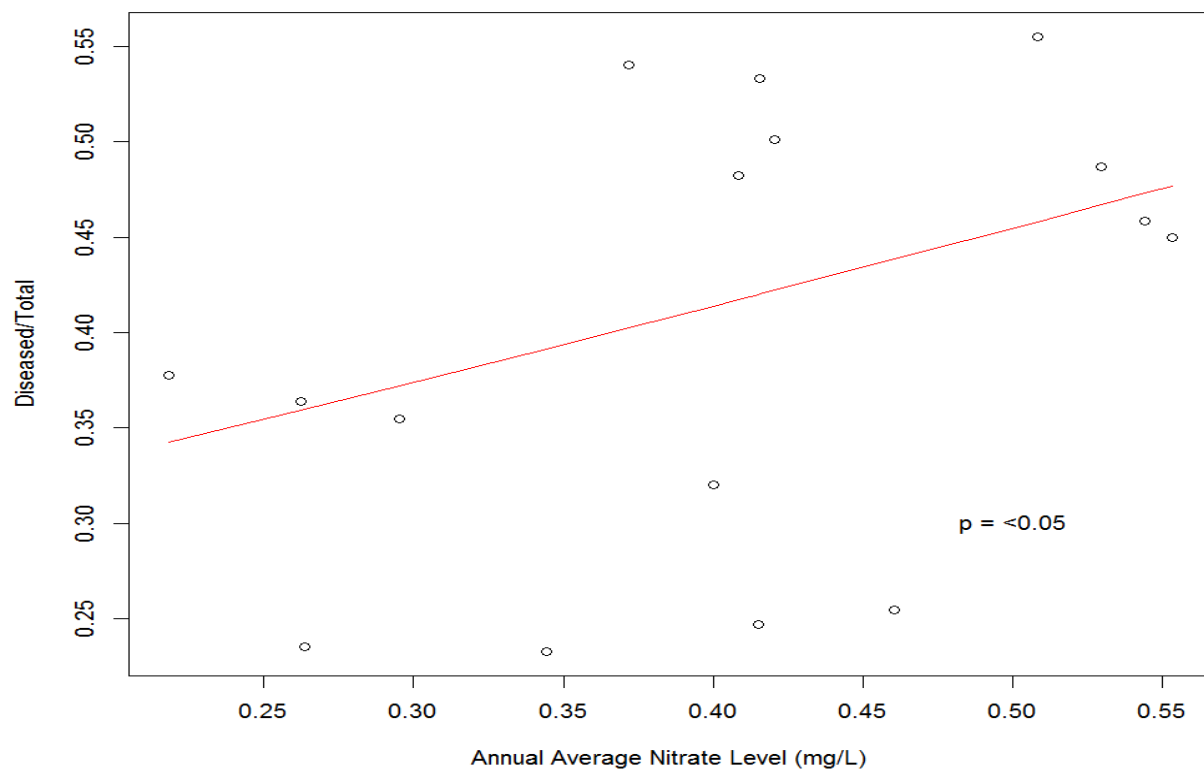
In an effort to address general water quality concerns in the Bay we looked at the three factors targeted by the EPA's Total Maximum Daily Load plan; nitrogen, phosphorus, and sediments in comparison with mycobacteriosis prevalence ("Maryland's Phase I..."). We examined both nitrates and total nitrogen in the water using Chesapeake Bay Program data for the segments of the Bay in which sampling occurred (Figure 2). Both average annual nitrate and total nitrogen values were positively correlated with disease prevalence in the sample population, with higher disease prevalence being associated with both increased nitrate and total nitrogen values (Figures 17, 19). Both of these relationships were found to be statistically significant (Figures 18, 20).

The regression output suggests a 53% increase in the odds of a fish becoming disease positive for every 0.1 mg/L increase in nitrates, and a 55% increase in odds for every 0.1 mg/L

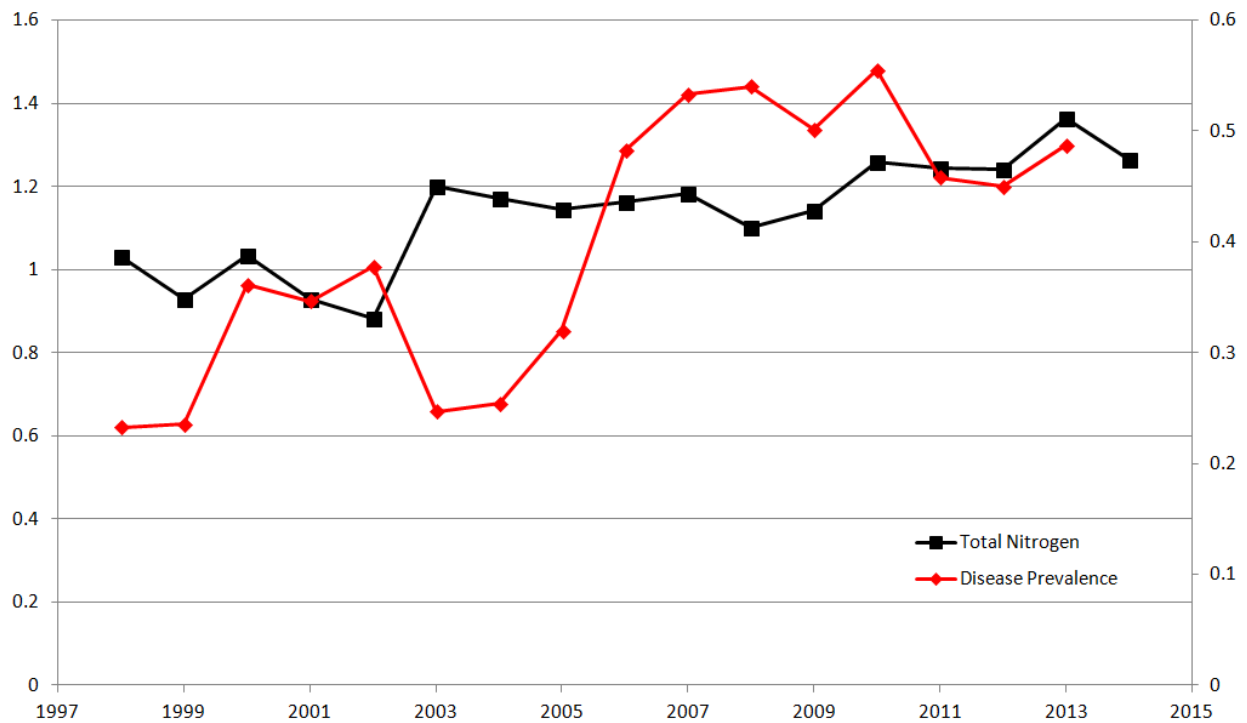
increase in total nitrogen. Since the beginning of Maryland DNR's survey (1998) nitrate and total nitrogen levels have fluctuated by approximately 0.33 mg/L and 0.5 mg/L, respectively.



