**Abstract:**

Cyanobacteria blooms have immediate impacts on ecosystem and human health. They introduce toxins into drinking water, and cause fish kills in both freshwater and marine systems (Dodds et al. 2009) leading to decreases tourism and aesthetic desirability. Though these direct impacts have been well researched () the more subtle impacts on nutrition and fitness of the organisms, that experience the blooms, are still poorly understood. We hypothesize that the poor nutritional value of BGA combined with its competitive dominance may create bottom-up pressure on higher trophic levels, reducing fish physical fitness and lowering the overall health of fish stocks. We plan to characterize the direct effects that BGA have on fish nutrition and health by combining fatty acid analysis and swimming performance Ucrit measurements on young of year yellow perch.

BGA blooms are common in eutrophic water bodies around the world. During a BGA bloom, other phytoplankton species can be competitively excluded limiting food source diversity for primary consumers forcing them to increase feeding on BGA that is normally avoided. BGA lacks many of the essential fatty acids (EFA) required for growth, development, and reproduction of zooplankton and fish ([Ahlgren, Lundstedt et al. 1990](#_ENREF_2); [Ahlgren, Gustafsson et al. 1992](#_ENREF_1); [Brett and Mὒller-Navarra 1997](#_ENREF_4)), a diet switch to BGA in zooplankton and primary consumers will cause a cascade effect up the trophic ladder causing declines in EFAs in secondary and tertiary consumers like fish ([Boersma, Aberle et al. 2008](#_ENREF_3)). To our knowledge, no research has directly assessed the non-acute effects that BGA has on higher trophic levels; a gap in knowledge that could lead to underestimations on the impacts that eutrophication and common BGA blooms may have on a system. Studies that directly assess these consequences will enable managers to quantify impacts of BGA on species fitness, community composition, and fish population health in varying states of eutrophication and climate ([Redfield 1958](#_ENREF_19); [Elser and Hassett 1994](#_ENREF_9); [De Senerpont Domis, Elser et al. 2012](#_ENREF_7)). These changes will have direct consequences

To determine if BGA could cause bottom up pressure on lake communities, young of year farm raised yellow perch will be fed diets with fatty acid signatures that are representative of (1) zooplankton from Missisquoi Bay, Lake Champlain, in the mid-summer (2) zooplankton from Missisquoi Bay during a cyanobacteria bloom or (3) a control diet of store bought fish pellets. Fish will be fed one of these diets for 2 months, and will be sampled at 4 weeks and 8 weeks. At the end of each time period 5 fish from each diet treatment will be placed in a swim tunnel where their swimming performance and respiration will be measured to acquire an Ucrit score as a determinant of health. 10 fish, including those used for Ucrit will be sacrificed and analyzed for EFA content. In this way we will be able to determine both how a diet of BGA zooplankton could affect resident perch, and the role temporal factors of bloom length on any effects.

Understanding these impacts will enable us to begin developing benchmarks for estimating BGA effects based on duration of the bloom, allowing for better fish management and understanding of the effects of eutrophication on a system.

**Title**: Fitness and fatty acid composition of yellow perch fed cyanobacteria derived diets

**Statement of regional or State water problem**:

Eutrophication has become a global problem causing widespread food web shifts in lakes and rivers. In North Eastern North America high population density and agriculture has resulted in extensive eutrophication of freshwater systems; impacting the Laurentian Great Lakes, Lake Champlain and many smaller lakes and ponds. In the past decade eutrophication has led to large harmful algae blooms (HABs) in many of the shallow and/or sheltered bays of Lake Champlain. These HABs have introduced toxins into drinking water, and caused fish and mussel kills in Missisquoi Bay. These impacts led to decreased tourism and aesthetic desirability of the region. Many of the organizations that are operating within the Lake Champlain watershed such as the Lake Champlain Committee and Lake Champlain Basin Project, have made understanding and controlling eutrophication, one of the primary objectives for their organizations. A recent model of Missisquoi Bay (Limnotech 2012) shows that even with a 50% reduction in current phosphorous loading we might not see a discernible decrease in average phosphorous content for 30 years. These data, along with predicted climate impacts for the state of Vermont suggest that eutrophication will continue in some of Lake Champlain’s basins for an extended duration. These findings make it imperative that we begin to look not only at reduction measures and better predictive models for annual cyanobacteria blooms but also work to better understand long term impacts on other organisms in our ecosystems.

