**Limnology & Oceanography Research Exchange (LOREX) Application**

**Project Title:** Mode of trophic fractionation by the aquatic carnivorous plant, *Utricularia australis*

**Student Name and Email:** Emmi Kurosawa, emmi.kurosawa001@umb.edu

**Current Institution:** University of Massachusetts, Boston  
**Advisor(s) Name:** Prof. Richard Kesseli  
**Program and Year in Program:** PhD, 7th year as of Jan 2024  
**Thesis Title:** How carnivorous is a carnivorous plant?

**ASLO Member ID:** 35308

**Potential exchange site and collaborator:** The Centre for Coastal Biogeochemistry at Southern Cross University (SCU) at Lismore, New South Wales, Australia. Prof. Joanne Oakes would be the collaborator for the SCU component.
Abstract

*Utricularia* spp. is a rootless aquatic carnivorous plant that occurs in nutrient-poor wetlands. *Utricularia* spp. is particularly sensitive to changes in nutrient concentration in its habitat. During a previous LOREX internship at Centre for Coastal Biogeochemistry (CCB), Lismore, New South Wales (NSW), Australia in 2020, I developed a new model using stable isotope ratios to quantify diet shift in *Utricularia* spp. from carnivory to autotrophy. During a second LOREX internship in 2023, I measured the trophic fractionation factor of *Utricularia* spp., a required parameter to refine the model I generated in LOREX 2020. This was the first time trophic fractionation has been quantified for botanical carnivory. However, how, and where within the plant the botanical fractionation occurs has not yet been addressed. *Utricularia* spp. is known to secrete various enzymes to digest trapped zooplankton within its bladder traps and feeds prey-derived carbon to bacterial communities that presumably exist to help digest prey within its traps. It is possible that *Utricularia* spp. also feed nitrogen from carnivory to these bacterial communities. We hypothesize that these nitrogen transfers are the source of trophic fractionation in *Utricularia* spp. and that fractionation therefore occurs within *Utricularia* spp. traps. In LOREX 2024, we propose to test this hypothesis by measuring the $\delta^{15}$N values of bladder contents and comparing them against the $\delta^{15}$N values of other plant tissues. This work will 1) elucidate for the first time, the mode and location of the trophic fractionation in botanical carnivory, 2) giving insight into N uptake processes by carnivorous plants, complementing our two previous collaborative works.

**Keywords:** *Utricularia*, carnivorous plants, stable isotopes, fractionation, nitrogen


Project Description

**Introduction**

Oligotrophic wetlands are an important source of drinking water and support a rich biodiversity. However, they are increasingly threatened by anthropogenic nutrient enrichment (Eyre et al., 1997). *Utricularia* is a genus of carnivorous plants that occurs in such wetlands (Taylor 1989). When nutrients are elevated, morphological evidence suggests that the contribution of carnivory to nutrient uptake by *Utricularia* decreases (Jobson et al., 2000; Guisande et al., 2004; Kibriya & Jones 2007; Adamec 2008). In LOREX 2020, we hypothesized that changes in proportion of carnivory ($N_{\text{prey}}$) would be reflected in $\delta^{15}\text{N}$ of *Utricularia* spp. Thus, $N_{\text{prey}}$ in *Utricularia* spp. could be a potential bioindicator of nutrient enrichment and early wetland degradation. We tested these hypotheses through a LOREX internship at the Centre for Coastal Biogeochemistry (CCB), New South Wales (NSW), Australia in 2020. New models we developed suggested that carnivory in *Utricularia* spp. is negatively correlated with $\text{PO}_{4}^{3-}$, $\text{NH}_{4}^{+}$, and dissolved organic carbon concentrations, and that the $N_{\text{prey}}$ below 0.4 (40% carnivory) might indicate early eutrophication (Kurosawa et al. in prep). In LOREX 2023, we measured N trophic fractionation factors (TFF) during prey consumption (botanical carnivory) in *Utricularia gibba*, estimating for the first time the value and the range of TFF and providing strong evidence that trophic fractionation does occur in botanical carnivory (Kurosawa and Oakes, in prep). However, how and where within the plant the trophic fractionation occurs is still largely unknown.

