Honours Application Proposal

Identification and quantification of Major Carotenoids in *Mysis diluviana* by Capillary Electrophoresis

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INTRODUCTION

Mysis diluviana is a pelagic shrimp-like crustacean found in freshwater lakes of northern North America and was introduced into Okanagan and Kalamalka lakes in the mid-1960s (1). It has become a highly abundant and invasive species in those lakes. It has been suggested *M. diluviana* may have been responsible for a sharp decrease in the Kokanee salmon population of Okanagan Lake (2). This decrease in Kokanee salmon would have been caused by competition between the two species for food (zooplankton, and particularly cladocerans).

As part of a plan developed by the BC government in the 1990s to help the Okanagan Lake Kokanee population recover, the BC government authorized the harvesting of the invasive *M. diluviana* from Okanagan Lake. The harvested *M. diluviana* is primarily being used as fish food in the aquarium industry; however, reports suggest *M. diluviana* from Okanagan Lake might also constitute a superior source of nutrition for captive fish reared in hatcheries relative to other commercially available fish foods (3,4).

Carotenoids are one of the most common types of natural pigments and can influence the colors of living organisms (5). More than 750 kinds of carotenoids have been identified and have been separated into two major types: carotene, which only contains hydrocarbons, and xanthophyll which contains oxygen. Many carotenoids are known to improve the antioxidative stress and immune system of an organism, resulting in improved disease resistance, growth performance, and survival. Carotenoids are also responsible for the orange-red colouration in the muscle tissues of trout and salmon.

Species in the genus *Mysis* are known to feed on phytoplankton and zooplankton. In Lake Michigan, *M. diluviana* were found to commonly ingest diatoms, chrysophytes, and calanoid copepods (6). Diet proportions by weight were dominated by copepods, although this shifted towards cladocerans in autumn. A study that analyzed zooplankton and phytoplankton in *Mysis* of Okanagan Lake supported the importance of diatoms and cladocerans in the diet of *Mysis* (7).

A study of carotenoids in marine animals suggested that the principal carotenoid in crustaceans such as *Mysis* is astaxanthin (8). In crustaceans, astaxanthin exists as carotenoproteins such as crustacyanin, and exhibits purple and yellow colors. Many crustaceans can synthesize astaxanthin from beta-carotene ingested from dietary algae. Furthermore, it suggests that carotenoids found in phytoplankton such as diatoms are metabolites of fucoxanthin and alloxanthin.

Previously, separating and analyzing carotenoids were done mostly by high performance liquid chromatography (HPLC) and high-resolution gas chromatography with flame ionization detector (GC-FID) (8, 9). Some disadvantages of the HPLC method are the requirement of costly materials and low-resolution. The main disadvantage of GF-FID is that the FID detector does not respond well to organic compounds containing oxygen, which would make the detection of certain carotenoids such as astaxanthin difficult.

Capillary electrophoresis (CE) is an ideal method for carotenoid analysis due to its high resolution and efficiency, small sample volumes, low risk of contamination, and reproducibility. An article in the Journal of Chromatographic Science discusses a CE method for the separation

of photosynthetic pigments such as chlorophyll derivatives and carotenoids (10). We intend to develop a method that will be accurate and sensitive for detecting low concentrations of carotenoids in *M. diluviana*. Our method will examine common carotenoids as well as specific carotenoids that affect tissue colouration in salmonids.

Carotenoids in *M. diluviana* will be analyzed by capillary electrophoresis (CE). CE is an analytical method that is used for the separation of analytes in solution. The separation is achieved by passing a solution through a silica capillary in the presence of an electric field. The ions will differ in their mass and charge, meaning they will migrate at different rates through the capillary. In CE, the solution is passed from the anode to the cathode. In general, small cations are eluted first, followed by large cations, neutral analytes, large anions, and finally small anions. The ions will be detected by UV spectrophotometry, in which light is passed through the sample and the absorbance at UV wavelengths is recorded. The detector response is recorded on an electropherogram where analytes can be identified based on migration time.

