MES Thesis Fund Committee
The Evergreen State College
Olympia, Washington 98505

To the MES Thesis Fund Committee,

Please accept the attached materials as my application for a MES Thesis Fund Award for the 2019 period. I have received credit for Case Studies and Thesis Design in the Fall of 2019. I have already collected data and have started processing samples for my thesis work as describe in my thesis prospectus. I expect to graduate in Spring 2020.

The goal of my thesis is to assess the mercury concentrations in zooplankton from three lakes in Washington. Arriving via atmospheric deposition from offshore industrial sources, mercury bioaccumulates from primary consumers (zooplankton) to fish and subsequently to humans, causing severe health impacts. Some factors affecting bioaccumulation, including thermal stratification are not well understood. Some seasonal variation in mercury load has been observed in fish, but little research has been done on mercury variation in zooplankton. I chose zooplankton because of their ubiquity in aquatic systems, their status as keystone species, and the role they serve as the entry point for mercury into the trophic system. I sampled zooplankton from Lake Ozette, Failor Lake, and Black Lake in three consecutive months spanning the fall de-stratification of each lake. Zooplankton samples will be analyzed for mercury concentrations as well as identified to the genus level. Temperature profiles will assess the thermal stratification of each lake at each sampling event.

Results from my research would validate the methodology of studying zooplankton as a proxy for species higher up the food chain, offering a more economical way for managers to measure mercury levels. A finer understanding of seasonal variation in mercury levels could inform safety advisories for fish consumption. The National Park Service and the State Department of Ecology have both expressed their interest in my findings as well as their desire to support the project.

I am requesting $1000 to cover mileage expenses and the rental of a kayak to aid in data collection. As the attached budget indicates, the total cost of the project is $1,135.98. I will cover the remainder out of pocket.

Thank you for your support and consideration,

Emilia Omerberg
Masters of Environmental Studies Program
The Evergreen State College

MES Thesis Fund Application:
Thesis Prospectus
Applicant, Emilia Omerberg

The Evergreen State College Graduate Program on the Environment

Thesis Prospectus

Name Emilia Omerberg **ID Number** A00309373 **4720 1**
Mailing Address **Lacey**

Telephone (415) 609 1215 **eomerberg@gmail.com**
E-mail

STUDENT AGREEMENT:

SIGNATURE: **DATE**

FACULTY READER APPROVAL:

SIGNATURE: **DATE**

MES DIRECTOR APPROVAL:

SIGNATURE: **DATE**

1) **Provide the working title of your thesis[1].**
   Assessment of Mercury Contamination in Zooplankton of Three Washington Lakes.

2) **In 250 words or less, summarize the key background information needed to understand your research problem and question.**
   Mercury is toxic to many forms of life. In humans, exposure to high levels of mercury can lead to tremors, cognitive and hearing losses, hallucinations, and even death (Azevedo et al., 2012). Similar effects have been noticed in small mammals and birds (Department of Agriculture, 2013).
   Mercury emitted from fossil fuel boilers, waste incineration, old and current mining operations, and other point sources have polluted the air, then contaminated water sources through atmospheric deposition (Eagles-Smith,

Suchanek, Colwell, Norman, & Moyle, 2008a; Fleming, Mack, Green, & Nelson, 2006; McIntyre & Beauchamp, 2007). While all forms of mercury are toxic, the organic form of mercury, methylmercury, is the bioavailable form and is the focus of much of the literature.

Bioaccumulation is the phenomenon by which persistent contaminants accumulate within individuals and within food webs with potentially devastating effects on those in upper trophic positions, including humans (McIntyre & Beauchamp, 2007). In aquatic ecosystems, bioaccumulation occurs primarily from consumption of prey; this process is called trophic transfer (McIntyre & Beauchamp, 2007).

Daphnia is a common herbivorous zooplankton (Pickhardt, Folt, Chen, Klaue, & Blum, 2002). They are known to be a major food source for many planktivorous fish, and they have even been called a keystone species (Eagles-Smith et al., 2008a; Mittelbach et al., 1995)

Studies by Farkas, Salánki, and Specziár, (2003) and Niimi (1983) have indicated that fish show a seasonal variation in mercury contaminant load. Pickhardt et al. (2002) suggest that this variation could be due to varying levels of contamination in prey. Additionally, it has been suggested that shifts in diet or thermal stratification might play a role as well (McIntyre & Beauchamp, 2007; Slotton, Reuter, and Goldman, 1995). Since there are seasonal variations in fish, it is feasible to think that zooplankton also show this seasonal variation.

