Response of the Common Mummichog (*Fundulus heteroclitus*) to Water Quality Changes in Long Island Sound Tributaries

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Abstract

Long Island Sound is a complex watershed which lies between Connecticut and New York. Surrounded by high concentrations of industrial and residential properties, Long Island Sound is extremely susceptible to water quality changes. The complexity of the watershed makes it difficult to accurately measure real time impacts to water quality and the subsequent impacts to aquatic species. One method of assessing water quality is the monitoring of indicator species. In particular, the fish species mummichog may be viable for monitoring water quality parameters. The common mummichog is an abundant species within Long Island Sound that possesses several qualities which may make them suitable as an indicator species. This project aimed to identify which water quality parameters, if any, for which the mummichog would be suitable for use as an indicator species. To investigate this, water quality parameters and mummichog population density were monitored at two separate sites. Of the parameters monitored as part of this project, it was found that salinity levels exhibit a non-linear correlation to the presence and density of mummichogs. Specifically, mummichogs were present at all observed salinity levels above 1.003 specific gravity. However, the maximum number of mummichogs was observed at a specific gravity of 1.019. Above this salinity, the number of mummichogs collected began to decrease. The Long Island Sound Study reports that the average salinity of the Long Island Sound is 1.026 specific gravity. The influx of freshwater into the watershed likely plays a vital role in diffusing salinity to levels within the estuaries that are tolerable by the mummichog. Further research is needed to better quantify the mummichog’s response to changing salinity levels and the potential water quality impacts due to anthropogenic sources.

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1. Introduction

Long Island Sound is a unique estuary which is situated between the coast of Connecticut and the north shore of Long Island, New York. Covering an area of 1,320 square miles and with over 600 miles of shoreline, Long Island Sound is an extremely important ecological resource to the New England area (DEEP 2016). Long Island Sound is considered an estuary as it receives a steady influx of salt water from the Atlantic Ocean and fresh water from several large river basins, including the Connecticut River, which carries water from as far as Quebec, Canada. Along the shores of Long Island Sound, there are many salt marshes and smaller, brackish estuaries which serve as vital cover, feeding, and spawning areas for numerous aquatic species (including mammals, fish, crustaceans, and shellfish) (Stierhoff et al. 2009). Since the earliest recorded history, estuaries have been important for settlement and resource use; resulting in their subjection to numerous point and non-point human-induced pollution sources. Estimates indicate that 67% of wetlands and 65% of sea grasses have been lost as a result of human-generated cumulative impacts since colonial establishment of New England (Lotze et al. 2006). Marine estuary preservation continues to be the focus of management groups around Long Island Sound.

One tool which can be utilized for the measure of ecosystem quality is the monitoring of an indicator species (Schlacher et al. 2014). Indicator species are organisms which, by way of their abundance, chemical composition, or habits, illustrate an aspect of a character or quality of an ecosystem (Freedman 2014). The most critical factor when selecting an indicator species is ensuring that the selected organisms accurately reflect the condition(s) being monitored (Schlacher et al. 2014).

The northern mummichog (*Fundulus heteroclitus macrolepidotus)* is a subspecies of *Fundulus heteroclitus* found from Connecticut to Newfoundland and may be an excellent candidate for use as an indicator species in Long Island Sound because of its limited range of movement and tendency to rely on the same locations for spawning. In addition, population density of the mummichog has been shown to be responsive to changing water conditions (Finley et al. 2013). This project is aimed at determining which specific water quality parameters (pH, salinity, temperature, dissolved oxygen, nitrogen, and phosphate) have a direct, measurable impact on the population density of the mummichog in the estuaries of Long Island Sound; for the purpose of identifying parameters for which the northern mummichog may serve as a viable indicator species and recommendations for further research work.

2. Review of Literature

There are several key water quality parameters which are intensively monitored across Long Island Sound; including dissolved oxygen, nitrogen, phosphate, salinity, and temperature. Traditionally, these parameters are monitored through the analytical analysis of water samples taken by various organizations, such as the Connecticut Department of Energy and Environmental Protection, University of Connecticut, and the Long Island Sound Study group**.**

The Mummichog (Fundulus heteroclitus)

The mummichog is a small fish of the *Fundulus* genus which can grow up to 7 inches in length and is olive green to blue with white undersides. Males of the species exhibit irregular silver bars on their sides. Research has shown that there are two subspecies of the common mummichod (*Fundulus heteroclitus)*. The northern mummichog (*Fundulus heteroclitus macrolepidotus)* is found from Connecticut to Newfoundland and the southern mummichog (*Fundulus heteroclitus heteroclitus*) ranges from New Jersey to Florida (Finley at al. 2009; Able and Felley 1986). The northern mummichog is a year round resident of the marshes and tidal creeks along Long Island Sound but is most active in the spring, summer, and fall. They are omnivorous and are known to feed on plankton, insect larvae, crustaceans, and dead fish (URI 2017).

The mummichogs limited range of regular movement and continual use of the same reproductive area provide a consistent population from which samples can be taken (Finley et al. 2013; Theriault et al. 2007**;** Skinner et al. 2005). This also reduces the likelihood of external impacts due to migration outside the area of study. Further, the mummichogs limited range of movement throughout the course of the year allow for the selection of a smaller sample area for monitoring versus a species which can travel great distances (Finley et al. 2013; Theriault et al. 2007).

Nutrient Loading and Dissolved Oxygen

Dissolved oxygen levels within Long Island Sound have been thoroughly investigated and documented. Of specific concern across most of Long Island Sound is hypoxia. Generally, a body of water is considered hypoxic when the dissolved oxygen level falls below 4 mg/l and severely hypoxic below 2 mg/l. Although hypoxia is naturally occurring and can fluctuate seasonally, human activities (such as the discharge of nutrient-rich waste materials) can greatly influence the dissolved oxygen levels of a given water body. The impacts of hypoxic conditions can range from direct mortality of aquatic organisms to disruption of the food chain (Stierhoff et al. 2009; Peterson et al. 2000). Within Long Island Sound and its estuaries, hypoxic conditions have been blamed for several large scale die-offs. Most notably, decreasing oxygen levels within Long Island Sound has been indicated as a possible cause of the rapid decline in the population of the American Lobster (Homarus americanus) (Mukherjee et al. 2016). In addition, Mukherjee et al. (2016) found that hypoxic conditions have long term impacts on marine species which last longer than the hypoxic period itself.

