Final Report

How Temperature and Phosphorus Loading Influence *E. coli* Growth on *Cladophora* in Lake Erie Beach Waters of Presque Isle State Park

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Introduction

The thirteen public Lake Erie beaches of Presque Isle State Park have been particularly affected by water pollution. In 1991, Presque Isle Bay was designated as the 43rd Great Lakes Area of Concern by the U.S. Department of State. While the bay has now been listed as an Area of Recovery, bacterial contamination of the lake-side beach waters is still present, resulting in anywhere from five to an excess of forty beach action notifications annually, posing a recurring health risk to park guests swimming in these waters.

As part of previously funded Pennsylvania Sea Grant initiatives, we have determined that the nuisance algae *Cladophora* can harbor very high levels of *E. coli*. This can pose a serious health risk to recreational swimmers who might come into direct contact with this algae. Also, *Cladophora* can serve as a source of *E. coli* contamination to the beach waters in which it resides, thereby indirectly putting beachgoers at risk of getting ill and contributing to the beach closings observed at Presque Isle State Park.

Previous monitoring efforts by many laboratories have identified phosphorus levels and water temperature as the two main factors that mitigate *Cladophora* abundance in Great Lakes beach waters. While temperature and phosphorus concentration might promote high levels of *Cladophora* in beach waters on certain days, the role that these variables have in mediating *E. coli* abundance on the *Cladophora* has yet to be determined in Presque Isle, or other, Great Lakes beach waters. Thus, determination of the health risk that *Cladophora* has in recreational waters on any given day, irrespective of *Cladophora* abundance, remains unknown.

To fully understand how *E. coli* concentration on *Cladophora* relates to *Cladophora* abundance, we undertook a series of experiments that tested *E. coli* concentration under different temperature and phosphorus concentration under both laboratory and environmental conditions in the beach waters of Presque Isle State Park. The results, presented below, provide insight into how these variables impact the survival of *E. coli*, and possibly other bacterial types, adhering to *Cladophora* mats in recreational freshwater.

Methods

Water and *Cladophora* Collection- Water or detached *Cladophora*, which represents the kind most likely to come into direct contact with recreational swimmers, was collected in the surf zones of Presque Isle using sterile bottles following standard procedures for the collection of water and solids from aquatic environments during the 2013 bathing season. For experiments involving laboratory manipulation of *Cladophora*, water and *Cladophora* were mixed into separate holding bowls and then incubated under the specified temperature or phosphorus concentration by adding KH_2PO_4. *Cladophora* or water that was not manipulated in the laboratory was immediately processed in the lab as described below.

Determination of *E. coli* concentration on *Cladophora*- 10 grams of the collected *Cladophora* was suspended in 50 mL of a buffered saline solution. The *Cladophora* was vigorously agitated by hand for 1 minute to release the *E. coli* from the *Cladophora*. Immediately following

agitation, 10 mL of the saline solution, or a volume that gave countable colonies, was filtered onto a 0.45 µm pore size filter and incubated overnight at 44.5° Celsius on modified mTEC agar. The resulting *E. coli* colonies were counted following the manufacturer's instructions to determine the colony forming units per gram of *Cladophora*.

Determination of phosphorus concentration on *Cladophora*- 10 grams of *Cladophora*, or an amount that provided results within the linear range of the standards, was processed for total phosphorus using the ascorbic acid procedure outlines in http://www.umass.edu/tei/mwwp/acrobat/sm4500P-E.PDF. Standards were created by dissolving various concentrations of KH_2PO_4 in water and processing in the same way as the samples. Absorbance values obtained from the samples were normalized to the standard curve to determine the total phosphorus concentration of the sample. All of these experiments were done under room temperature.

Results

E. coli concentration on *Cladophora* as a function of temperature under laboratory conditions

In a series of experiments, *Cladophora* was incubated in water obtained from Presque Isle State Park under a broad range of temperatures over a series of four days. The purpose of this test was to narrow down the optimal range in which *E. coli* growth on *Cladophora* occurs (data not shown). From these results, it was determined that the temperatures between 42°C and 48°C were optimal for growth of *E. coli* on *Cladophora*. Within this more defined range, our results indicate that *E. coli* growth on *Cladophora* is optimal at approximately 45.5°C, and that optimal growth of E. coli occurs at this temperature at after approximately 72 hours of incubation (Figure 1).