3) State your research question.

How does mercury contamination in zooplankton vary between lakes on the Olympic Peninsula? What causes this variation?

4) Situate your research problem within the relevant literature. What is the theoretical and/or practical framework of your research problem?

Due to the heavy impact on human health and other top predators, many researchers focus on the bioaccumulation of mercury in aquatic systems. Bioaccumulation is the phenomenon by which persistent contaminants accumulate within individuals and within food webs (McIntyre & Beauchamp, 2007). Some detail the many ways to test for mercury bioaccumulation at different levels of the trophic system, including examining feathers of birds (Solonen et al. 1990), adult mosquitoes (Hammerschmidt et al. 2005), varying fish tissues (McIntyre and Beauchamp 2006), and whole bodies of zooplankton (Pickhardt et al. 2002, Long et al. 2018) for mercury concentration.

Consuming fish is a direct pathway for mercury poisoning in humans. Consequently, examining mercury levels in fish is an important facet of bioaccumulation (Pickhardt et al. 2002). However, catching and testing large quantities of fish can be difficult, requiring a lot of resources and person power. Because of this, testing other parts of the ecosystem that can act as indicators of the levels of mercury in organisms in higher trophic positions may be a more efficient option (Mittelbach et al. 1995). Pickhardt et al. (2002) describe *Daphnia* as playing a fundamental role in the

bioaccumulation of mercury and suggest *Daphnia* can be used as an indicator for mercury load up the system.

The theoretical system of bioaccumulation beginning with *Daphnia* can be described as follows. *Daphnia* are at the bottom of the trophic system and are consumed by organisms above them (Pickhardt et al. 2002, Mittelbach et al.1995, Eagles-Smith et al. 2008, and McIntyre and Beauchamp 2006). As plankton-eating fish consume *Daphnia*, they acquire and accumulate the mercury load of the *Daphnia*. As piscivorous fish then consume the planktivorous fish, this transfer and accumulation of mercury continues up the trophic ladder. It continues on with apex predators such as birds of prey and humans (McIntyre and Beauchamp 2006 and Solonen et al. 1990).

Pickhardt et al. (2002) found that increased algal blooms decreased the amount of mercury that accumulates up the trophic system. Others found similar results (Chen et al. 2005 and Chen et al. 2012). Researchers also found that increased algae concentrations spread out the mercury load within the system. When the zooplankton consumed the algae, they consumed a reduced load of mercury and this pattern is continued up the trophic system.

This fundamental study conducted by Pickhardt et al. (2002) showed how to measure mercury in *Daphnia*; however, it did not address the seasonal variation that has been observed in mercury contaminant load of fish (for example, in Farkas et al. 2003, and Ward et al. 1999). Studies of mercury contamination in fish have pointed to seasonality as a factor that changes mercury load (Farkas et al. 2003 and Niimi 1983). Interestingly, in these studies, the effect of seasonality on mercury contamination in fish is best explained by the condition factor of the fish (Fulton's condition factor, $! = \frac{!'''' \times !}{! \times !}$ where w and l are the recorded as net weight and total length of a fish, respectively (Farkas et al. 2003)). It was suggested that increased levels in the spring correlated with increased feeding rates and, therefore, increased condition factor of the fish. It is unclear if the seasonal variation of mercury load in fish is increased food consumption, or if the food itself has increased mercury load. It has also been suggested that fall spikes in methylmercury availability after lake turnover increases mercury load in prey and up the trophic ladder (Slotton et al. 1995).

5) Explain the significance of this research problem. Why is this research important? What are the potential contributions of your work? How might your work advance scholarship?

Mercury is a neurotoxin that accumulates up the trophic ladder (Eagles-Smith et al. 2008). In aquatic systems, the larger predators accumulate more mercury, which in turn becomes a problem for human and other consumers of aquatic species.

Mercury is a naturally accruing element, but the bioavailable form (methylmercury) is perpetuated by human activity (Fleming et al. 2006). Continual drawdown of water levels and refilling of aquatic systems such as rivers, wetlands and lakes create prime habitat for iron and sulfate reducing

bacteria to convert mercury into methylmercury (Fleming et al. 2006). Atmospheric deposition and run-off from industrial sites, including clear-cut logging sites, (Van Furl, Colman, Bothner, & Furl, 2010) also distributes this toxic element throughout the environment. Due to some combination of these types of factors, Lake Ozette on the Olympic Peninsula has very high levels of mercury in fish populations and even has a health advisory for all fish (Washington Department of Health).