Although salt marshes have been found to be very effective at fixing excess nutrients, extreme or long term loading can reduce the efficiency of this process (Vivanco et al. 2015). Excess nutrients which enter the water column can result in the rapid and excessive growth of algae. Once the excess nutrients are consumed, the algae begin to die and bacteria then facilitate the decomposition process; which in turn consumes oxygen. This can directly result in the rapid generation of hypoxic conditions (Mukherjee et al. 2016). In addition to acute affects, dissolved oxygen levels were found to impact the rate of growth of several species which seasonally frequent the studied marshes (Stierhoff et al. 2009). While hypoxic conditions can be extremely detrimental to the habitat as a whole, it has been found that mummichogs often thrive in the nutrient rich conditions which precede hypoxia (Finley et al. 2009). Increased nutrient loading can lead to increased algae growth.

A strong preference to habitation within and around vegetation has been observed with mummichogs; which serves as an explanation for their increase in numbers during nutrient loading (Finley et al. 2009). However, a study conducted in Canada found that the mummichog was significantly impacted once eutrophication occurred (Finley et. al 2013).

Salinity

While nutrient levels within salt marshes along Long Island Sound are of constant focus of research and regulatory bodies, less attention has been given to the fluctuation of salinity and its associated impacts to aquatic organisms. Long Island Sound experiences diurnal tides; meaning there are generally two periods of low water and two periods of high water in each 24-hour period. The infiltration of sea water into marsh and estuarine areas as the tide rises causes an increase in salinity (Marshall et al. 2016). The average salinity of Long Island Sound in the vicinity of the sample sites is 1.026 specific gravity (Long Island Sound Study 2018). The fluctuation of salinity is also driven, in part, by human activities. Discharges of fresh water to marsh areas occur due to many causes, including storm water run-off from impervious surfaces, malfunctioning septic systems, intentional water discharge, etc. Incoming tidal salt water and freshwater inflow to these estuarial areas results in the maintenance of a brackish aquatic environment. In previous studies, it has been found that the mummichog tends to prefer areas of greater salinity over those closer to fresh water (Marshall et al. 2016; Fangue et al. 2006; Fritz and Garside 1975). The exception to this is that ovulating female mummichogs tended to spend more time in lower salinity during laboratory trials (Marshall et al. 2016).

Temperature

A parameter which is frequently monitored in Long Island Sound is water temperature. Surface and bottom water temperatures have recently increased and have been indicated as a possible cause of a wide spread American Lobster die-off which began in 1999 (Rice and Stewart 2016; Wilson and Swanson 2005; Howell et al. 2005). Increasing water temperatures have also allowed for the northward spread of several finfish and crustaceans which were historically confined to more temperate areas (Howell et al. 2005). The mummichog spawning period is initiated by increasing water temperatures in the spring, with the ideal temperature for reproductive potential between 7°C and 11°C (Shimizu 2003). Further research may indicate that artificial fluctuation of water temperatures due to runoff entering estuaries could result in impacts to the reproductive cycle.

pH

Increasing emissions from human activities and the resulting elevated atmospheric carbon dioxide levels are resulting in a slow acidification (lowering of pH) of the world's oceans (Perry et al. 2015). Another cause of acidification is the microbial decomposition of organic matter. As discussed previously, this is the primary cause of hypoxic conditions. However, the decomposition process also releases carbon dioxide into the water column which decreases the pH over time (Wallace et al. 2014). The effects of reduced pH levels within Long Island Sound alone were found to have only minor impacts to juvenile scup (Stenotomus chrysops), a finfish who frequently inhabits the same estuaries as the mummichog (Perry et al. 2015).

Pollution

During previously conducted studies, the mummichog has been found to be resistant to many aquatic pollutants and contaminants. Specifically, the mummichog was found to be most tolerant of soaps and oils while insecticides were found to be most toxic (Eisler 1986). In addition, it has been found that water pollution can affect the mummichog's ability to forage. Mummichogs residing in waterbodies contaminated with mercury were found to exhibit a slower prey capture rate than mummichogs in non-polluted areas (Smith and Weis 1997). Due to the wide array of possible marine pollutants, this project cannot account for all variables.

The identification of a viable indicator species for the above discussed water quality parameters could increase the information available to all interested parties (such as regulatory bodies, advisory groups, and environmental advocacy groups) and reduce the need for frequent and costly chemical analysis. Specifically, the presence and density of the mummichogs in response to changes in the discussed parameters will be documented with the goal of focusing future research work.

3. Project Methodology and Design

This project was designed to determine if there is a correlation between the presence/density of the mummichog in relation to changes in water chemistry. This information could be used for identifying if the mummichog is potentially a viable indicator species and if so, for which parameters.

Site Selection

Two sampling sites were selected for the collection of water and mummichog population samples (Figure 1). These sites were selected for their proximity to developed areas and the known presence of mummichogs. Pre-sampling was conducted during the spring of 2017 to ensure that there was an adequate population of mummichogs to support this study.

Figure 1 - Sampling sites used for this study (image courtesy of Google Earth).

Site A is located on Ragged Rock Channel, a man-made canal which runs 0.34 miles west from the Connecticut River at a point 2.6 miles north of Long Island Sound. The exact location of sampling is near a marina and marked as "A" in Figure 2. The site receives run-off from areas surrounding the marina basin, discharges from vessel operations, and tidal inundation. The lowest water depth observed at low tide is 2 feet at the site of sampling. At this site, there was little observed water movement. The creek is lined with vegetation on both banks, which extend approximately 2 feet into the water at low tide. The vegetation and overall creek width increased with tidal inundation. Sampling was conducted at position 41°18'38.64"N 72°21'33.62"W.

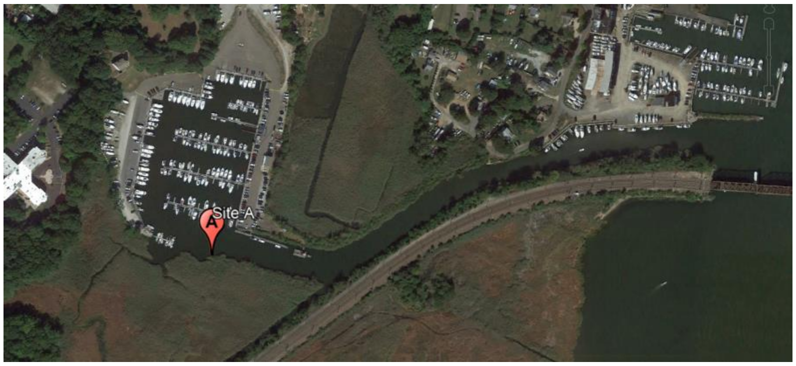


Figure 2 - Ragged Rock Creek sampling location (image courtesy of Google Earth).