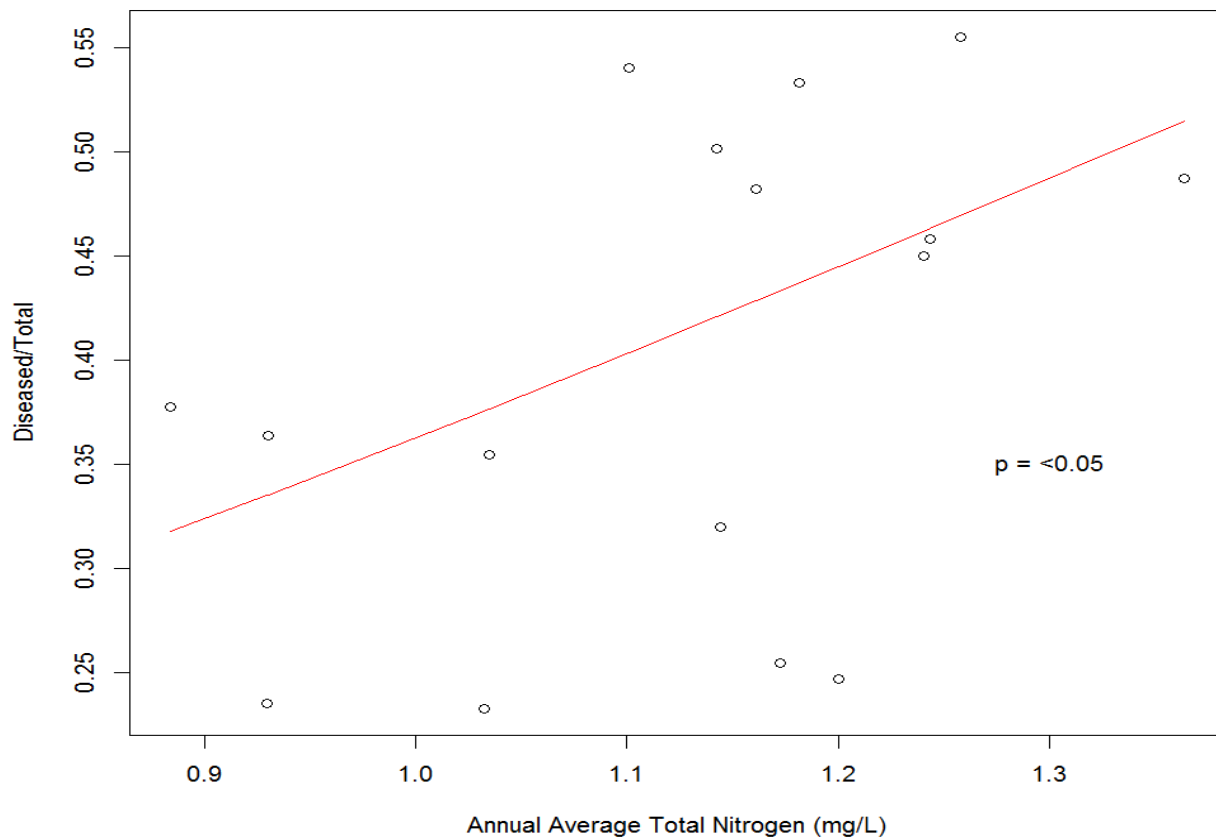
**Figure 17** A comparison between average annual nitrate levels (mg/L) and disease prevalence (age 1-6 fish).



**Figure 18** A logistic regression comparing average annual nitrate levels with disease prevalence in age 1-6 fish. The regression yielded statistical significance with  $p < 0.05$ .



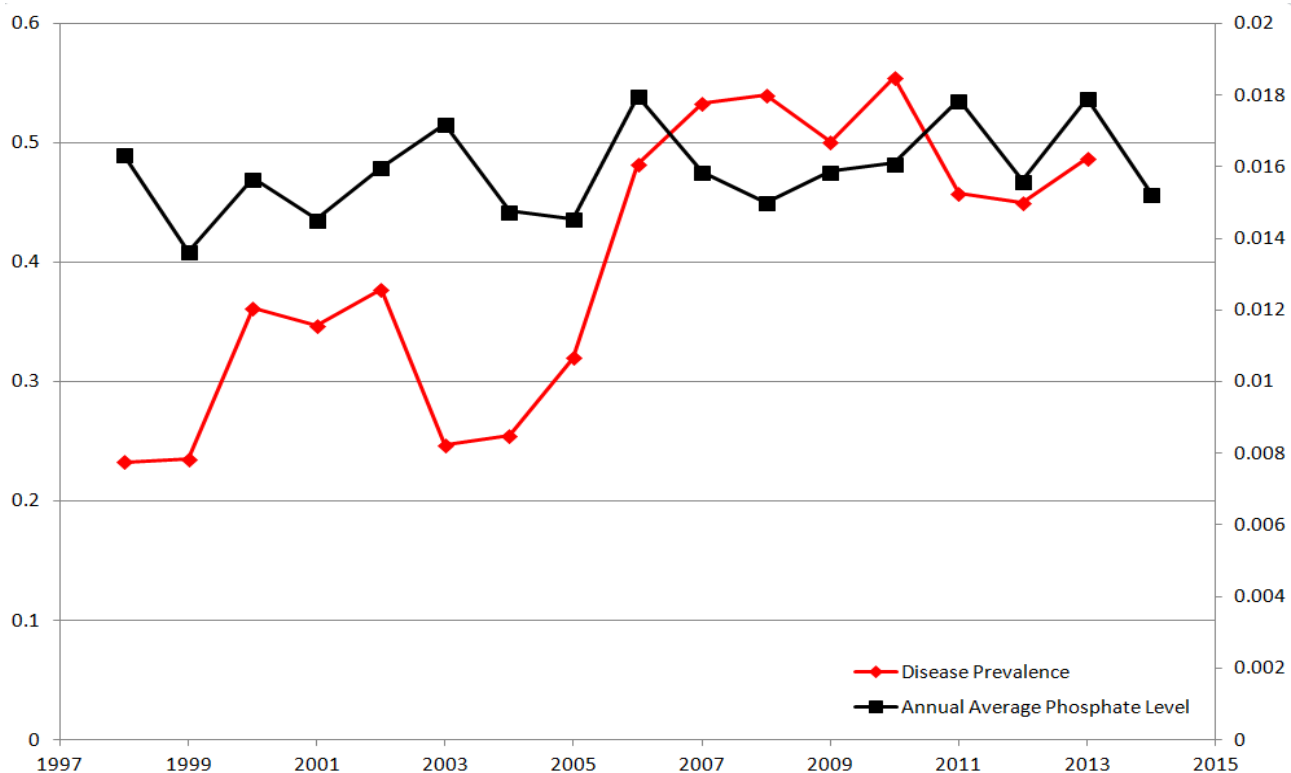
**Figure 19** A comparison between average annual nitrogen levels (mg/L) and disease prevalence (age 1-6 fish).



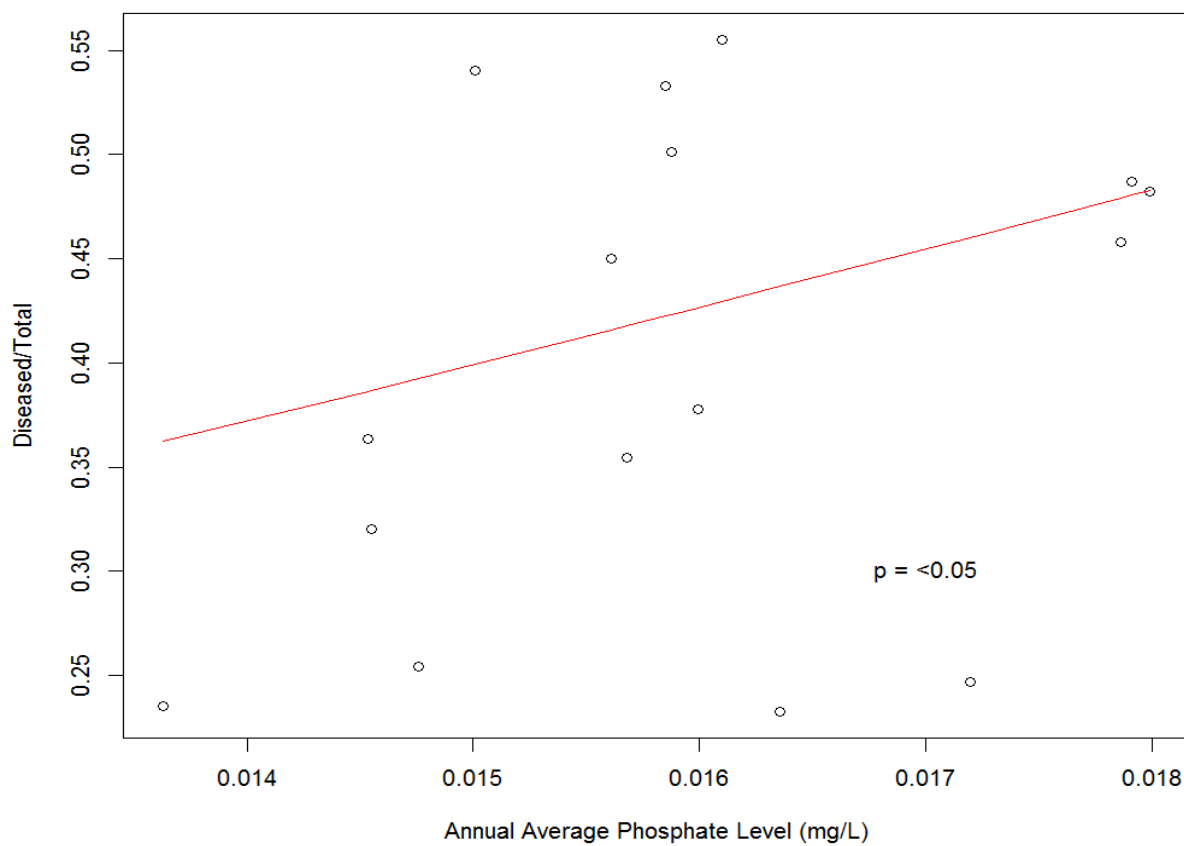
**Figure 20** A logistic regression comparing average annual nitrogen levels with disease prevalence in age 1-6 fish. The regression yielded statistical significance with  $p < 0.05$ .