More research on how this lake ecosystem functions and changes between seasons will be valuable for sport fishing, recreation and ecosystem conservation due to the previously observed abnormality. I will compare total mercury concentrations in *Daphnia* from Lake Ozette to that found in two other lakes, one to establish a control in the area and the other to compare this known outlier to another lake in the region. I will be using lakes that share similar characteristics, such as adjacent wetland complexes, in order to minimize variation due to lake location alone. Failor Lake on the Olympic Peninsula, just north of Aberdeen, is much smaller than Lake Ozette; however, it is in a similar geographic area with an adjoining wetland complex and a large amount of recreational use, including recreational fishing.

Results from my study could influence policy on fishing regulations and have implications for human health and safety. If a relationship between *Daphnia* and mercury levels in a particular season is seen, fishing regulation could be adapted to protect human health but also allow for consumption if seasonal variation is great enough. If no relationship is visible, but mercury is still detectible in the *Daphnia,* then my results could indicate to future resource managers, a facilitated mechanism for testing mercury levels in these aquatic ecosystems. This is valuable for managers because testing of *Daphnia* is far less resource-intensive than collecting fish for analysis. Additionally, research has been done on mercury levels and thermal stratification in other parts of the country, but these results have not been duplicated on the Olympic Peninsula.

6) Summarize your study design[2]. If applicable, identify the key variables in your study. What is their relationship to each other? For example, which variables are you considering as independent (explanatory) and dependent (response)?

This study will examine mercury levels in *Daphnia* in summer and fall. The explanatory variable will be season and the response variable will be mercury level.

My study sites are Lake Ozette, Failor Lake and Black Lake. Each lake was sampled in August, September, and October. The lakes are on a gradient from wilderness to urban with varying amounts of human impacts. They all have wetland complexes, but the lakes vary in size quite dramatically.

At each lake, I also collected data on other variables, including water clarity (Secchi depth), temperature, dissolved oxygen, conductivity and chlorophyll a. Chlorophyll a is a proxy measure for the productivity of a lake,

as it is a by product of photosynthesis. Additionally, I took a lake temperature profile each time I sampled.

7) **Describe the data that will be the foundation of your thesis. Will you use existing data, or gather new data (or both)? Describe the process of acquiring or collecting data[3].**

For my study I will collect new data in the form of lake temperature profiles and zooplankton tows. I will find out the mercury concentrations in zooplankton from three lakes over a seasonal and stratification change. There will be 3 samples from each lake (9 in total) that will be run in triplicate in the lab if the amount of biological material allows.

Zooplankton collection was done by vertical tows through the water column using a 20 diameter 500 micrometer mesh net (following Mittelbach et al. 1995). These samples were taken from a boat at the deepest part of the lake. Samples were taken monthly at each lake August-October, encompassing the fall turnover of the lake (based on temperature). Presence of fall turnover was identified by a lack of thermocline in the lake.

Samples will be frozen until processing in the winter. Processing will include thawing, collecting a wet weight, freezing to -80 degrees C, freeze-drying, collecting a dry weight and then running the samples on the mercury analyzer to ascertain the ng/g of mercury in the zooplankton collections.

I will use the Nippon Instruments Corporation Mercury Analyzer 3000, which is available for use by Evergreen students. This instrument has a detection limit of ~.002ng and can measure samples such as water, soil, minerals, airborne particulates and biological materials. I will be using biological materials (zooplankton).

Mercury levels in zooplankton range from 16.5ng/g - 693 ng/g (Long et al., 2018). Therefore the minimum dry weight I can use on the machine will be .125 mg of zooplankton. .125 mg of zooplankton corresponds to 16 ng/g of mercury. This is the lowest quantity that a machine with a detection limit of .002ng can detect.

Taxonomy samples were also taken, using a subsection of the zooplankton tow stored in ethanol. These will be used to identify the species of zooplankton present in each lake and which species are present over the seasonal change. Measurements of size and abundance will also be taken. I aim to use a compound light microscope (available to Evergreen students) with photo capabilities to aid in this process.

Phytoplankton samples were also taken using a 10 cm diameter 20 micrometer mesh net. Both vertical and horizontal tows were done through the water column and these samples are frozen until analysis occurs.