Biological N transfers within an organism are likely to cause trophic fractionation of nitrogen isotope ratios, due to differences in the rate of processing of $^{14}\text{N}$ and $^{15}\text{N}$; faster processing of $^{14}\text{N}$ results in a higher proportion of heavier $^{15}\text{N}$ remaining within the body of the organism (Fry, 2006). The possible N transfers in *Utricularia* spp. which may cause fractionation is that 1) they secrete various enzymes to digest trapped zooplankton within its bladder traps (Adamec and Ellison, 2019), and 2) *Utricularia* supports and feeds prey-derived carbon to bacterial communities that exist presumably to help digest prey within its traps (Sirová et al., 2009). The plants may feed nitrogen obtained from carnivory to the bacterial communities in a similar manner. This suggests that the bladder traps of *Utricularia* spp. are the site of fractionation. In LOREX 2024, we will test this hypothesis by measuring the bladder contents of $\delta^{15}\text{N}$ values and comparing these values against the $\delta^{15}\text{N}$ of other plant tissues. This work will 1) elucidate for the first time, the mode and the location of the trophic fractionation in botanical carnivory, and 2)
provide insight into N uptake processes by carnivorous plants, complementing our two previous collaborative works.

Fig. 1. *Utricularia australis*

**Work plan during the internship and project feasibility**

During an eight-week exchange period I will 1) undertake field sampling of *U. australis* (Fig. 1), 2) dissect plants into five tissues: bladder contents, bladder skin, apical growth, stems, and leaves, and 3) prepare and analyse samples for nitrogen stable isotope ratios. The isotope work will be carried out at the Centre for Coastal Biogeochemistry (CCB) at Southern Cross University (SCU) in Lismore, NSW, Australia. SCU is the ideal institute for this project due to their state-of-the-art stable isotope facilities, and broad expertise in stable isotope biogeochemistry. SCU also has good proximity to the sites where *Utricularia australis* samples were collected for our previous work, allowing us to determine the location of fractionation for the same species we worked on previously. At SCU I will be mentored by Prof. Joanne Oakes, who is a leading scientists in assessing environmental stable isotopes.

**Outline of Methods**

Specimens of live *Utricularia australis* will be harvested from previously identified sites in northern New South Wales, within a two hour drive of SCU. Plants will be cleaned free of debris and epiphytes and dissected into five tissue parts: bladder contents, bladder skins, apical growth, stems, and leaves (n=5 for each tissue). The tissues of *U. australis* will be dried at 60°C until stable weight is achieved. The dried samples will then be packed into tin capsules, and
measured for $\delta^{15}$N using EA-IRMS. The $\delta^{15}$N will be compared among five plant tissues. Changed $\delta^{15}$N values will indicate sites where fractionation occurs.

**Timeline**

Week 1: Plant collection  
Week 2-5: Plant dissection  
Week 6: Sample preparation for EA-IRMS  
Week 7-8: Measurement of $\delta^{15}$N in plant tissues using EA-IRMS, and data analysis

**Dissemination of the results**

I aim to publish the results obtained from this research exchange in a high impact peer reviewed journal, such as Limnology and Oceanography or Limnology and Oceanography: Letters. I will present my work at the ASLO conference and will also publish on the multimedia blog as well as social media through ASLO’s Communication and Science Office and also via the CCB Twitter account run by Prof. Bradley Eyre (@biogeochemSCU). I will also give a seminar on my research while hosted at Southern Cross University, as well as when I return to University of Massachusetts Boston.
Project Justification

Why it is important for me to conduct this research

Australia has the highest species richness and endemcity of *Utricularia* in the world. Australian species also exhibit unique characteristics that cannot be found in any other parts of the world. However, these unique plants and their habitats are disappearing due to the anthropogenic destruction. In the previous LOREX projects, we developed novel models and estimated the degree of botanical carnivory and measured botanical trophic fractionation for the first time. This work will further investigate how and where the fractionation for the botanical carnivory occurs, giving insight into N uptake processes by carnivorous plants.