#### Phosphates/Total Phosphorus

Another nutrient of concern for Bay water quality, both total phosphorus and phosphates were examined as covariates affecting striped bass disease status. Both average annual phosphate and total phosphorus values were shown to have a positive correlation with disease prevalence, with higher prevalence of mycobacteriosis associated with increased levels of phosphorus components (Figures 21, 23). Both of these relationships were proven to be statistically significant, and the resulting logistic regression plots are shown in Figures 22 and 24. Phosphorus values were taken from the Chesapeake Bay Program's Water Quality Data Hub for the segments in which fish were sampled (Figure 2). The regression output suggests a >100x increase in the odds of a fish becoming disease positive for every 0.01 mg/L increase in both phosphates and total phosphorus. Since 1998, phosphate and total phosphorus levels have fluctuated by approximately 0.004 mg/L and 0.01 mg/L, respectively.

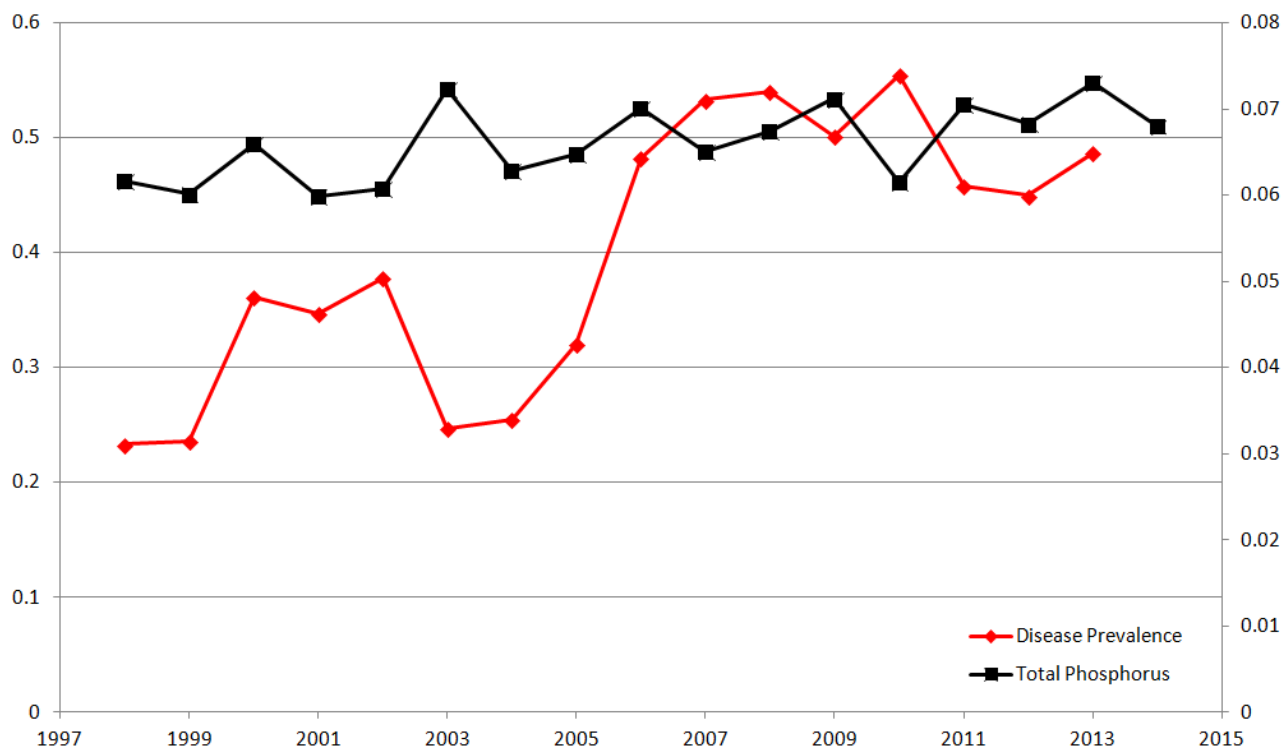


**Figure 21** A comparison between average annual phosphate levels (mg/L) and disease prevalence (age 1-6 fish).

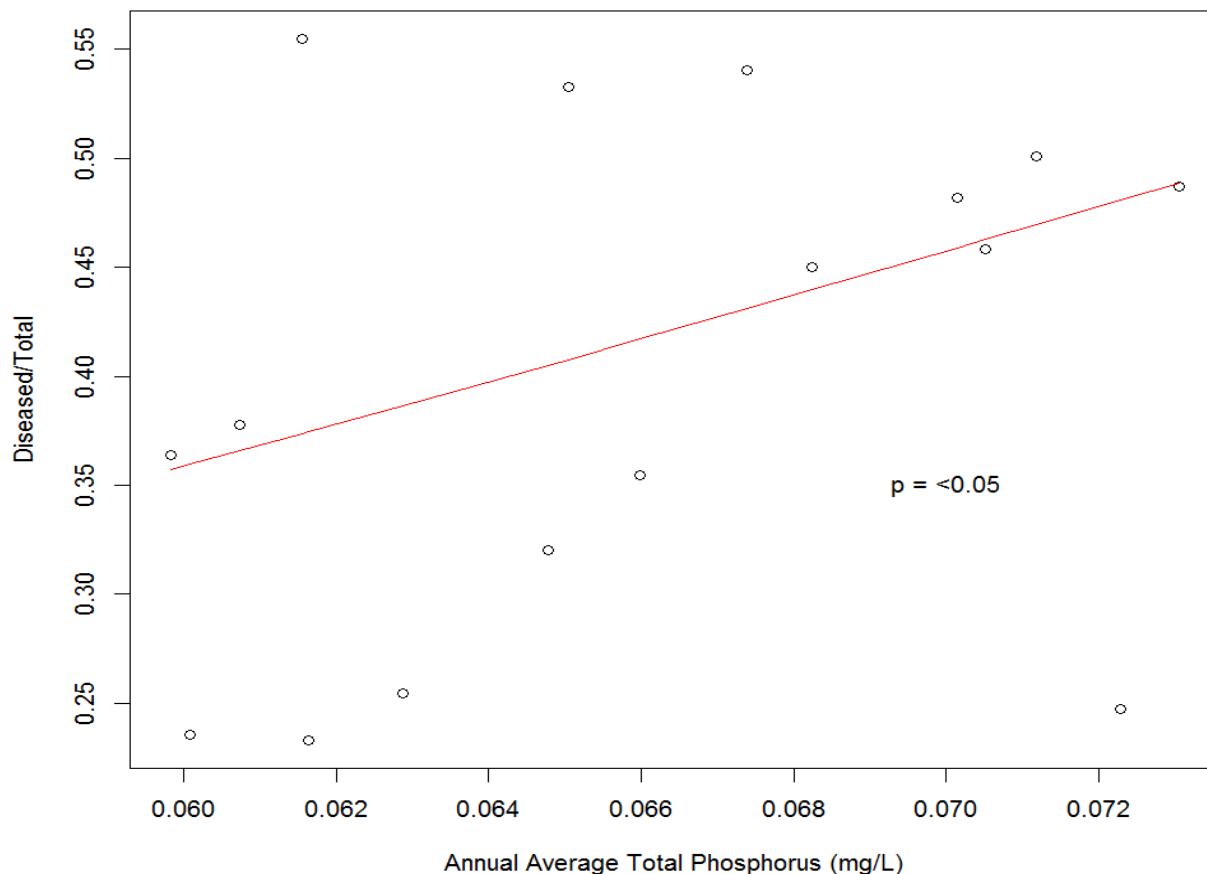


**Figure 22** A logistic regression comparing average annual phosphate levels with disease prevalence in age 1-6 fish. The regression yielded statistical significance with  $p < 0.05$ .