Each sampling excursion also included a lake temperature profile, including total depth and temperature, dissolved oxygen, and conductivity measurement at each meter (or half meter for lakes shallower than 5 meters) below the surface. This will allow us to assess the stratification and destratification of the lakes.

Each water sample was collected in a 500 ml dark plastic bottle. The sample will be frozen until processing. The sample will be processed to find

chlorophyll a levels in the lakes in each month. Processing will include defrosting, filtering and running on a spectrophotometer. (Mittelbach et al., 1995), (Jordan, Stewart, Eagles-Smith, & Strecker, 2019), (Long et al., 2018) (Chiapella, Eagles-Smith, & Strecker, n.d.)

Table 1: Data Collected

Sample	Location	Month	Lake temperature profile Is the lake stratified?	Mercury in zooplankton (ng/g hg in zooplankton	Chlorophyll a ($\mu g\ ml^{-1}$)	Depth (meters)	Secchi depth (meters)
1	Ozette	August	yes	63	12.1	11	1.5
2	Failor	August					
3	Black	August					
4	Ozette	September					
5	Failor	September					
6	Black	September					
7	Ozette	October					
8	Failor	October					
9	Black	October					

This table shows the type of data that I collected and the units they will be in. The values inserted in the first line are examples of values I expect to see and not actual values. Blank cells represent values that will be filled in as analysis takes place.

Table 2: Zooplankton Species

	Species a	Species b	Species c	Species d
August				
Ozette	PM	A	A	PS
Failor	A	PS	A	PS
Black	A	A	PL	PS
September				
Ozette				
Failor				
Black				
October				
Ozette				
Failor				
Black				

This is a presence-absence table. Each species will be noted with an absence (A), present and small sized (PS), present and medium sized (PM), or present and large sized (PL). August has been filled out with hypothetical data as an example.

8) **Summarize your methods of data analysis. If applicable, discuss specific techniques that you will use to understand the relationships between variables (e.g., interview coding, cost-benefit analysis, specific statistical analyses, spatial analysis) and the steps and tools (e.g., lab equipment, software) that you will take to complete your analyses.**

In the lab, I will be using the lyophilizer, mercury analyzer 3000, spectrophotometer, and a compound microscope. In the computer lab, I will use R studio.

For statistical analysis I will use a chi-squared test for comparing presence absence data of zooplankton. I will also use a Kurskal-Wallis test for the ordinal data of small, medium, and large sized zooplankton.
I use a diversity metric such as Shannon's diversity index for zooplankton taxonomy.

I will use nonmetric dimensional scaling (NMDS) or some kind of ordination like principal coordinate analysis (PCS) to compare similarities and di-similarities between mercury concentrations.
I will use multivariate analysis of variance (MANOVA) to compare mercury values both within lakes and between lakes.

9) **Address the ethical issues raised by your thesis work. Include issues such as risks to anyone involved in the research, as well as specific people or groups that might benefit from or be harmed by your thesis work, perhaps depending on your results. List any specific reviews you must complete first (e.g., Human Subjects Review or Animal Use Protocol Form), and specific permits or permissions you need to obtain before you begin collecting data (e.g. landowner permissions, agency permits).**

There are minimal ethical issued raised by my thesis work. I am not conducting research on humans therefore no vulnerable parties will be implicated. One ethical issued raised is based on the reporting of my findings. I have a duty to the public to disclose my findings on mercury concentration in Lake Ozette, Lake Failor, and Black Lake as they could have harmful impacts on human health and wellbeing. My findings could also have impacts on homeowners and impact property values. Additionally, my findings could negatively impact recreational businesses and government agencies that profit from fishing licenses sold in the area.

In order to avoid misrepresentation about my findings I will write a blog post for the lay reader and submit it to the Evergreen MES blog. I will also email a copy of my thesis and the lay blog post to the people I have made contact with along the way, including staff at the National Park Service and at the water treatment plant at Hoquiam.

For the collection of my final sample at Failor Lake, I needed access from the Water Department of the City of Hoquiam. I was able to obtain access to the lake after its closure by working with the people at the water treatment plant and arranging for them to unlock the gate for me. They have

indicated they are willing to open it again if need be and they are also interested in the outcome of my research. I also spoke to the city administrator (an MPA graduate), and he indicated he would be interested in knowing the results of my findings on behalf of the City of Hoquiam.

I was unaware of the need for a special permit for collecting scientific specimens. I am now in the process of retroactively applying for permits from the National Park Service and from Washington Fish and Wildlife.