Site B is located on Oyster Creek, a tidal tributary which leads directly to Long Island Sound. The specific site of sampling is 0.65 miles north of Long Island Sound. Sampling was conducted at marker "B" as shown in Figure 3. Oyster Creek receives run-off from a variety of property uses, including industrial, residential, roadways, open space, and vessel operations. The lowest water depth observed at low tide was 1 foot. This site was observed to have significant water flow during the incoming and outgoing tides. All sides of the creek are lined with vegetation which was out of the water at low tide. Sampling was conducted at position 41°17'9.57"N 72°23'39.50"W.

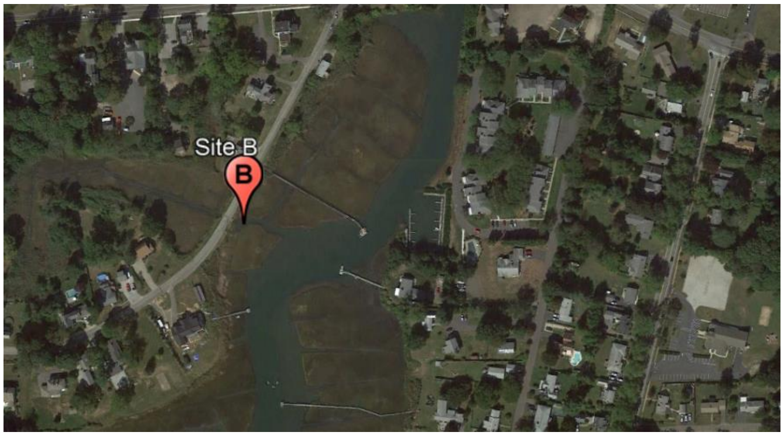


Figure 3 - Oyster Creek sampling site (image courtesy of Google Earth).

Sample Collection

During each sampling event, water samples and mummichog population samples were taken at each site. Twenty-one (21) sample collection events were conducted at each site between July 2017 and September 2017, for a total of 42 data points between both sites.

All water samples were collected by grab sampling. A clean, two-gallon plastic bucket was used as the primary collection vessel. The bucket was triple rinsed with water from the site of sampling to reduce cross contamination from previous sampling events. After triple rinsing, the bucket was fully submerged in the water and then removed. All testing, with the exception of dissolved oxygen, was conducted with water drawn from this primary sample. Once testing was complete, the water samples were disposed of (not returned to the bucket). Due to the sensitivity of dissolved oxygen testing, water samples for this parameter were collected directly from the sampling site. A designated sample bottle was triple rinsed with water being sampled. This bottle was then completely submerged, filled, and capped underwater. This prevents the introduction of oxygen and the invalidation of test results.

Mummichog population sampling was conducted with the use of a wire minnow trap (Frabil Model No. 1268, Plano, IL, USA). The rectangular trap was constructed of coated metal mesh and measured 8” x 8” x 18”. The trap had one entrance door with an opening of 1” in diameter. For each sampling event, the trap was baited with a can of wet cat food (Friskies brand pate). The can was punctured four times around the perimeter and on the top. The baited trap was placed into the water and allowed to soak for exactly 1 hour. At the end of the hour, the trap was retrieved and the contents were emptied into a bucket with water from the sample site. The mummichogs were quickly counted and returned to the water. For this project, only the quantity of mummichogs caught were analyzed and recorded.

Sample Analysis

Commercially available testing devices were utilized for the field analysis of the water samples. All tests were conducted in accordance with the manufacturer's instructions and containers were rinsed with distilled water before and after testing to prevent cross contamination. Table 1 details the equipment and analytical methods utilized for this project.

Table 1 - Monitored Parameters and Equipment

Parameter	Test Device	Method
Temperature and pH	Lab Safety Supply Model 49G969 temperature compensating handheld pH meter	Direct read handheld device (calibrated to pH 4.01, 7.01, and 10.01 prior to each use) automatically corrected for water temperature
Salinity	Handheld refractometer ETvalley Model Number: COMINHKPR127198	Direct read handheld device (zeroed with distilled water prior to each use)
Dissolved Oxygen	LaMotte Test Kit 5860-01	Azide modification of the Winkler Method
Nitrogen (as Nitrate/Nitrite)	Industrial Test Systems 480009	Colorimetric zinc reduction
Phosphate	Taylor Technologies K-1106	Colorimetric Stannous chloride technique

The procedures utilized for the analysis of samples are detailed in Appendix A.

Data Analysis

Statistical analyses was conducted on the data collected for the purposes of determining if a correlation exists between any monitored parameter and the number of mummichogs collected. No transformations were applied to the data. The Grubbs' Test was applied to the data using a significance level of 0.05 in order to detect outliers and excessive residual. All analysis was conducted by use of the processing software "Minitab® Statistical Software v. 17" (Minitab Inc., State College, PA).