**Figure 23** A comparison between average annual phosphorus levels (mg/L) and disease prevalence (age 1-6 fish).

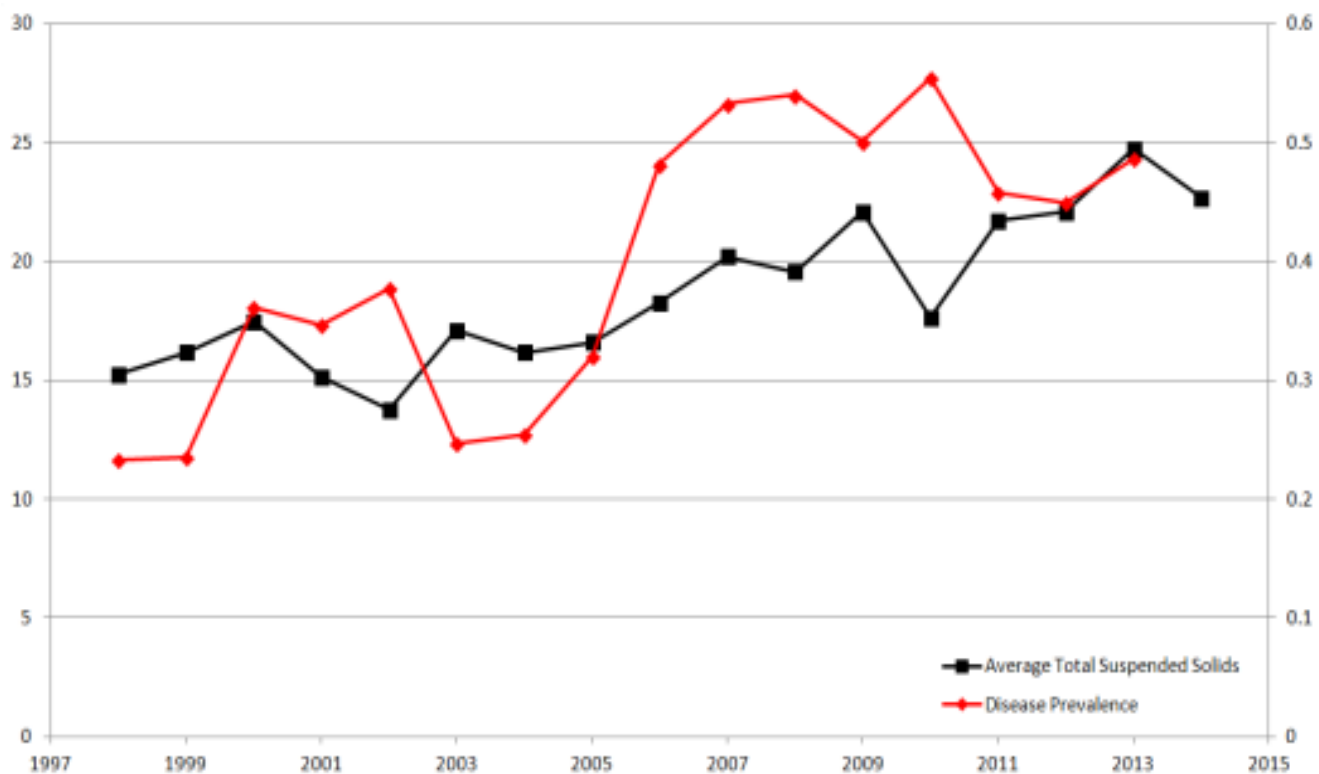


**Figure 24** A logistic regression comparing average annual phosphorus levels with disease prevalence in age 1-6 fish. The regression yielded statistical significance with  $p < 0.05$ .

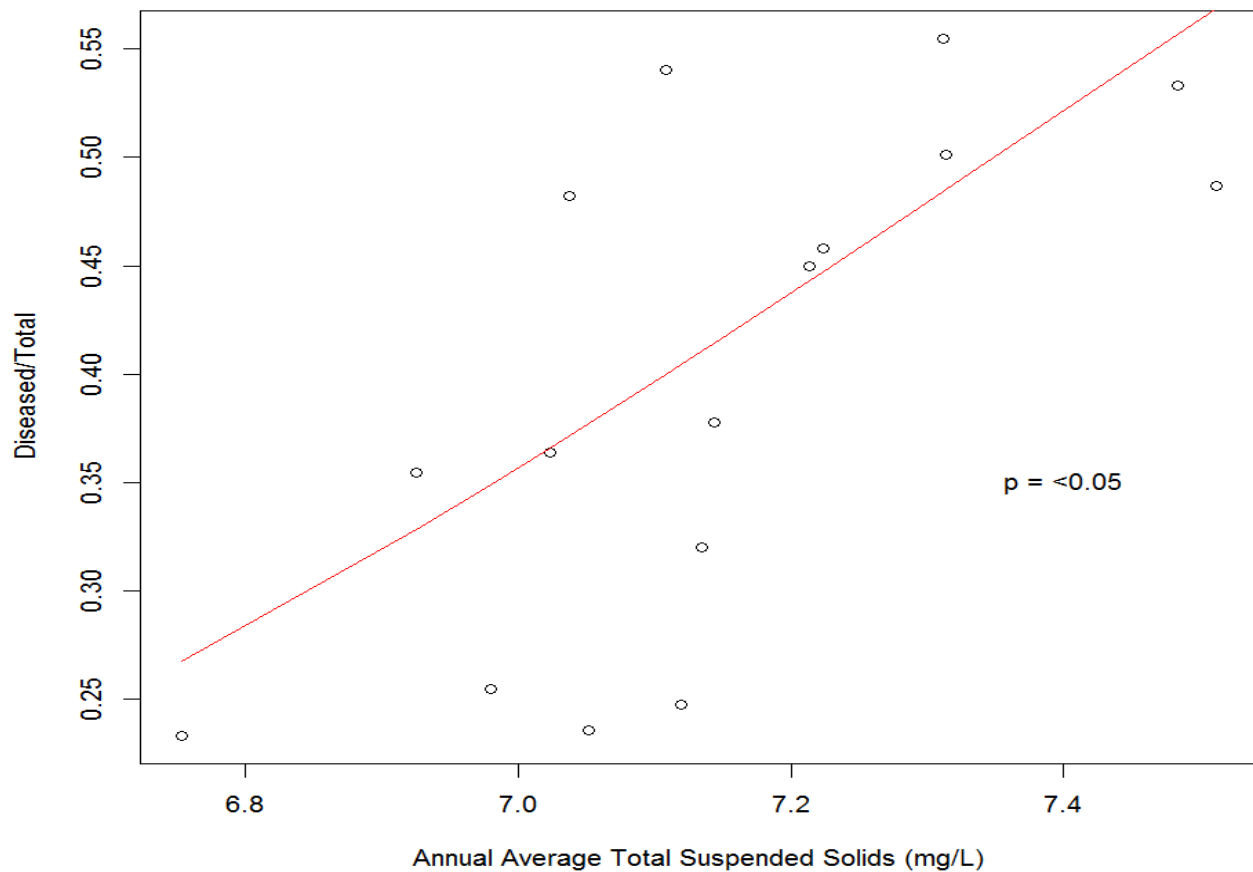
### Total Suspended Solids

In addition to nutrients, sediment loading is a targeted issue of the TMDL program (“Maryland’s Phase I...”). Using Total Suspended Solids (TSS) data taken from the Chesapeake Bay Program at sampling sites, we were able to demonstrate a strong positive relationship between average annual TSS values and the prevalence of mycobacteriosis in striped bass (Figure 25). This was the strongest of all relationships found by this study and was found to be statistically significant by binomial logistic regression (Figure 26).

The regression output suggests a 5.4x increase in the odds of a fish becoming disease positive for every 1 mg/L increase in total suspended solids. Since the start of the survey (1998), total suspended solids levels have fluctuated by approximately 11mg/L.