Why it is important to do it at the host institution

The Centre for Coastal Biogeochemistry (CCB) has a world-class stable isotope laboratory and expertise in the techniques and interpretation required to successfully complete this project. Our LOREX 2020 collaboration has become a chapter in my dissertation, as well as a manuscript planned to be submitted in late January, 2024 to *Annals of Botany*. The LOREX 2023 collaboration estimated the trophic fractionation factor in *Utricularia* spp. – the first time fractionation has been measured for botanical carnivory. We are currently drafting the manuscript for this work. The LOREX 2024 collaboration will cement our professional interdisciplinary relationships as well as allowing access to the sites I previously used *Utricularia* for model development; it is ideal to use the same species from the same sites to measure and compare $\delta^{15}$N values of plant tissues.

How it will contribute to my thesis

The project completed through my LOREX 2020 collaboration is one of the main chapters in my dissertation. The results from the current project will address how and where fractionation occurs during the trophic transfer in carnivorous plants, thus strengthening my dissertation by providing additional insight into N uptake process in botanical carnivory.

How it will contribute to my future career goals

My project through the LOREX internship is a valuable opportunity to continue working with stable isotope ecologists and other potential future collaborators. Australia is a hotspot for carnivorous plant species, and high-profile work in this area will help to establish me as an international expert in my field. Stable isotopes have applications in many areas of environmental research, and in fact, as a result of my 2023 LOREX internship, I identified a new collaborative opportunity with colleagues in Germany and Netherlands who are also interested in investigating botanical carnivory through compound-specific isotope analysis. Gaining new cross-disciplinary network opportunities like this will be a huge asset to my future opportunities as a leader in carnivorous plant research and conservation.
References


Dear Prof. Paytan and members of the LOREX review panel,

Re: Letter of support – Emmi Kurosawa

I enthusiastically support Emmi Kurosawa and her proposal to elucidate trophic isotope fractionation factors for the carnivorous plant Utricularia gibba. The Centre for Coastal Biogeochemistry (CCB) and Faculty of Science and Engineering (Southern Cross University) will host Emmi for the duration of the LOREX exchange, and provide further mentoring thereafter. CCB is well placed to support Emmi’s project. We have a world-class stable isotope facility, expertise in aquatic biogeochemistry, and have consistently achieved the highest possible ranking in geochemistry in national research reviews since 2010.

Emmi previously visited our research group through the LOREX program in 2020 and 2023. Her exchange in 2020 led to a conference presentation that received a student award at the Australasian Environmental Isotope Conference (Nov. 2022) and a manuscript expected to be submitted for publication within the next few weeks. We are now working on drafting a manuscript based on Emmi’s 2023 project, which measured trophic fractionation during botanical carnivory for the first time. A LOREX opportunity in 2024 would provide Emmi with a valuable opportunity to further investigate this fractionation, providing insight into nitrogen processing by Utricularia. This would cement Emmi’s position as an expert in carnivorous plants and help to further her career. Emmi would also have the opportunity to provide samples to colleagues in Germany and the Netherlands, who are using compound-specific isotope analysis to investigate botanical carnivory. This would value-add to Emmi’s LOREX 2024 experience. It is essential that Emmi completes this work in Australia, as this is where her research species is located.

Emmi’s proposal is well aligned with the research expertise of CCB in stable isotope techniques, and builds upon our existing areas of research in aquatic environments. Previous exchanges provided the opportunity for Emmi to improve her understanding of stable isotopes and for us at SCU to learn about carnivorous plants. A further LOREX opportunity in 2024 will cement our collaboration with Emmi, leading to an additional manuscript, and will positively impact our research group as well as Emmi. Within our research group we currently have 10 postdoctoral fellows and academics and 12 PhD students (many working in freshwater habitats), providing a dynamic environment for exchange of ideas and expertise.

I am confident that Emmi has the drive, knowledge, and capability to successfully complete her proposed project. Furthermore, the project would be a valuable contribution to her dissertation and field of research. I look forward to working with Emmi should her application be successful.

Sincerely,
A/Prof. Joanne Oakes