Beyond the immediate impacts on ecosystem and human health (Dodds et al. 2009) there are more subtle impacts of Cyanobacteria on nutrition and fitness of the organisms that experience the blooms and these impacts are still poorly understood. We hypothesize that the poor nutritional value of BGA combined with its competitive dominance creates bottom-up pressure on higher trophic levels, reducing fish physical fitness and lowering the overall health of fish stocks. To evaluate this hypothesis we will directly quantify the effects that a BGA based diet has on fish nutrition and health by combining fatty acid analysis and swimming performance Ucrit measurements on young of year yellow perch.

During a BGA bloom, other phytoplankton species can be competitively excluded from a system limiting food source diversity for primary consumers and force them to feed increasingly on BGA that would normally be avoided. This is an issue because BGA lacks many of the essential fatty acids (EFA) required for growth, development, and reproduction of consumers ([Ahlgren, Lundstedt et al. 1990](#_ENREF_2); [Ahlgren, Gustafsson et al. 1992](#_ENREF_1); [Brett and Mὒller-Navarra 1997](#_ENREF_4)). Thus a diet switch to BGA in zooplankton and primary consumers will have direct developmental effects on these basal organisms and likely also cause a cascade effect up the trophic ladder triggering declines in EFAs in secondary and tertiary consumers like fish ([Boersma, Aberle et al. 2008](#_ENREF_3)). To our knowledge, no research has directly assessed the non-acute effects that BGA has on higher trophic levels; a gap in knowledge that could lead to underestimations on the impacts that eutrophication and common BGA blooms may have on a system. Studies that directly assess these consequences will enable managers to quantify impacts of BGA on species fitness, community composition, and fish population health in varying states of eutrophication and climate ([Redfield 1958](#_ENREF_19); [Elser and Hassett 1994](#_ENREF_9); [De Senerpont Domis, Elser et al. 2012](#_ENREF_7)). These changes will have direct consequences long term predictions for fisheries productivity, and tourism development.

**Statement of results or benefits**:

We will collect data on fatty acid composition and swimming performance (Ucrit) for yellow perch at three different exposure levels of diet, (1) T0, before exposure to treatment diet, (2) T1, after two weeks of the treatment diet, and (3) T2, four weeks of treatment diet. We expect to see a change in both EFA content and Ucrit at between treatments and exposure levels (fig 1). By colleting a fatty acid profile of the fish immediately following Ucrit measurments we will be able to directly compare EFA composition and swimming performance allowing us to quantify the effects that BGA based diets has on fish health. By using multiple exposure levels we can determine how the duration of a bloom event factors into fish health.

Through this data we will be able to conclusively assess the impacts that consistent BGA blooms could have on fish EFA content and health. This information can then be used in conjunction with a new integrated assessment model that is being formulated by Vermont EPSCoR’s RACC grant, to model how land use changes and policy will impact nutrient delivery to Missisquoi Bay.

The laboratory nature of this project allows for the opportunity to work with ECHO Lake Aquarium and Science Center (ECHO) to show current laboratory techniques of swim-tunnel respirometry to the public. ECHO is attached to the Rubenstein Ecosystem Science Laboratory (RESL) where the experiments will take place and a large window facing into ECHO joins the RESL wet lab to ECHO allowing for demonstrations of experiments to take place in the lab to be observed by the public. We will conduct our experiments in front of this window and develop and interactive demonstration of swim tunnel respirometry which can be presented to the 150,000 people who pass through ECHO yearly. Our demonstration will focus on teaching teens and adults the value of science and technology in the conservation of our water resources and basic information of bioaccumulation, nutrition, eutrophication and BGA blooms.