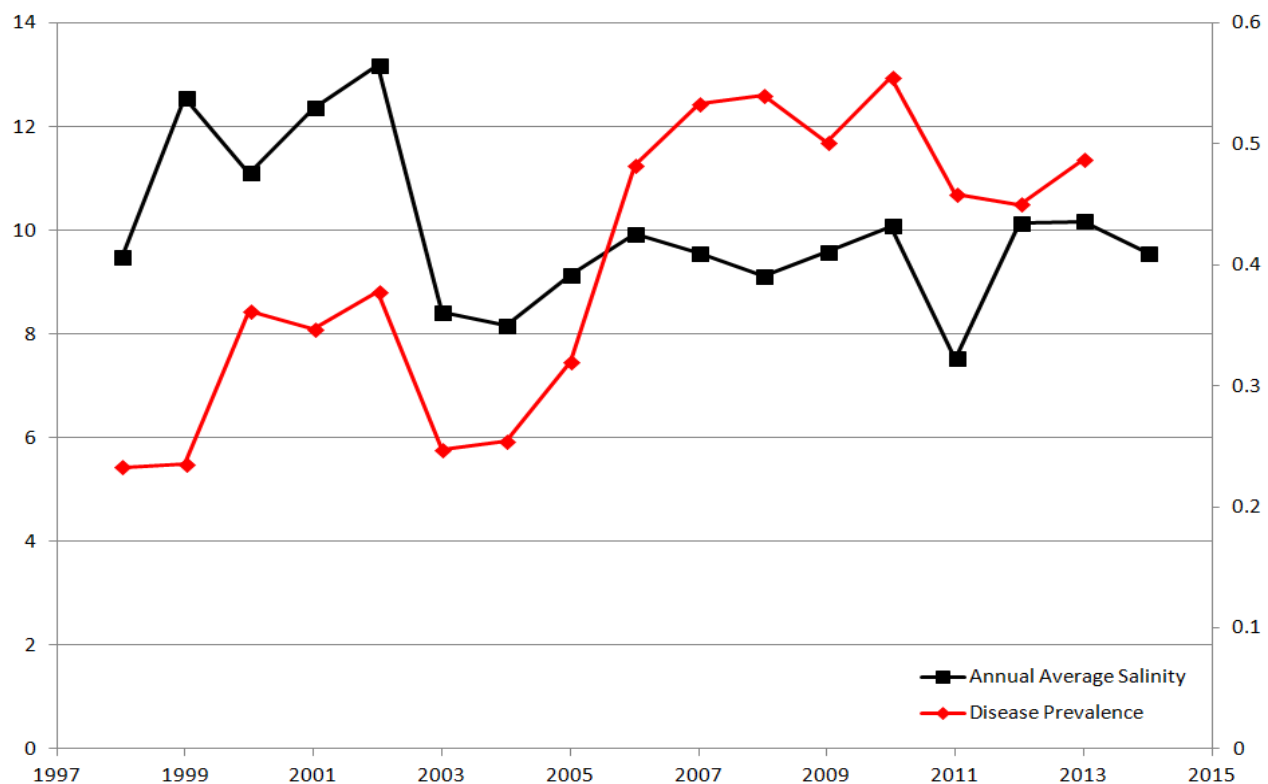
**Figure 25** A comparison between average annual total suspended solids (mg/L) and disease prevalence (age 1-6 fish).



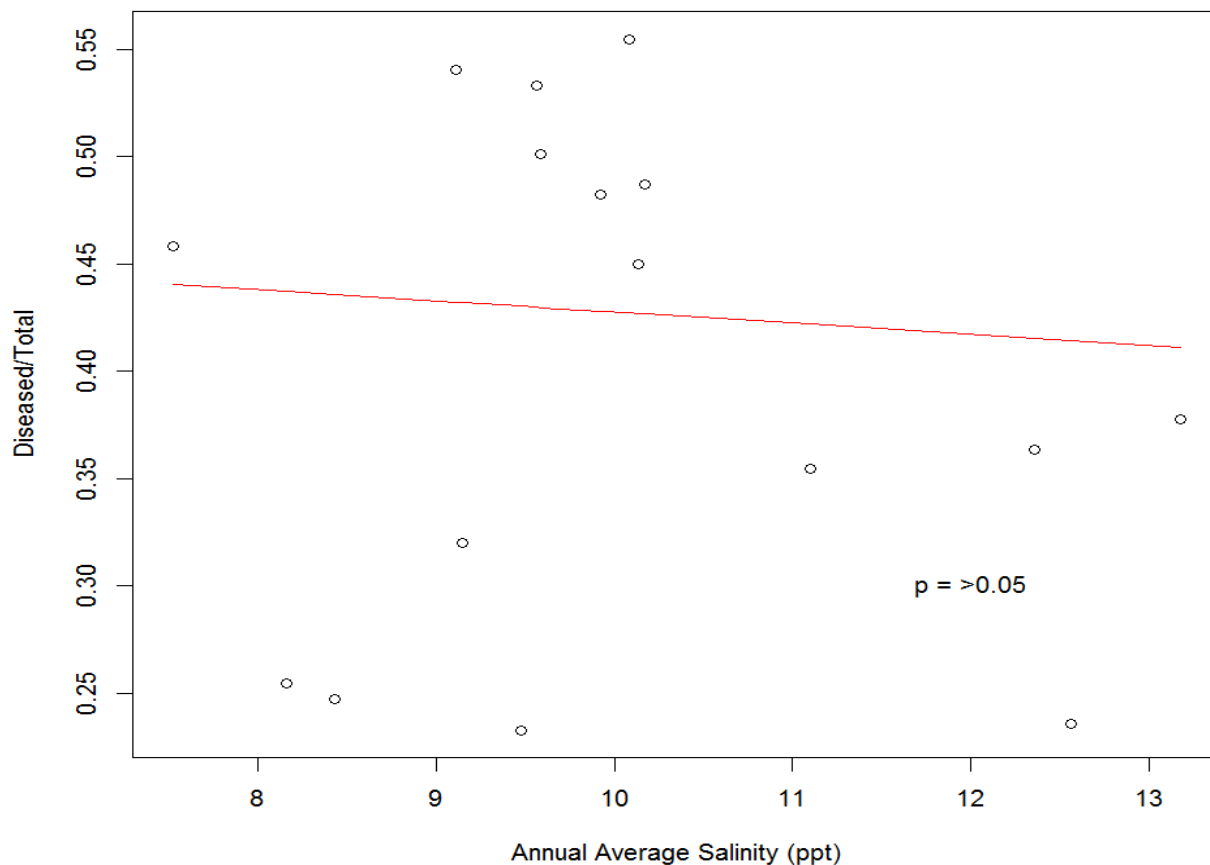
**Figure 26** A logistic regression comparing average annual total suspended solids with disease prevalence in age 1-6 fish. The regression yielded statistical significance with  $p < 0.05$ .

### Salinity

Salinity was examined as a possible covariate in order to address increasing runoff and stream flow input as potentially important drivers of mycobacteriosis. Using salinity data from the Chesapeake Bay Program's water quality data hub for all Bay Segments in which sampling occurred (Figure 2), we were able to conclude that there was no statistically significant relationship between salinity and the prevalence of mycobacteriosis (Figures 27 and 28)



**Figure 27** A comparison between average annual salinity (ppt) and disease prevalence (age 1-6 fish).

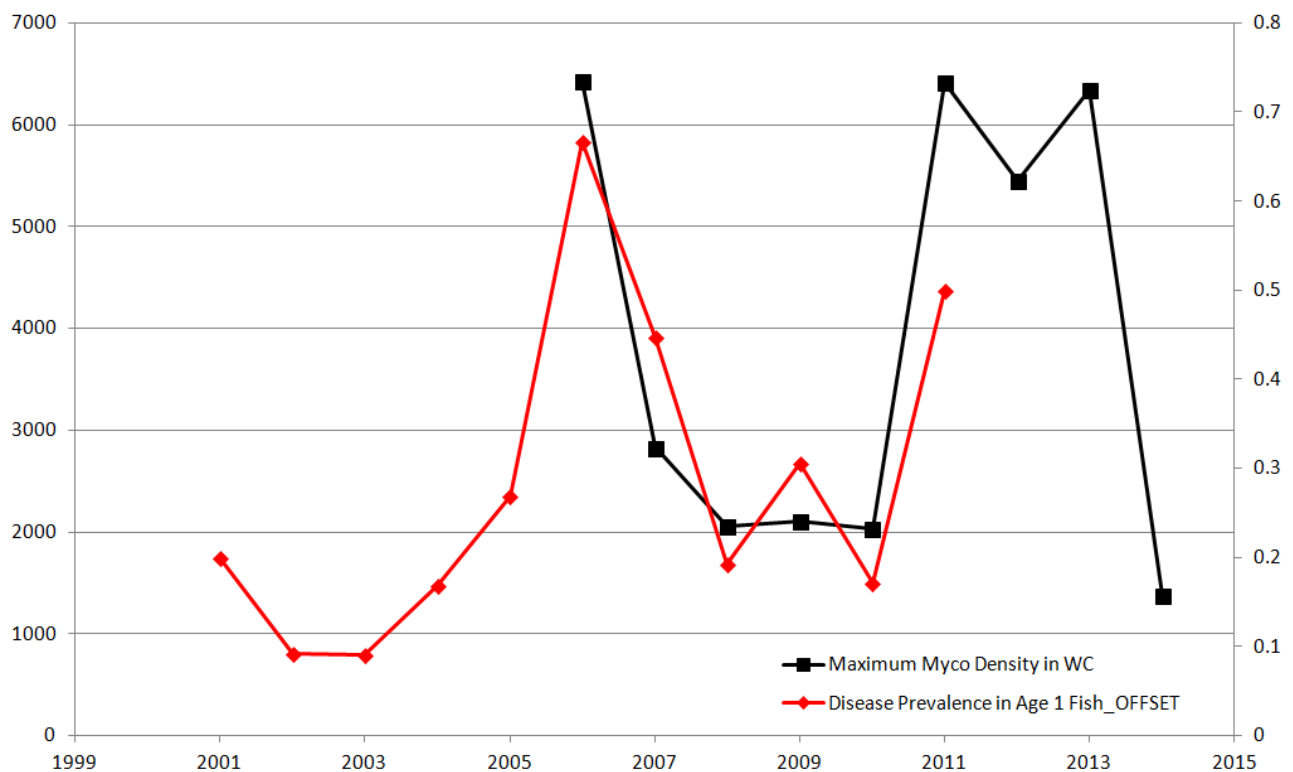


**Figure 28** A logistic regression comparing average annual salinity with disease prevalence in age 1-6 fish. The regression did not yield statistical significance with  $p > 0.05$ .