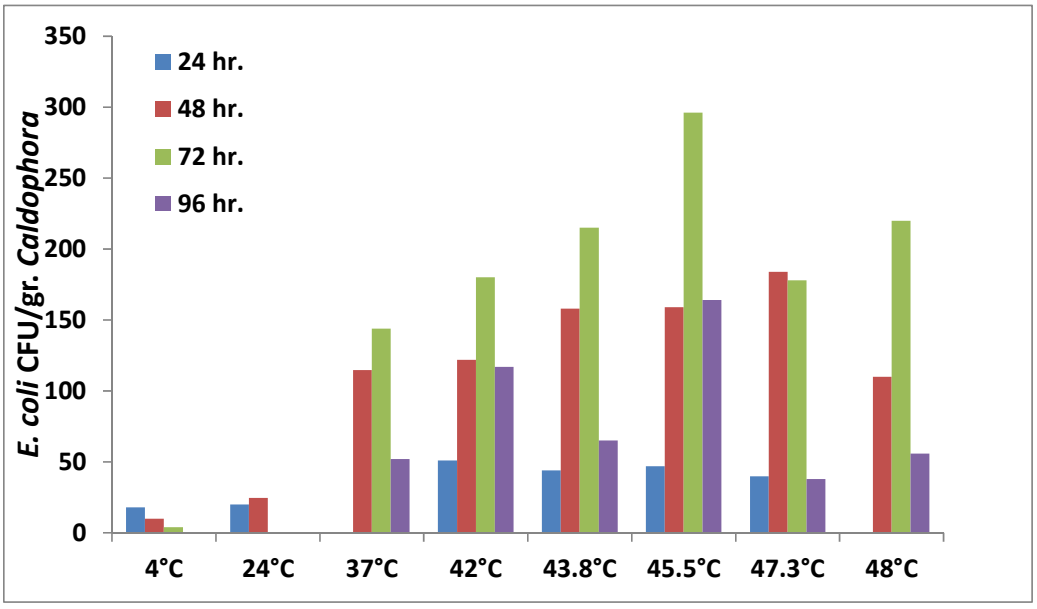

Fig. 1- The impact of temperature on *E. coli* growth on *Cladophora* under laboratory conditions

E. coli concentration on *Cladophora* as a function of phosphorus concentration under laboratory conditions

In addition to temperature, we also measured the concentration of *E. coli* on *Cladophora* under various concentrations of phosphorus under laboratory conditions. Initial experiments covered a broad range of phosphorus concentrations between 0.1mM and 1,000mM (data not shown). From this, we were able to refine the concentration of phosphorus incubated with *Cladophora* to be optimal between 50mM and 500mM (Fig. 2). These results indicate that the optimal concentration of phosphorus conducive to *E. coli* growth under laboratory conditions is 100mM (Fig. 2). At this phosphorus concentration, *E. coli* concentration on *Cladophora* reached its highest level at 96 hr. incubation at a concentration of 151 E. coli CFU/gr. of *Cladophora*.

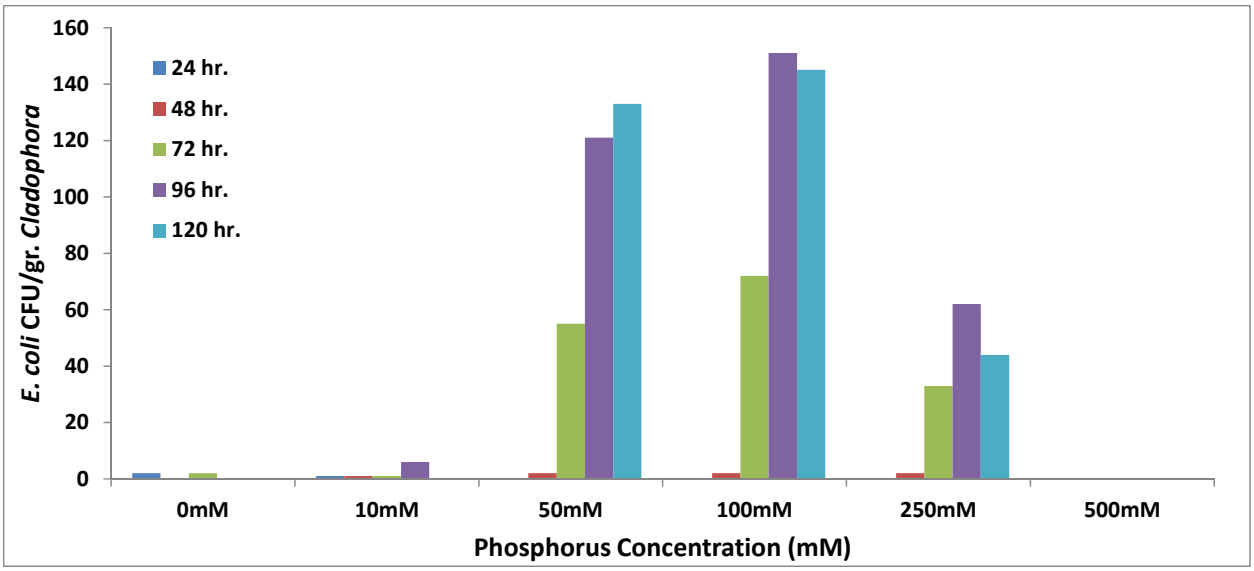

Fig. 2- The impact of phosphorus concentration on *E. coli* growth on *Cladophora* under laboratory conditions