**Nature, Scope, and Objectives of the project, including a timeline of Activities:**

**Nature**: ?

**Scope**: We will assess the impacts that consistent BGA blooms have on the health and fitness of YOY yellow perch in Lake Champlain.

**Objectives**:

To determine the effect that a BGA based diet has on YOY yellow perch swimming performance and EFA content

To determine how the amount of exposure to BGA based diets factors into EFA content and swim Performance

To expose the public to current research, and educate them on the diverse impacts that nutrient loading can have on an ecosystem.

**Timeline**:

Acquire fish

Acclimate fish

Start treatment

First sampling

March

Jan

Dec

Nov

Oct

Sept

July

June

May

April

August

Feb

2014

2015

ECHO demo

Manuscript preparation

Publication

Second sampling

Analysis

ECHO demo

**Figure 1. Predicted timeline for experiment and manuscript publication.**

**Methods, procedures, and facilities:**

Yellow perch will be fed diets with fatty acid signatures that are representative of zooplankton from Missisquoi Bay in the mid-summer, or during a cyanobacteria bloom. We will analyze fish for fatty acid content and Ucrit upon arrival, after acclimation and post feeding trials. All procedures involving live fish will take place at the Reubenstein Ecosystem Science Laboratory at 3 College st. Burlington Vermont. This location processes facilities for proper fish husbandry, including de-chlorinated water, ample tank space, and proper water filtration. Lipid chemistry will take place the the Kraft laboratory in Terrel Hall on the University of Vermont’s main campus.

Experimental diets:

Diets for this experiment will be formulated based on the observed fatty acid composition of zooplankton greater then 300µm collected in July and August of 2013. Zooplanktons in this size class represent the main food source for pelagic planktiverous fishes, and are also of a size most likely able to consume cyanobacteria cells and small colonies. This makes them the best size class for modeling trophic transfer of fatty acids from primary producers to yellow perch.

All diets will be produced by (COMAPANY) and will have standard levels of protein, carbohydrates, minerals, vitamins and fats. The only difference in each diet will be the composition of essential fatty acids. The diets based on pre-HAB and peak HAB zooplankton will be formulated with the essential fatty acid values as reported in Table 1.

|  |  |  |  |
| --- | --- | --- | --- |
|  | EPA | DHA | ALA |
| Diet | µg/mg | % comp. | µg/mg | % comp. | µg/mg | % comp. |
| Pre-HAB |  |  |  |  |  |  |
| Peak HAB |  |  |  |  |  |  |
| Control |  |  |  |  |  |  |

Table 1. Essential fatty acid composition of the three experimental diets to be used .

Experimental animals and feeding

Yellow perch will be obtained through a commercial supplier (name) and maintained in the lab in groups of 8 individuals to single 10 g aquaria under a natural photoperiod of 14 hours of light per 24 hour cycle. All tanks will be individually filtered and kept at a standard 18C throughout feeding and experimentation. Groups of fish will be randomly selected to receive one of 3 diets pellets with a BGA FA ratio, pellets with a pre-bloom FA ratio or a control diet. Fish will be fed these diets for 2 months and then Ucrit of individual fish will be measured. Following Ucrit measurements all fish will be sacrificed, measured to the nearest millimeter and mg then we will take tissue for FA analysis.

Swim performance:

Swim performance and respiration of the three different treatments will be measured using intermittent flow respirometry with Blazka-type LoligoSystems Mini Swim Tunnel Respirometers, a DAQ-M control device and the AutoResp software version 2.2.0 (Loligo Systems, Denmark). Prior to measurements, fish will be starved for 8 hours to obtain a post absorptive state respiration rate and swim performance (). Following this period one fish from each treatment group will be placed in separate swim tunnels where they will be allowed to acclimate at the lowest flow rate (approximately 1body length/s) for 2 hours to eliminate factors of handling stress. Swimming performance will then be evaluated by exposing fish to stepwise increases in water velocity in increments of 1body length/s increase every 5 minutes until the fish is too fatigued to continue swimming and rests against the rear of the chamber. Critical swim speed (Ucrit) will then be calculated using the equation:

$$U\_{crit}=V\_{p}+((t\_{f}/t\_{i})×V\_{i})$$

Where Vi is the velocity increase per increment, Vp is the final velocity swam, ti is the increment time length and tf is the duration of the last velocity increment until fatigue.