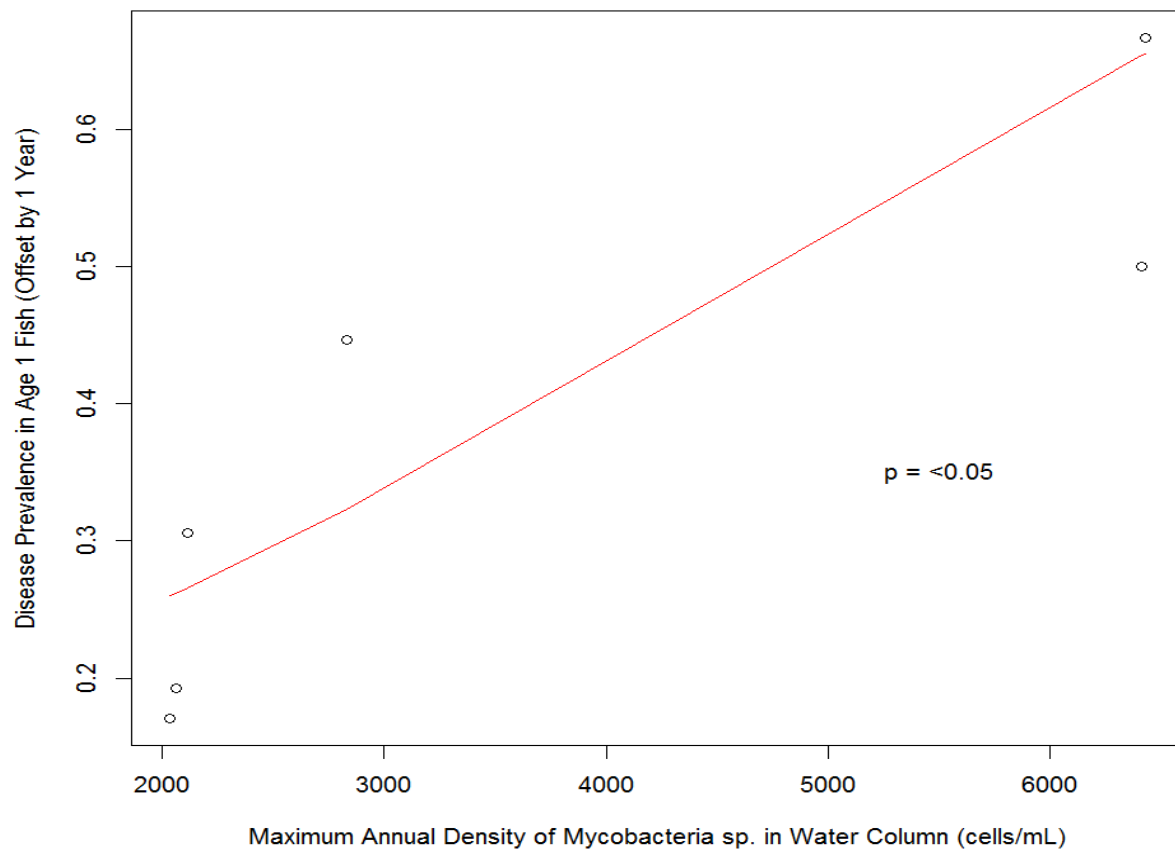
### Bacterial Density in the Water Column

Once statistical significance had been found between mycobacteriosis prevalence and water quality covariates, we quickly came upon the hypothesis that increased nutrient loading may be increasing the density of bacteria in the water column, and therefore increasing the amount of *Mycobacterium* spp. available for infection of striped bass. This was confirmed by a study by Jacobs et al (2009) in which molecular assays were used to evaluate the density (cells/mL) of *Mycobacterium* species in Chesapeake Bay waters, which was then compared to water quality covariates. In accordance with our results, this study found positive statistical relationships between the density of free swimming *Mycobacterium* spp. and nitrates, total nitrogen, total phosphorus, total suspended solids, and water temperature. In contrast to our results, this study determined a negative correlation with dissolved oxygen and salinity, and did

not find any significant correlation with average phosphates. We used the data from this study to examine whether the disease prevalence is correlated with the availability of *Mycobacterium* in the water column. While not correlated with overall prevalence in the striped bass population (ages 1-6), a strong relationship was determined between disease prevalence in age 1 fish and the density of the bacteria in the water column the previous year (Figure 29 and 30). It is important to note that in Figure 29, the data for disease prevalence in age 1 fish has been offset or pushed back by one year; the actual data followed the trend of bacterial density, but lagged behind in response by a year, suggesting the trends of free swimming bacteria were being reflected in the striped bass population once they reached age 1 the following year. The regression output suggests a >100x increase in the odds of an age 1 fish becoming disease positive for every 1000 cell/mL increase in *Mycobacterium* spp. present in the water column the year before. Since the first available data from Jacobs et al (2009), in 2006, the number of *Mycobacterium* in the water column has fluctuated by approximately 5000 cells/mL.



**Figure 29** A comparison between the annual maximum density of *Mycobacterium* spp. in the water column (cells/mL) and disease prevalence (age 1-6 fish). Note that disease prevalence is offset by one year to more easily demonstrate the relationship.



**Figure 30** A logistic regression comparing the annual maximum density of *Mycobacterium* spp. in the water column (cells/mL) and disease prevalence (age 1-6 fish). Note that disease prevalence data was offset by one year to more easily demonstrate the relationship. The regression yielded statistical significance with  $p < 0.05$ .



### Combined Water Quality Model Results

Covariate	Units (scaled)	Odds Increase per Unit	Historical Fluctuations
Nitrates	0.1 mg/L	0.53x	~0.33 mg/L since 1998
Nitrogen (Total)	0.1 mg/L	0.55x	~0.5 mg/L since 1998
Phosphates	0.01 mg/L	>100x	~0.004 mg/L since 1998
Phosphorus (Total)	0.01 mg/L	>100x	~0.01 mg/L since 1998
Temperature	1 °C	1.33x	~2.5 C since 1998
Total Suspended Solids	1 mg/L	5.4x	~11 mg/L since 1998
* <i>Mycobacterium</i> spp. Density	1000 cells/mL	>100x	~5000 cells/mL since 2006

**Table 1** The beta analysis outputs are shown from the regression model run for each environmental covariate. Historical trends in covariate fluctuations are included. Units have been scaled to represent a reasonable increase given historical trends. **Note:** \*Data shown for the density of *Mycobacterium* spp. in the water column represents only the relationship with the following year's age 1 fish.