E. coli concentration on *Cladophora* under various Presque Isle State Park water temperatures

To understand how water temperature in Presque Isle State Park beaches impacts *E. coli* concentration on *Cladophora*, we analyzed the concentration of *E. coli* on *Cladophora* at times when the temperature of the water was also taken. The results are shown in Fig. 3. The results indicate very little correlation between the actual temperature of the water and the concentration of *E. coli* found on *Cladophora* which was taken from the water (Fig. 3). A linear regression of 20 data points indicates only a modest inverse relationship between water temperature and *E. coli* concentration on *Cladophora*, which is not statistically significant.

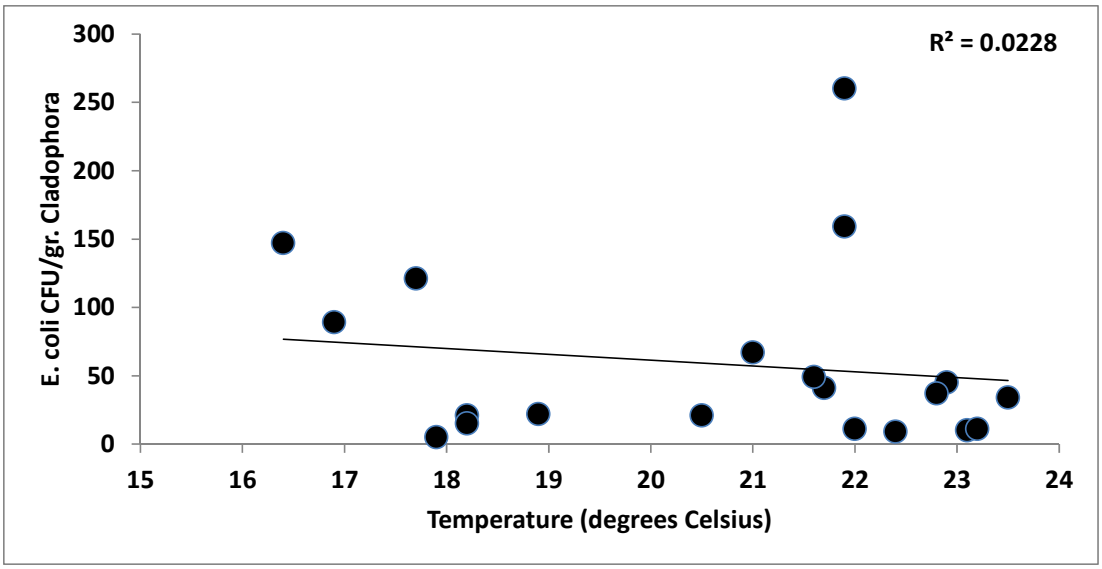

Fig. 3- The relationship of Presque Isle water temperature to concentration of *E. coli* on *Cladophora*

E. coli concentration on *Cladophora* under various phosphorus concentrations in Presque Isle State Park beach water

As we did with temperature, we also determined the concentration of phosphorus in Presque Isle State Park water to determine how it related to concentration of *E. coli* on *Cladophora* (Fig. 4). When examining 20 samples taken from Presque Isle water of determined phosphorus concentration, we found very little correlation between these two variables (Fig. 4). While the linear regression indicated a slight inverse relationship between *E. coli* concentration on *Cladophora* and the phosphorus concentration of the water which it resided (r2=0.0066), it is not statistically significant (Fig. 4).