Oxygen consumption

Oxygen consumption (MO2) during swim performance trials will be measured using intermittent respirometry at 18oC with Pt100 fiberoptic probe connected to a Oxy-4 Mini device and AutoResp software version 2.2.0 (Loligo Systems, Denmark). Independent measurements of MO2 will be recorded on 5 minute intervals and all chamber water will be flushed with oxygenated water following each interval. Then by plotting the logarithm of oxygen consumption versus swimming speed, a regression equation can then be determined for each fish; estimating the basal MO2 (y intercept), maximum MO2 (at Ucrit) and aerobic scope (maximum — basal MO2) for each fish (Mager and Grosell, 2011).

Tissue fatty acid analysis:

Fish lipids will be extracted from liver and muscle tissues and fatty acid methyl esters (FAME) will be prepared. Tissue homogenates (Ultra-Turrax T25 homogenizer) will be extracted for total lipids with chloroform-methanol (2:1; v/v) according to Bligh and Dyer (1959). The total lipids obtained will be saponified by refluxing with 0.5 M sodium methoxide (NaOCH3) for 20 min at room temperature. Subsequently, the saponifiable lipids will be converted to their methyl esters by using 10% boron trifluoride in methanol for 10 min at 60C (Nuernberg et al. 2007). The resultant fatty acid methyl esters will be extracted into hexane and analyzed by gas chromatography- Mass Spectrometry (GC-MS) using a Shimadzu GC 2010 gas chromatograph (Shimadzu, Osaka, Japan) equipped with a Rtx-2330 capillary column (30m long × .25 mm diamter × .20μm film thickness; COMPANY) connected to a Shimadzu GCMS-QP2010plus. Fatty acid peaks will be identified based on comparing retention times and mass/charge ratios with external standard solutions (37 component mix, Supelco, PA, USA, #463 Nu-Chek Prep., MN, USA). Relative proportions will be deterimened from peak intensity, and total mass of individual fatty acids will be determined with calibration curves derived from external standards

**Related research**:

**Training potential**:

This experiment will provide training and experience to one PhD and one MSc student and 2 to 4 undergraduate researchers in the area of physiological ecology and lipid analysis. The graduate students will conduct or direct all of the fatty acid sampling, processing and analysis and the swim performance tests and respirometry. In addition the graduate students will supervise and train to 2 to 4 undergraduate volunteers to help with fish husbandry and sampling. The experiment will result in at least one manuscript submission to a peer reviewed journal and a chapter in a PhD student dissertation. Finally, the methods and results of the study will be disseminated to the public at ECHO Lake Aquarium and Science Center (ECHO), a public aquarium that welcomes >150,000 visitors every year. We will work with the Science Education Specialist, Linda Bowden to develop an interactive presentation to showing the techniques of swim tunnel respirometry and educating the public on lake eutrophication and the harmful effects of BGA blooms on the ecosystem. To further the projects exposure to the public, the majority of the sample processing and experiments with take place in front of the large viewing window that links the Rubenstein Ecosystem Science Laboratory to ECHO allowing visitors to watch science in action in a working laboratory.

**Investigator Qualifications**

The price of individual samples is broken down as follows:

|  |  |  |
| --- | --- | --- |
| Item | Cost per Other Sample |  |
| Chemical/reagent1 | $5.95 |  |
| Consumables2 | $2.30 |  |
| Other supplies3 | $1.73 |  |
| Total | $9.97 |  |

1 Includes chemicals and reagents used in the protocol (hexane, methanol, chloroform, sodium methoxide, sodium sulfate, potassium bicarbonate, boron trifluoride, and sodium sulfate)

2 Includes Pasteur pipets, glass wool, tin foil, and vials w/septa and caps

3 Includes gas tanks (air, hydrogen, nitrogen) and accessories (septa, liner, O-rings) for the gas chromatograph