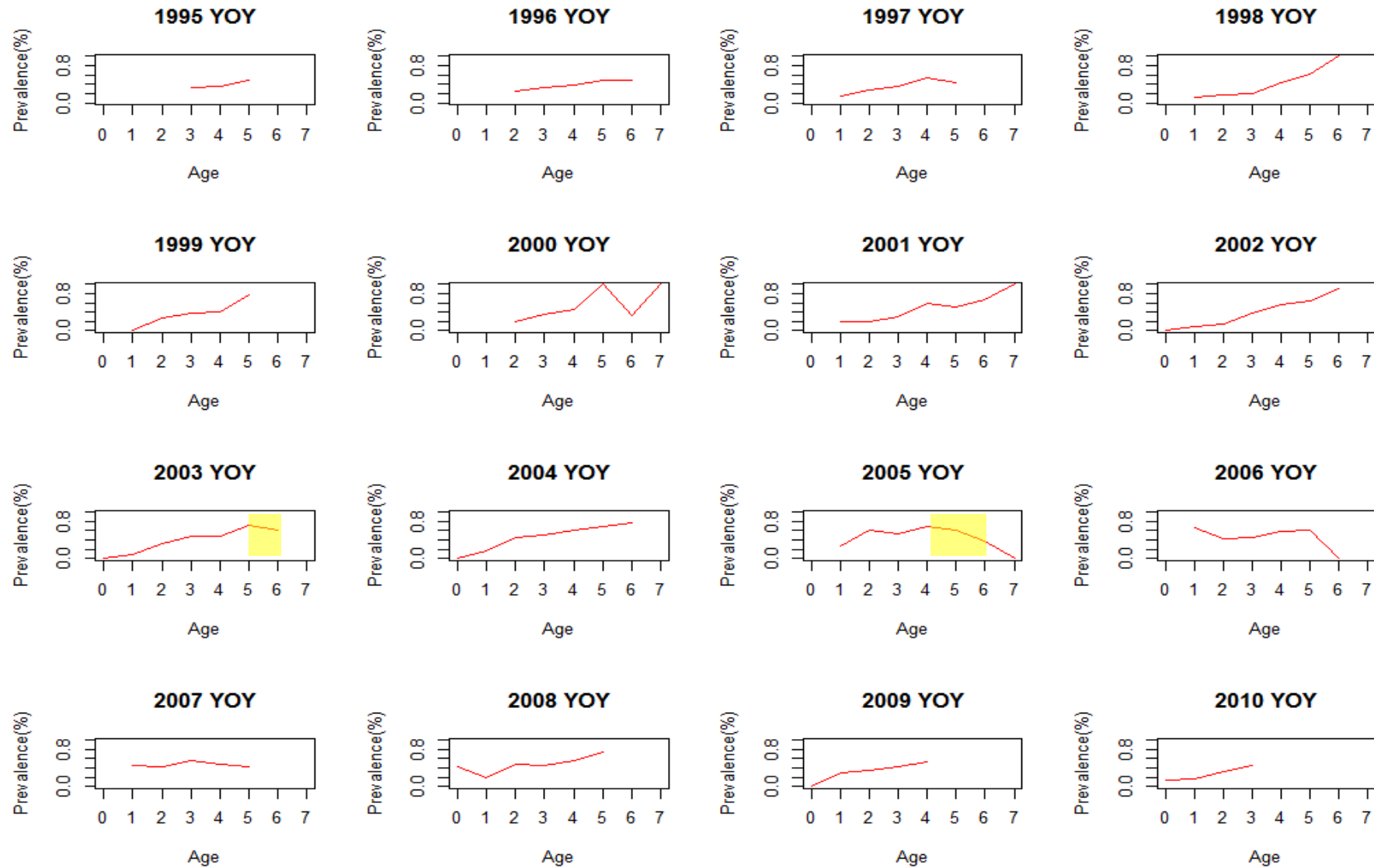
## A Simple Estimation of Disease-Associated Mortality

With the current data from MD DNR Fisheries Service where adequate data on older age classes was available (number sampled  $\geq 20$ ), we were able to calculate  $A_I$  as a first step towards estimating the impacts of mycobacteriosis on the production of striped bass in the MD portion of the Chesapeake. These values are conservative estimates of the proportion of annual mortality associated with mycobacterial infection. Confidence intervals around the point estimates were calculated from Fleiss et al's (2003) binomial approximation of the normal curve (Table 2). The cohort data used for these calculations is highlighted in Figure 31. While additional ages within cohorts demonstrated the decrease in infection interpreted as mortality, these were not analyzed due to small sample sizes.

Cohort	Age Range	$A_I$	LCI (95%)	UCI (95%)
2003	5 to 6	<b>0.1382</b>	0.0509	0.2256
2005	4 to 5	<b>0.0999</b>	0.0554	0.1444
2005	5 to 6	<b>0.3906</b>	0.3029	0.4784

**Table 2** Simple estimates of disease-associated mortality are shown for three age classes from two different cohorts (see Figure 31).  $A_I$  can be interpreted as the proportion of mortality that can be attributed to infection with mycobacteriosis.

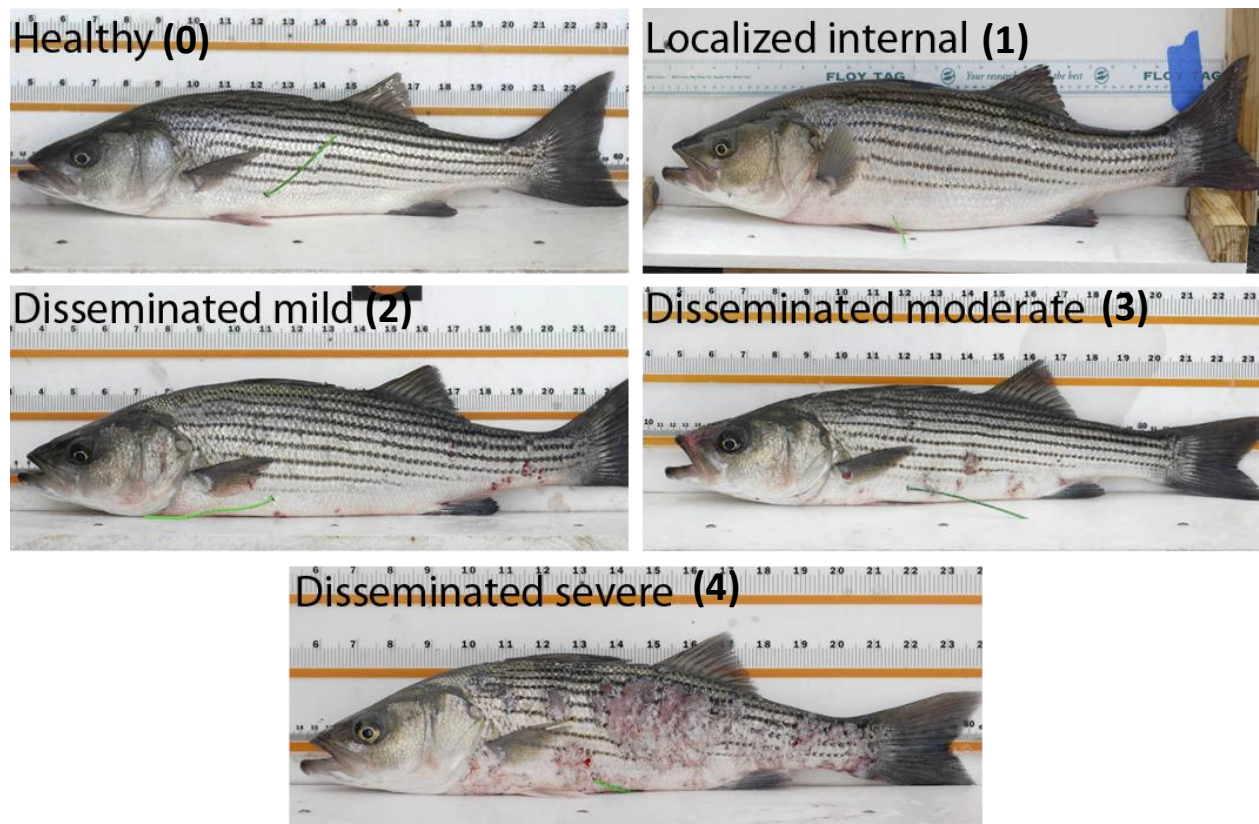
### Proportion Infected Over Time by Cohort



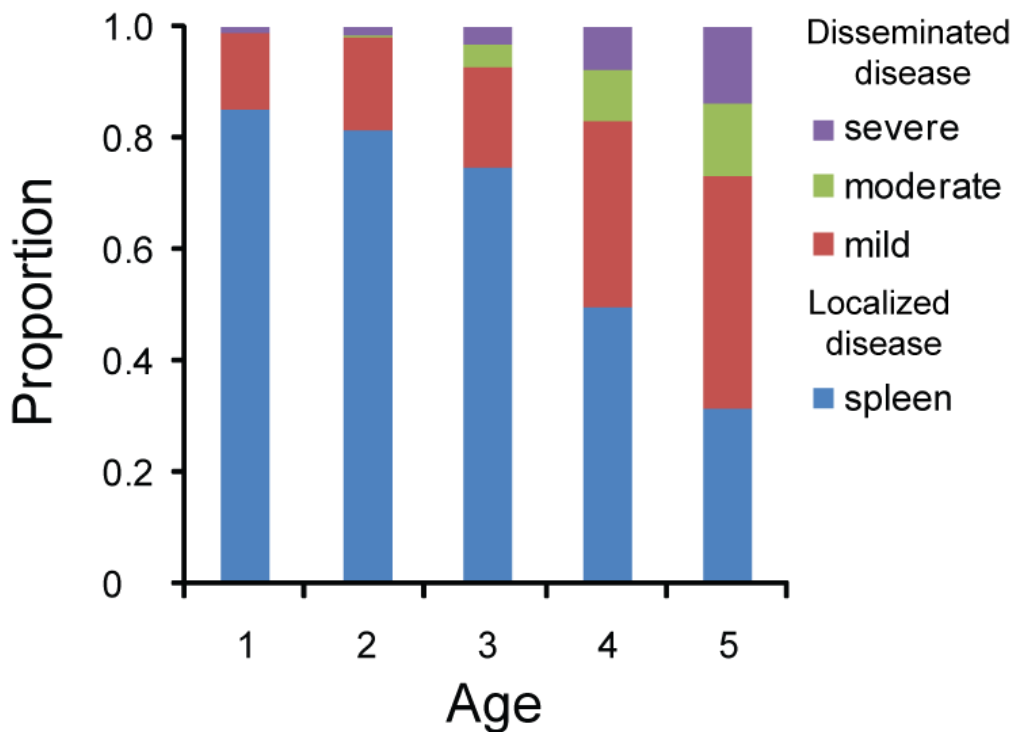
**Figure 31** Disease prevalence over time, by cohort class. Note the highlighted decreases in prevalence used to provide disease-associated mortality estimates (Table 2).