The carotenoid content of *M. diluviana* will be analyzed in samples from Okanagan Lake that had previously been collected for Dr. Gosselin's research. The wavelength of the UV detector, pH and concentration of the buffer, as well as voltage and injection time conditions on the CE will be optimized to obtain high resolution peaks for each carotenoid. The linear relationship between the concentration of the carotenoids and their corresponding peak area on the electropherogram will be used to validate the method by showing reproducibility of the peak area, migration time, precision, and accuracy.

Objectives

This research aims to develop an accurate and sensitive method to determine the total amount of selected carotenoids present as well as the forms of carotenoids present in *M. diluviana* using capillary electrophoresis (CE). The project specifically aims to determine: (1) the total amount of carotenoids present in *M. diluviana*; (2) the forms of carotenoids present in *M. diluviana*; and (3) the seasonal variation in amount and forms of carotenoids present in *M. diluviana*. The third goal will be addressed if time allows.

MATERIALS AND METHODS

Instrumentation

Capillary electrophoresis investigations will be performed using a Beckman Coulter P/ACE System MDQ capillary electrophoresis system equipped with a photodiode-array UV detector. The system is controlled at 25 ^{o}C with 32 Karat software and uses a fused silica capillary of 50-µm I.D. x 375-µm O.D. with total and effective lengths of 50 cm and 40 cm, respectively.

Sample Preparation

The samples *M. diluviana* shrimp were previously collected for Dr. Gosselin's research from Okanagan Lake and are held in a -80 ^{o}C freezer. Samples may need to be homogenized by grinding using a mortar and pestle. Samples will be dissolved in methanol and diluted to the desired volume for analysis.

Conditioning Capillary

A new capillary will be flushed and conditioned before use. This is accomplished through sequential flushing (20 psi for 30 min) of 0.1 M NaOH and run buffer for 30 min at 20 psi. During analysis, the column will be rinsed with 0.1 M NaOH at 20 psi for 3.0 min, followed by water at 20 psi for 1.0 min, and finally with rinse buffer at 20 psi for 3.0 min. Injection pressure, temperature, voltage, and run time will be optimized during method development.

Analysis

Carotenoid content in *M. diluviana* shrimp samples will be analyzed using the method of external standard calibration. Five carotenoid standards were purchased (Astaxanthin, Beta carotene, Canthaxanthin, Lutein, Zeaxanthin). A calibration curve will be created for each standard and be used to determine the line of least squares which will be used to determine the amount and forms of carotenoids present in the *M. diluviana* shrimp samples.

Expected Results

The results of this research will be beneficial to the effort to enhance the growth, survival, and reproduction of captive fishes, particularly salmonids. Carotenoids are a type of organic compound that can be important in salmonid diets. Salmonid hatcheries often include a carotenoid supplement such as astaxanthin in the diets of their fish during the months preceding the release of catchable-sized fish. This dietary supplement, however, increases production costs. Crustaceans in the genus *Mysis* are known to contain natural carotenoids and therefore might constitute a partial or complete source of carotenoids for salmon diets. It would also be beneficial to verify if capillary electrophoresis is an effective method to identify carotenoids in *M. diluviana* samples.

Timeline

Period	Plan
May 11 – May 31	Background Literature Research Ordering reagents and carotenoids standards CE Instrumentation and Laboratory training
June 1 – June 30	Method design for CE Carotenoid sample preparation
July 1 – July 31	Optimization of CE experimental conditions
Aug 1 – Aug 31	Method Validation
Sept 1 – Oct 31	Carotenoid Sample Analysis
Nov 1 – Nov 30	Begin Writing Thesis
Jan 1 – Jan 31	Revise Thesis Update Presentation
Feb 1 – March 31	Work on Poster Presentation Present at Poster Conference Revise Thesis
Beginning April	Final Revisions Thesis Defense

Budget

Laboratory Supplies	Cost
- Buffer Reagents	$ 140
- CE Cartridge	$ 410
- Carotenoid Standards	
o Astaxanthin	$ 306
o Beta carotene	$ 392
o Canthaxanthin	$ 454
o Lutein	$ 650
o Zeaxanthin	$ 1130
Total	**$ 3482**

The source of funding will be the $1000 from my UREAP grant and any research costs exceeding the $1000 provided by UREAP will be covered by the NSERC Discovery grant of my primary supervisor, Dr Louis Gosselin**.**

Literature Sources

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