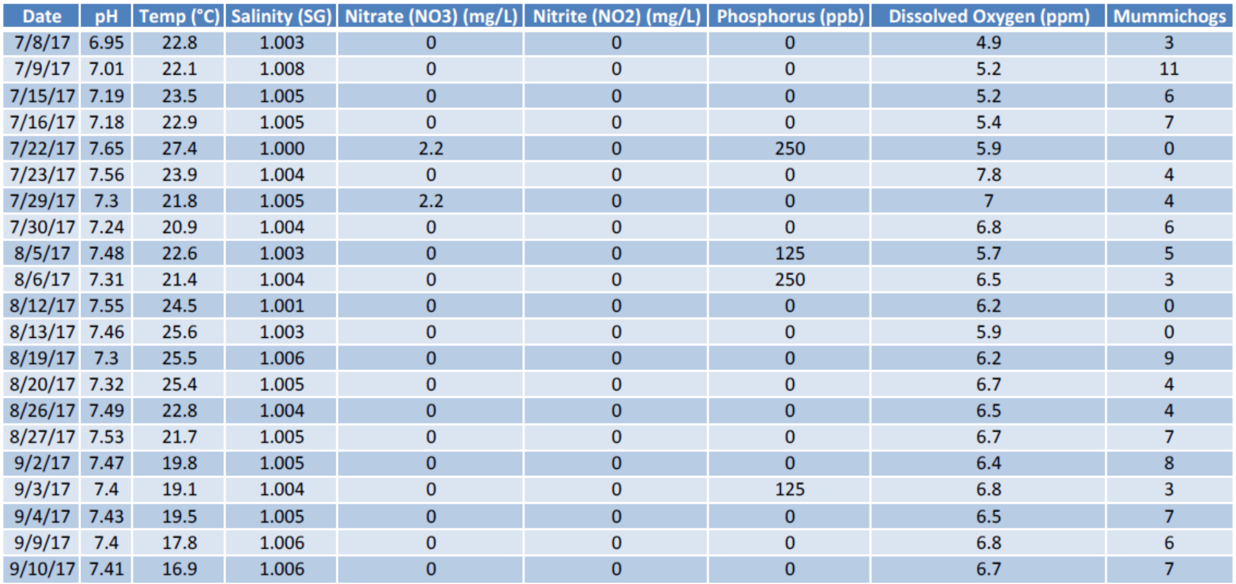
In order to determine if a significant correlation existed, the Pearson correlation coefficient "r-value" and P-value was calculated for each parameter. Statistical regression analysis was then conducted on all parameters with a P-value below the significance level established for this project (0.05). A scatter plot of the data was used as an aid in determining if linear or non-linear regression would be applied to each data set. The method of least squares was utilized for calculation of the regression equation. The resulting best fit line was graphed and visually assess for goodness of fit. The coefficient of determination (R^2; R-squared) was used as the basis for the selection of the line of best fit for each data set; including whether linear or non-linear regression was most appropriate. Both linear and non-linear regression was conducted on the selected data set. The fitted line with the best fit as indicated by the R-squared was chosen.

4. Summary of Outcomes, Discussions, and Recommendations

Results of Population and Water Quality Monitoring

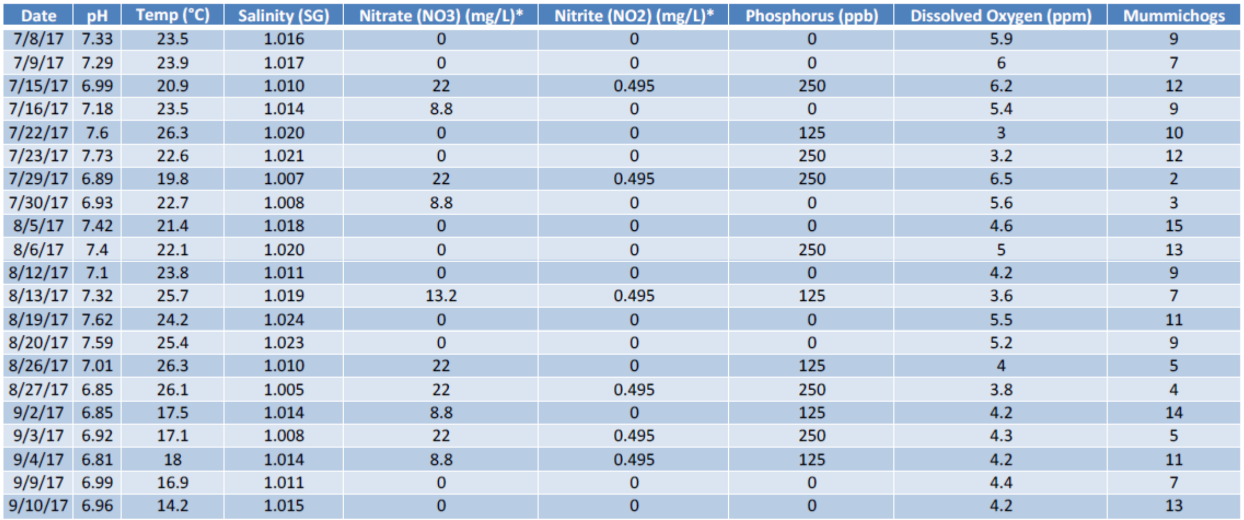
The data collected at each sampling site is reported in Table 2 and Table 3. Data is listed in chronological order for each site.

Table 2 - Data collected at the Ragged Rock Creek site.



Date	pH	Temp (°C)	Salinity (SG)	Nitrate (NO3) (mg/L)	Nitrite (NO2) (mg/L)	Phosphorus (ppb)	Dissolved Oxygen (ppm)	Mummichogs
7/8/17	6.95	22.8	1.003	0	0	0	4.9	3
7/9/17	7.01	22.1	1.008	0	0	0	5.2	11
7/15/17	7.19	23.5	1.005	0	0	0	5.2	6
7/16/17	7.18	22.9	1.005	0	0	0	5.4	7
7/22/17	7.65	27.4	1.000	2.2	0	250	5.9	0
7/23/17	7.56	23.9	1.004	0	0	0	7.8	4
7/29/17	7.3	21.8	1.005	2.2	0	0	7	4
7/30/17	7.24	20.9	1.004	0	0	0	6.8	6
8/5/17	7.48	22.6	1.003	0	0	125	5.7	5
8/6/17	7.31	21.4	1.004	0	0	250	6.5	3
8/12/17	7.55	24.5	1.001	0	0	0	6.2	0
8/13/17	7.46	25.6	1.003	0	0	0	5.9	0
8/19/17	7.3	25.5	1.006	0	0	0	6.2	9
8/20/17	7.32	25.4	1.005	0	0	0	6.7	4
8/26/17	7.49	22.8	1.004	0	0	0	6.5	4
8/27/17	7.53	21.7	1.005	0	0	0	6.7	7
9/2/17	7.47	19.8	1.005	0	0	0	6.4	8
9/3/17	7.4	19.1	1.004	0	0	125	6.8	3
9/4/17	7.43	19.5	1.005	0	0	0	6.5	7
9/9/17	7.4	17.8	1.006	0	0	0	6.8	6
9/10/17	7.41	16.9	1.006	0	0	0	6.7	7

Table 3 - Data collected at the Oyster Creek site.