## Additional Analyses

Although apparent prevalence was selected, the severity of mycobacterial infection was also examined as a potential indicator of fish health over time. Severity rankings are given by Maryland DNR's Fish and Wildlife Health Team as they process surveyed fish, and are based on the presence of AF+ bacteria and the appearance of external ulcerous lesions. Rankings of severity range from a score of 0, being a healthy fish, to a score of 4, being an obviously severely diseased animal with a large number of external lesions. When the disease is only present internally in the primary target organ, the spleen, we consider the disease to be 'localized'. Fish with localized disease are given a severity ranking of 1. As the disease progresses and spreads to other organs or presents itself in external lesions we consider the disease to be 'disseminated'. Fish showing disseminated disease are given severity rankings from 2-4, depending on the observed severity of infection. A visual representation of these rankings is provided by Figure 32. Figure 33 shows the frequency of each severity score across age classes for infected fish, and demonstrates the progression of disease with age.



**Figure 32** Visualizations of each severity ranking as assigned by Maryland DNR's Fish and Wildlife Health Program.



**Figure 33** Frequency distribution of each severity score across age classes for infected fish. Note the progression in severity with increasing age.

### Meetings and Workshops

In order to get feedback on project plans and to improve the final product, several meetings were conducted within the first few months of the project period with experts in mycobacteriosis and fisheries modeling. The first meeting was held March 31, 2015 at the Cooperative Oxford Laboratory in Oxford, MD. Attendees included Jim Uphoff and Alexei Sharov from Maryland DNR, John Jacobs from NOAA, and Chris Ottinger from USGS. Also from the Oxford Lab, Lonnie Gonsalves of NOAA was met with at a later date. A brief presentation was given regarding the background of the survey, initial data exploration, and thoughts on covariates to include in future analyses. The attendees provided incredibly helpful feedback including additional covariates and data sources to consider. Prominent feedback regarded the examination of forage fish availability over time as it relates to fish health and susceptibility to disease, and the species composition and pathogenicity of mycobacteria in the Bay.

A trip was also made to the Virginia Institute of Marine Science at the College of William and Mary in April 2015 to meet with Mary Fabrizio, Rob Latour and a graduate student, and Wolfgang Vogelbein. The statistical models used for estimating force-of-infection and disease-associated mortality were discussed at length with Rob Latour, and all meetings followed

the same general outline as the Oxford workshop. We were met with great enthusiasm for the project by all parties, and discussed the possibility of future collaboration in order to incorporate Virginia data into a Bay-wide indicator.

Once sufficient analysis was underway, a presentation of project progress was given to the Sustainable Fisheries Goal Implementation Team Semiannual meeting in June. Results presented included general prevalence data, trends in severity and prevalence within cohorts over time, and some preliminary examinations using water quality covariates. Many of these comparisons will be presented later in this report. In general the presentation was met with interest from attendees.

With the exception of the mortality estimates derived from cohort analysis, many final results were presented on a poster at the American Fisheries Society Annual Meeting in Portland, OR in August. The results presented on the poster focused on the relationships between overall disease prevalence and various water quality factors, which will be discussed in the next section.

## **Conclusions**

Through the statistical models employed in this study, we conclude that the prevalence of mycobacteriosis in Maryland's Chesapeake Bay striped bass is correlated with several water quality factors such as water temperature, nitrogen, phosphorus, and total suspended solids (see Table 1). In addition, through literature review we have determined that this increase in prevalence is likely due to an increase in the availability of *Mycobacterium* spp. in the water column (Jacobs et al 2009). More specifically, we believe that young of the year fish are contracting the disease from free-swimming bacteria in the water, leading to an observable increase in age 1 prevalence the following year.

Disease-associated mortality, or the proportion of mortality that can be attributed to mycobacterial infection, was estimated at ~10-40%. While these values are likely conservative, they provide a reasonable starting place towards estimating the impacts of mycobacteriosis on the production of striped bass in the Maryland portion of the Chesapeake Bay.

## **Recommended Next Steps**

Sampling should be continued through Maryland DNR's Striped Bass Survey in order to continue examining the trends and correlations between mycobacteriosis prevalence and water quality covariates. While this study has provided an interesting narrative regarding the progression of mycobacteriosis in Maryland Chesapeake Bay striped bass, this study should be combined with data from the Virginia portion of the bay in order to develop a Bay-wide story of infection. An ongoing survey conducted by the Virginia Institute of Marine Science collects very similar data to Maryland DNR's survey, but due to a lack of funding they are behind on

processing the data and laboratory samples necessary to have a comprehensive, up to date data set such as that in Maryland. Because of this, future funding for this work should be directed to the Virginia portion of the Bay in order to accomplish a Bay-wide data set.

In addition, future sampling work should be modified to include the collection of larger, older age class fish. Because of the lack of an appropriate sample size for fish aged 7 and older in this data set (see Figure 5), disease-associated mortality could not be estimated through a force-of-infection modeling approach, and so was estimated using a simple, conservative method. Efforts should be focused on improving the sample population of these older fish in order to work towards more comprehensive annual estimates of mortality associated with mycobacterial infection.

Final project results will be presented at the STAR monthly meeting on October 22<sup>nd</sup> in Annapolis, MD.

## Acknowledgements

Howard Townsend, NOAA Chesapeake Bay Office

Mark Matsche, Maryland Department of Natural Resources

John Jacobs, NOAA National Ocean Service





## Literature Cited

- Bruno, D. W., J. Griffiths, et al. (1998). "Pathology attributed to *Mycobacterium chelonae* infection among farmed and laboratory-infected Atlantic salmon *Salmo salar*". *Diseases of Aquatic Organisms* 33: 101-109.
- Fleiss, JL, B Levin, and MC Paik. 2003. *Statistical methods for rates and proportions*. 3<sup>rd</sup> ed. John Wiley & Sons Hoboken, New Jersey.
- Gauthier, D. T., M. W. Rhodes, et al. (2003). "Experimental mycobacteriosis in striped bass *Morone saxatilis*". *Diseases of Aquatic Organisms* 54: 105-117.
- Gauthier, D.T., R.J. Latour, et al (2008). "Mycobacteriosis-Associated Mortality in Wild Striped Bass (*Morone saxatilis*) from Chesapeake Bay, USA". *Ecological Applications* 18(7): 1718-1727.
- Heckert R, Elankumaran S, Milani A, Baya A (2001) "Detection of a new *Mycobacterium* species in wild striped bass in the Chesapeake Bay". *Journal of Clinical Microbiology* 39: 710-15.
- Hines, M., J. Kreegar, et al. (1995). "Mycobacterial Infections of Animals: Pathology and Pathogenesis". *Laboratory Animal Science* 45(4).
- Jacobs, John, Matt Rhodes, et al. (2009). "Influence of Environmental Gradients on the Abundance and Distribution of *Mycobacterium* spp. in a Coastal Lagoon Estuary". *Applied and Environmental Microbiology* 75(23): 7378-7384.
- Rhodes MW, Kator H, et al. (2001). "A unique *Mycobacterium* species isolated from an epizootic of striped bass (*Morone saxatilis*)". *Emerging Infectious Diseases* 7: 896-899.
- State of Maryland. "Maryland's Phase I Watershed Implementation Plan for the Chesapeake Bay Total Maximum Daily Load" (2010).