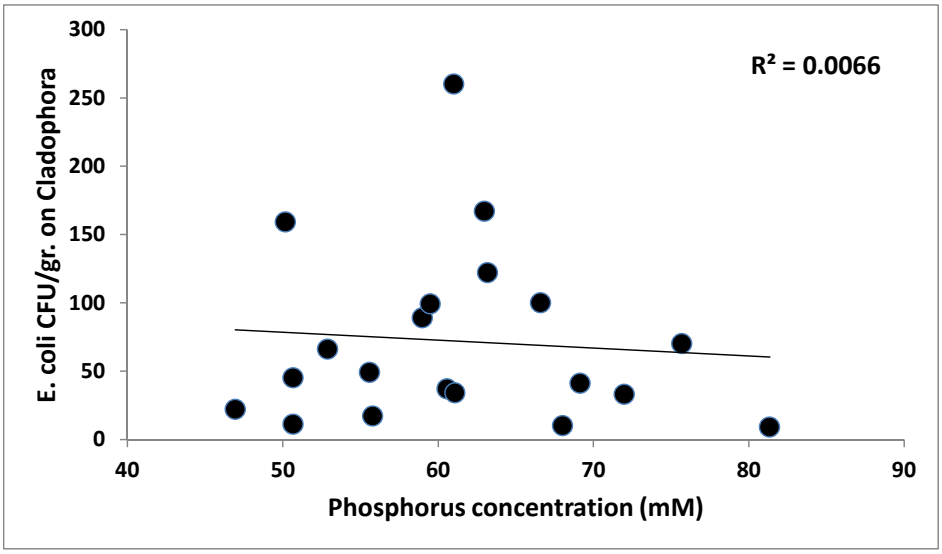

Fig. 4- The relationship between *E. coli* growth on *Cladophora* and phosphorus concentration in Presque Isle beach water

Discussion

While the relationship of water temperature and phosphorus concentration on *Cladophora* growth has been well documented in a number of cases, how temperature and phosphorus concentration of water impacts *E. coli* growth on *Cladophora* has not yet been explored. In this study, we have found that under laboratory conditions, there is a dose dependent responsiveness to *E. coli* concentration on *Cladophora* for both the temperature of the water and for the phosphorus concentration. Specifically, we found that 44.5°C is the optimum temperature for *E. coli* growth on *Cladophora*, while the optimum concentration of phosphorus for growth of this bacteria on this algal mat is 100mM.

However, an examination of the relationship of *E. coli* growth on *Cladophora* to water temperature and phosphorus concentration in its active environment yielded very different results. In these cases, we found no evidence that the temperature of the water or the concentration of phosphorus in that water had any direct impact on the concentration of *Cladophora*. Taken together, this data suggests that other variables besides water temperature and phosphorus concentration in the aquatic environment are acting to impact the concentration of *E. coli* on *Cladophora*.

What are the factors that might impact *E. coli* growth on *Cladophora* in the aquatic environment? Certainly, UV light could play a role, as could the presence of protest grazers or bacteriophages. Some of our more recent work has also shown that chemical of concern that are found in freshwater, such as fluoxetine, have the ability to influence the concentration of *E.coli* and other bacteria in aquatic environments. Future studies aimed at testing a greater number of factors that impact *E. coli* growth on algae will yield more concise results.

Regardless of what might be impacting *E. coli* growth on *Cladophora*, our results have important implications for considering *E. coli* presence in water and beach management. While previous studies have shown that higher water temperatures and phosphorus loading in freshwater leads to greater amounts of *Cladophora* blooms, this is not the case for the concentration of *E. coli* on the algae. Hence, it would be inappropriate to assume that a large algal bloom in water (precipitated by higher water temperatures and phosphorus loading) will lead to high concentrations of *E. coli* on that algal bloom. Since it is the *E. coli* and other pathogenic bacteria that concerns us when assessing water quality, our results indicate that the amount of an algal bloom by itself can't be used to make water management decisions.

Overall Findings

- Under laboratory conditions, the optimal temperature for *E. coli* growth on *Cladophora* is 44.5°C.
- Under laboratory conditions, the optimal concentration of phosphorus for *E. coli* growth

on *Cladophora* is 100mM.

- There was no clear evidence that water temperature in Presque Isle beaches related to *E. coli* concentration on the *Cladophora* present in that water.
- There was no clear evidence that phosphorus concentration in Presque Isle beach water related to *E. coli* concentration on the *Cladophora* present in that water.
- Other factors besides water temperature and phosphorus concentration act to mediate *E. coli* concentration on *Cladophora* in freshwater.

Recommendations

- Since factors that determine overall *Cladophora* abundance in freshwater (ie- water temperature and phosphorus loading) do not relate to *E. coli* concentration on *Cladophora*, beach management decisions should not be made using the overall abundance of *Cladophora* as an indicator.
- Further work is needed to understand what variables besides water temperature and phosphorus loads influence *E. coli* concentration on *Cladophora* in freshwater.
- Examination of how water temperature, phosphorus loading, or other variables impact the concentration of specific pathogenic bacterial types in water or *Cladophora* will yield more specific insight into water quality to make beach management decisions.