Date	pH	Temp (°C)	Salinity (SG)	Nitrate (NO3) (mg/L)*	Nitrite (NO2) (mg/L)*	Phosphorus (ppb)	Dissolved Oxygen (ppm)	Mummichogs
7/8/17	7.33	23.5	1.016	0	0	0	5.9	9
7/9/17	7.29	23.9	1.017	0	0	0	6	7
7/15/17	6.99	20.9	1.010	22	0.495	250	6.2	12
7/16/17	7.18	23.5	1.014	8.8	0	0	5.4	9
7/22/17	7.6	26.3	1.020	0	0	125	3	10
7/23/17	7.73	22.6	1.021	0	0	250	3.2	12
7/29/17	6.89	19.8	1.007	22	0.495	250	6.5	2
7/30/17	6.93	22.7	1.008	8.8	0	0	5.6	3
8/5/17	7.42	21.4	1.018	0	0	0	4.6	15
8/6/17	7.4	22.1	1.020	0	0	250	5	13
8/12/17	7.1	23.8	1.011	0	0	0	4.2	9
8/13/17	7.32	25.7	1.019	13.2	0.495	125	3.6	7
8/19/17	7.62	24.2	1.024	0	0	0	5.5	11
8/20/17	7.59	25.4	1.023	0	0	0	5.2	9
8/26/17	7.01	26.3	1.010	22	0	125	4	5
8/27/17	6.85	26.1	1.005	22	0.495	250	3.8	4
9/2/17	6.85	17.5	1.014	8.8	0	125	4.2	14
9/3/17	6.92	17.1	1.008	22	0.495	250	4.3	5
9/4/17	6.81	18	1.014	8.8	0.495	125	4.2	11
9/9/17	6.99	16.9	1.011	0	0	0	4.4	7
9/10/17	6.96	14.2	1.015	0	0	0	4.2	13

Analysis of Results

All data was reviewed for the presence of outliers. Two outlying data points were identified within the Ragged Rock Creek Nitrate data. While these points were identified as outlying by the Grubb's Test, they were not removed due to the observed skew on analytical results. No other outliers were identified by the Grubbs' Test or when plotted.

For each data set, the Pearson Correlation Coefficient and P-values were calculated. The formula utilized by the Minitab software for calculating the Pearson Correlation Coefficient (r-value) was:

$$\rho = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{(n-1)\ s_x s_y}$$

$\bar{x}$	sample mean for the first variable
s_x	standard deviation for the first variable
$\bar{y}$	sample mean for the second variable
s_y	standard deviation for the second variable
n	column length

For the purposes of this project, the variable "n" refers to the number of paired values (entries in the spreadsheet column).

The P-value was calculated using the formula: 2 x P(T > t) where T follows a t distribution with n-2 degrees of freedom. The value t was calculated as follows:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

r	correlation coefficient
n	number of observations

For the purposes of this project, a correlation is considered to be statistically significant when the P-value is less than or equal to 0.05.

Table 4 - Ragged Rock Creek p-values

	Temperature	pH	Salinity	Nitrate	Nitrite	Phosphorus	Dissolved Oxygen
Pearson Correlation	-0.431	-0.417	0.867	-0.336	N/A	-0.418	-0.085
P-value	0.051	0.060	0.000	0.137	N/A	0.060	0.713

Table 5 - Oyster Creek p-values

	Temperature	pH	Salinity	Nitrate	Nitrite	Phosphorus	Dissolved Oxygen
Pearson Correlation	-0.251	0.378	0.610	-0.550	-0.363	-0.130	-0.188
P-value	0.273	0.091	0.003	0.010	0.106	0.575	0.414

Table 6 - Entire Data p-values

	Temperature	pH	Salinity	Nitrate	Nitrite	Phosphorus	Dissolved Oxygen
Pearson Correlation	-0.296	-0.092	0.735	-0.072	-0.010	-0.019	-0.448
P-value	0.057	0.563	0.000	0.649	0.949	0.907	0.003

The number of mummichogs collected showed a strong, positive correlation to the salinity of the seawater at both sites individually (Table 4 and Table 5) and when the data is analyzed as a whole (Table 6). In addition, the p-value representing the correlation between the mummichogs and nitrate levels at the Oyster Creek site was below the statistical significance level. Further, the P-value for dissolved oxygen (when reviewed for both sites) was below the statistical significance threshold. As such, salinity dissolved oxygen, and nitrates were further analyzed.

A p-value could not be calculated for the correlation between the mummichogs captured and nitrate levels at the Ragged Rock Creek site. No nitrates were detected at the Ragged Rock site during the course of this sampling period.

Correlation between Mummichog Density and Salinity – Ragged Rock Creek

The fitted line representing the correlation between the number of mummichogs collected and the salinity of the water at the Ragged Rock Creek site is Y = -14.57 + 1456X, where Y represents the expected number of mummichogs and X represents the salinity (specific gravity). The data exhibited a moderately strong positive correlation across all salinity levels recorded at the Ragged Rock Creek site.

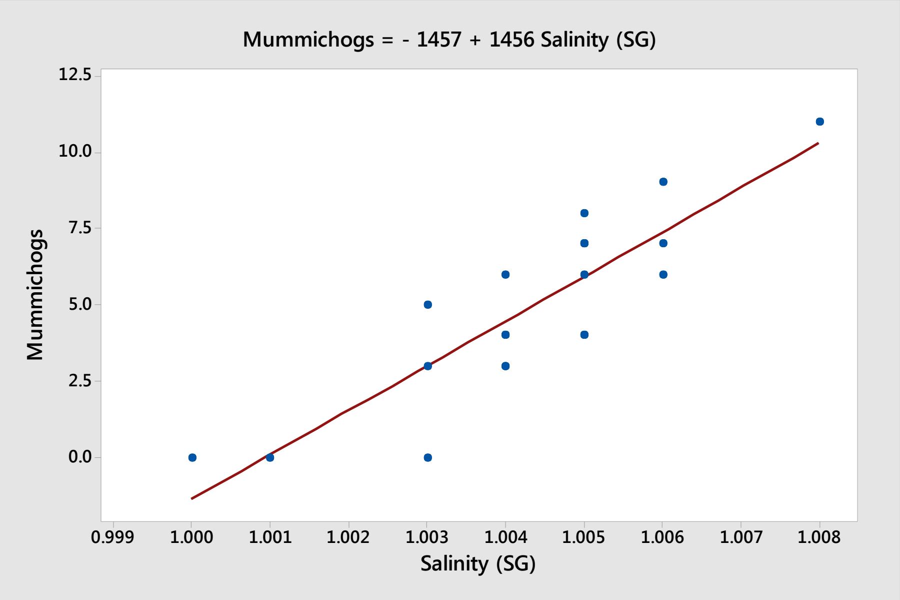


Figure 4 - Fitted line plot of the correlation between mummichogs and salinity at the Ragged Rock Creek site.

Goodness of fit of the linear regression equation was tested by calculating the R-Squared value, using the formula: $r^2 = (r^2) * 100$. The fitted line plot in Figure 4 (Y = -1457 + 1456X) explains 74.2% of the variation within the data.

Correlation between Mummichog Density and Salinity – Oyster Creek

Similar to the Ragged Rock Creek data, the data collected at the Oyster Creek site exhibited a positive correlation, although not as strong (see Figure 5). Further, it was found that a linear fitted line did not adequately represent the collected data. The model which is most contiguous with the data is a quadratic fitted line with an equation of $Y = -52585 + 103265X – 50687X^2$; where Y is the number of mummichogs and X is the salinity (in specific gravity).

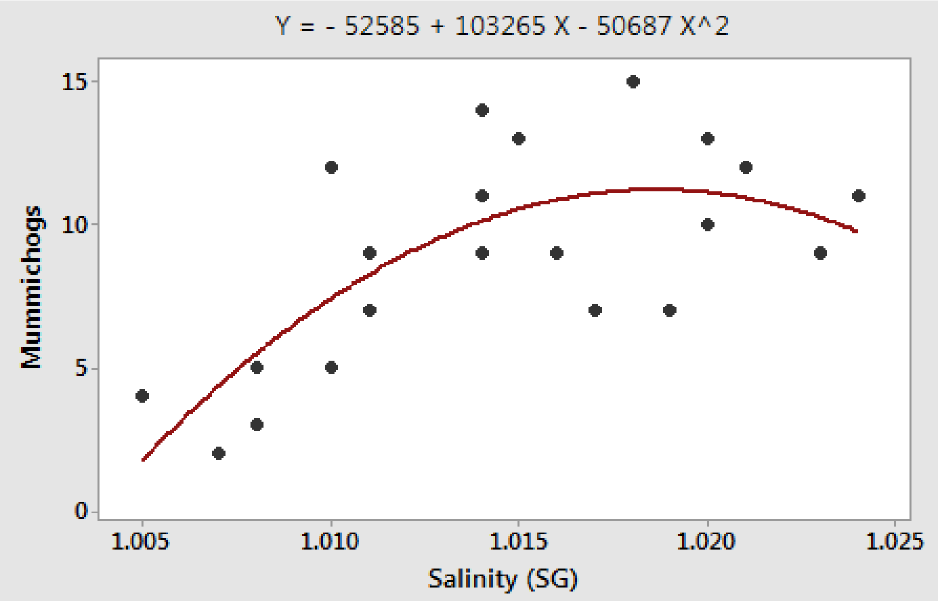


Figure 5 - Fitted line plot of the correlation between mummichogs and salinity at the Oyster Creek site.

The r-squared value for the linear model was calculated as 37.2% while the r-squared value of the quadratic best fit line is 76.6%. The number of mummichogs captured increased for salinity levels up to 1.019 specific gravity but then decreased as salinity levels continued to increase; indicating a preference for brackish water.

Correlation between Mummichog Density and Salinity – Both Sites

When analyzing the mummichog and salinity data from both sites together, it is found that the data follows a best fit line similar to that observed with the Oyster Creek site as shown in Figure 6.

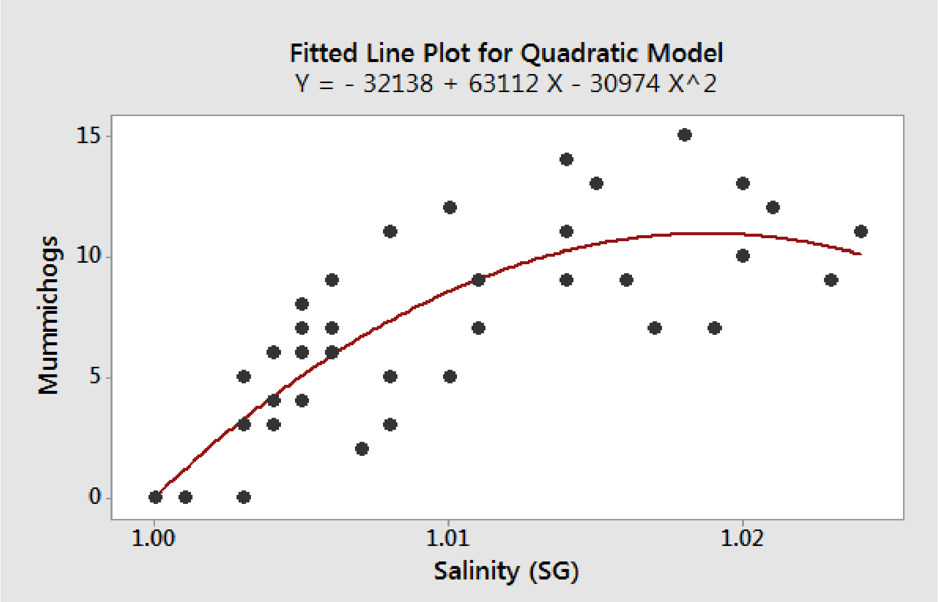


Figure 6 - Fitted line plot of the correlation between mummichogs and salinity at the both sites.

The number of mummichogs collected at both sampling sites varied in response to changing salinity levels. It was observed that higher salinity levels resulted in increased mummichog capture per hour of trap soak time. The data from both sites shows that the mummichog density increases as salinity increases. However, the data from the Oyster Creek site showed that the mummichogs captured began to decrease above a specific gravity of 1.019. This was not observed at the Ragged Rock Creek site as the specific gravity did not exceed 1.008 during the sampling period.

Correlation between Mummichog Density and Nitrates – Oyster Creek Site

Although nitrate levels were found to have a p-value of 0.010 at the Oyster Creek site, a further review of the data shows that it is not likely an accurate correlation which could explain the variation observed. A plot of the data (Figure 7) shows that the regression line does not accurately reflect the data. In addition, this correlation was not supported by the data collected at the Ragged Rock Creek site. There was wide variability in the nitrate data collected at the Oyster Creek Site. This may have been attributed to the accuracy of the testing equipment or other factors which were not monitored during this project. For these reasons, nitrate was not identified as exhibiting a strong correlation to the presence of mummichogs.

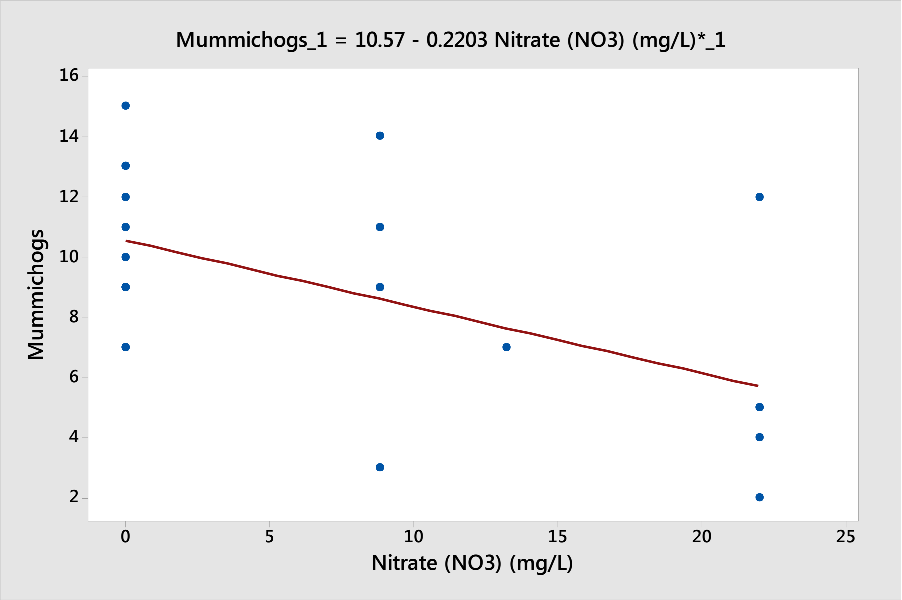


Figure 7 - Fitted line plot of the correlation between mummichogs and nitrate levels at the Oyster Creek site.

Correlation between Mummichog Density and Dissolved Oxygen

When the data collected at both sites is combined and analyzed, dissolved oxygen exhibited a moderately negative correlation to the number of mummichogs collected. When plotted, there is a visible, although loose, correlation between these two variables (Figure 8). Further, the R-squared value is quite low, 20.04%.

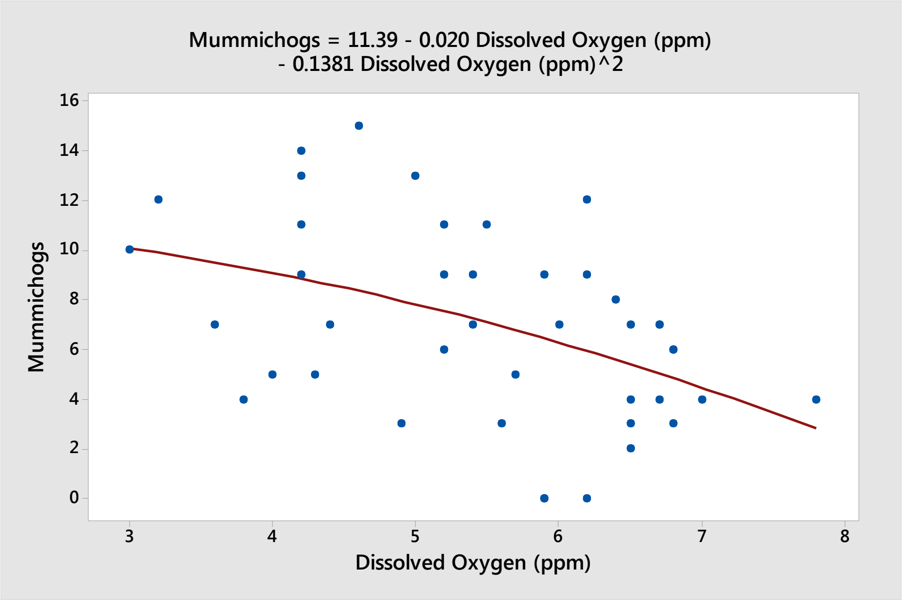


Figure 8 - Fitted line plot of the correlation between mummichogs and dissolved oxygen levels at both sites.

Discussion

This project was aimed at identifying if the mummichog could serve as a viable indicator species for certain water quality parameters within the Long Island Sound watershed. The data collected shows that salinity (recorded as specific gravity) had the strongest correlation with the population density of the mummichog. During this study, mummichogs were captured at salinity levels ranging from 1.003 specific gravity to 1.024 specific gravity. The observed response of the mummichog to salinity is in keeping with previously conducted research. It had been found that the mummichog could withstand short term exposure to fresh water and seawater in a laboratory setting. However, long term exposure to either extreme resulted in increased weight loss and mortality (Nead & Buttner 1987).

In addition, salinity fluctuations have the potential to impact reproductive viability of the mummichog. It has been found that during spawning periods, viable females prefer areas of lower salinity (Marshall et al. 2016) over areas of higher salinity. Additional research focusing on the impacts of salinity variation on mummichog reproductive success and viability of the offspring would provide insight into the magnitude of the impacts resulting from this issue.

A review of the dissolved oxygen data for all sites shows a moderately negative correlation with the number of mummichogs captured. However, the data collected does not necessarily present a strong argument for a direct correlation between the dissolved oxygen levels and the number of mummichogs captured during this study. The reduction in mummichogs collected as dissolved oxygen levels increased is likely the result of another parameter/source which was not monitored during this study. The results of multiple regression analysis with salinity and dissolved oxygen as the indicators and mummichogs as the response resulted in a low R-squared value of 33.57%. This is an indication that there are likely influences not accounted for which is driving the variation.

Although not analyzed during this project, pollution from sources other than nutrient loading could be impacting the presence or movement of the mummichogs. Sample site A was located in close proximity to a municipal marina which could introduce pollutants such as petroleum. Sample site B was in close proximity to residential properties which could introduce pesticides and other contaminants.

The remaining parameters that were monitored during this study did not individually show any significant correlation with the number of mummichogs collected during sampling events. Further, multiple regression analysis on other combinations of parameters were not found to be statistically significant.

The goal of this project was to identify which water quality parameters the mummichog could serve as a viable indicator species. The data collected during this study indicates that the mummichog responds in a predictable manner to changes in salinity. However, more data would need to be collected before a definitive conclusion could be drawn. Field reading instruments were utilized for the analysis of the water quality parameters. Field analysis was conducted on all samples. By nature, these devices are expected to have a higher error rate than analytical laboratory tests. In addition, there are many other variables which were not monitored, including: water pollutants, tidal influence, weather patterns, light conditions, presence of predators, and availability of forage for the mummichogs. Due to the resource constraints of this project, the number of sampling events was limited. It is for these reasons that the research must be specifically verified by additional research before a sound conclusion can be drawn.

Implications

Although the mummichog was not found to be a viable or effective indicator species during this project, there are other implications of the data collected. The mummichog is a very important forage fish for many birds and aquatic species that reside within Long Island Sound. As previously discussed, Long Island Sound is a watershed which receives fresh water from several major sources (including rivers and stormwater runoff). Continued development within the watershed creates more impervious surfaces, which in turn results in more runoff entering Long Island Sound. If the appropriate best management practices are not adhered to, fresh water entering Long Island Sound tributaries (such as the two monitored) could reduce the salinity to a point which is not desirable for the mummichog. This could potentially cause impacts to the predator species which rely on the mummichog as a food source.

The Long Island Sound Study reports that the average salinity of eastern Long Island Sound is 1.026 specific gravity (Long Island Sound Study 2018). It is likely that the inflow of fresh water from runoff plays a vital role in maintaining a brackish environment within the estuaries monitored during this project. However, the results of this project also indicate that salinity levels approaching fresh water (1.0 specific gravity.) are less than ideal. Stormwater management programs under the Clean Water Act utilize total maximum daily load (TMDL) as a means of identifying the maximum levels of a pollutant which may be discharged into an impaired waterway. However, little focus is given to the discharge of clean water. While non-contaminated fresh water will not increase the contaminant levels within a receiving waterway, the discharge can still cause impairment (in this case in the form of salinity decrease).

Conclusions

The mummichog could prove to be a viable indicator species for salinity within the Long Island Sound watershed. However, more detailed and focused research is needed before a definitive conclusion can be made. Research should be conducted over a longer period of time than this project in order to identify seasonally induced changes. In addition, any future research should work to identify sampling sites that allow the isolation of more variables; including tidal inundation, seasonal migration, pollution, etc.

In addition, the use of laboratory accuracy testing methods may provide different results than what was observed during this project. This could identify other correlations which were not detectable with the field measurement devices which were utilized.

Although this project was aimed at assessing if the mummichog could serve as an indicator species, it also highlights the fact that water quality changes can have significant influences on the aquatic food chain. Changes in water quality due to anthropogenic causes can have significant impacts on the mummichog and their availability as a prey species within the estuaries of Long Island Sound.

Dissemination of this Project

It would be beneficial to disseminate this information to individuals and organizations which could initiate further, more detailed research into the impacts of salinity on the mummichog. As the research conducted was preliminary in nature (with the intent of identifying the need for more focused research), informal means of dissemination would be most appropriate. An example of an organization which would benefit from this information is the Long Island Sound Study. The Long Island Sound Study is a multi-state collaborative research organization aimed at protecting the resources of the Long Island Sound.

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Appendix A – Sample Procedures

Temperature and pH:

1. Containers filled with calibration buffers for 4.01, 7, and 10.01 pH.
2. Storage solution rinsed from sensor with distilled water
3. Meter placed into 3 point calibration mode
4. Sensor placed in 4.01 pH buffer
5. Reading adjusted, if necessary, to read 4.01
6. Sensor placed into 7 pH buffer
7. Reading adjusted, if necessary, to read 7.00
8. Sensor placed into 10.01 pH
9. Reading adjusted, if necessary, to read 10.01
10. Adjustments saved and meter placed into measurement mode
11. Sensor rinsed with distilled water
12. Disposable beaker filled with water from the grab sample
13. Sensor placed into the water to be tested
14. Temperate and pH measurements recorded once stabilized (pH and degrees Celsius)
15. Senor removed from sample water and meter power secured
16. Sensor rinsed with distilled water
17. Sensor immersed in storage solution to prevent drying
18. Sample disposed of

Salinity

1. Using a disposable plastic pipette, three drops of distilled water was placed on the glass measurement surface
2. Reading adjusted to 1.0 specific gravity
3. Measurement surface wiped dry
4. 3 drops of water from the grab sample placed on the measurement surface with a disposable pipette
5. Reading taken as specific gravity
6. Measurement surface rinsed with distilled water and wiped dry

Nitrogen (as nitrate and nitrite)

1. Water from grab sample placed into disposable plastic beaker
2. Test strip removed from container and placed into sample water for 2 seconds
3. Strip removed from sample and allowed to rest for 1 minute
4. After 1 minute, test strip patches compared to colorimetric guide to determine nitrate (NO_3 in mg/l) and nitrite (NO_2 in mg/l)
5. Sample disposed of

Phosphate

1. 5 ml test cell rinsed with sample water and refilled by way of a disposable pipette
2. 5 drops of Phosphate reagent #1 added to sample
3. Container capped and inverted to mix
4. Sample allowed to stand for 2 minutes
5. 1 drop of Thiosulfate N/10 added to sample
6. 2 drops of Phosphate reagent #2 added to sample
7. Container recapped and inverted to mix
8. Sample allowed to sit for 1 minute
9. Sample color compared to colorimetric scale provided by the manufacturer
10. Results recorded in parts per billion

Dissolved Oxygen

1. 60 ml glass sample bottle rinsed with water to be sampled. Dissolved oxygen samples taken directly from the water body to ensure accurate results
2. Sample bottle immersed in water body with cap on
3. Cap removed while under water
4. Sample bottle allowed to fill
5. Sample bottle tapped to release any air bubbles
6. Sample bottle capped under water to prevent skewed results due to oxygen exposure
7. Immediately upon uncapping, 8 drops of Manganous Sulfate Solution and 8 drops of Alkaline Potassium Iodide Azide added
8. Sample bottle recapped and mixed by inversion
9. Precipitation allowed to settle below the shoulder of the sample bottle
10. Once settled, 8 drops of 1:1 Sulfuric Acid added to sample bottle
11. Sample bottle capped and gently inverted until the precipitate and reagent are fully dissolved (at this point the oxygen is fixed)
12. Titration tube filled with 20 ml of the stabilized sample
13. Titration plunger filled with Sodium Thiosulfate 0.025N titrating solution
14. Slowly add one drop of titrating solution at a time while gently mixing
15. Continue until sample changes from yellow-brown to pale yellow
16. Remove titrator and add 8 drops of Starch Indicator Solution (sample turns blue)
17. Continue titration until blue color fully disappears
18. Reading taken from titrator in part per million dissolved oxygen
19. Sample disposed of