10) Reflect on how your positionality as a researcher could affect your results and how you will account for this in the research process[4].

My positionality coming into this research is that I have had lots of training in the scientific method. I consider myself a conservation biologist and actively work to conserve habitats and native species. I will be looking at the data I collect with a scientific eye and will be using the best scientific practice in my methods and analysis. I am a resident in this area but I am not a property owner, and my life is in transition as I move from school to work; therefore, I may not be here long after finishing this research. I am not conducting this work on the behalf of any agency therefore no bias should result from my data collection or analysis. I may partner with the Washington Department of Ecology to use some of their fish samples. If this is the case then I may have some bias based on the institutional goals of the Department of Ecology.

Another bias I have is that I am expecting to see a significantly higher amount of mercury in one of my study sites compared to the others.

Additionally, I have the bias that I would like to complete this project in a projected time frame and therefore will not have more than one season in which to collect data. This could skew the results; however, any initial findings will still be valuable to the scientific community and the resource managers in the area.

11) Provide at least a rough estimate of the costs associated with conducting your research. Provide details about each budget item so that the breakdown of the final cost is clear.

Travel

Location	Distance (mi)	Round trip (mi)	Times traveled	Total miles	Cost (.59/mile)
Ozette	204	408	3	1,224	$722.16
Failor	70	140	3	420	$247.80
Black Lake	13	26	3	78	$46.02
				Subtotal	$1015.98

Equipment

Item	Price	Times Rented	Cost
Kayak	$40.00	3	$120.00
		Subtotal	$120.00

Other

Item	Application fee	Permit cost	Total
Collection permit	$105.00	$12.00	$117.00
		Subtotal	$117.00

Total Cost
$1252.98

All collection and processing equipment was borrowed from the Science Support Center at Evergreen State College or from the Washington State Department of Ecology. Abir Biswas also donated time slots and use of the mercury analyzer.

12) Provide a detailed working outline of your thesis.

- **Cover**
- **Title page**
- **Table of contents**
- **Key terms and definitions**
- **Abstract**

- **Introduction/background information**

- **Literature review**
  - Importance of mercury

Mercury (Hg), in its elemental form is characterized by a malleable silver liquid at standard temperature and pressure (toxnet). Human use of mercury dates back to more than 2500 AC where early humans used cinnabar (mercury sulfide) as paint for both the body and cave walls due to its distinct red and gold coloring (Azevedo et al., 2012).

Currently, most mercury comes from degassing of the Earths crust however there are many human practices that increase the availability of mercury such as mining (old and current), fossil fuel extraction and waste incineration (Department of Agriculture, 2013; Eagles-smith, Suchanek, Colwell, Norman, & Moyle, 2008b; Fleming et al., 2006; McIntyre & Beauchamp, 2007)(toxnet). In current times mercury is used in the electrical industry as cathodes, in the dental industry in silver amalgams, and in many medical instruments and scientific tools such as thermometers and barometers (toxnet)

- Negative impacts of mercury

While mercury has many practical uses it is also a danger to human health. In humans, exposure to high levels of mercury can lead to tremors, cognitive and hearing losses, hallucinations, and even death (Azevedo et al., 2012). , Similar effects have been noticed in small mammals and birds (Department of Agriculture, 2013) see Abirs sources)
Inhaling mercury vapor can cause immediate chest pain, coughing, hemoptysis, and interstitial pneumonia which can be deadly (toxnet). Ingestion of mercury compound such as mercury chloride can cause ulcerative gastroenteritis and acute tubular necrosis which results in death if dialysis is not available (toxnet).

- Inorganic vs organic form
    - Methylation
    - Sulfur and iron reducing bacteria
    - Other inputs- mining, cinnabar, dental

Mercury can be found in its elemental form (metallic), in the inorganic form, and in the organic form. The organic form is of large concern for human health because it is most readily absorbed into the human body( ). The inorganic form can be transformed into the organic form in the presence of sulfate and iron reducing bacteria (Fleming et al., 2006). This process is called methylation. This addition of the methyl group to the mercury (making it into methylmercury ($CH3Hg^{+)}$)) allows the compound to be able to move across the blood brain barrier. When humans consume food that contains methylmercury, that methylmercury can make its way through normal barriers to toxins and lodge in cells within the body. Once there, it can cross into the brain and blood and impact neurological systems and the kidneys (Government of Canada 2013). Mercury is more soluble in plasma, whole blood, and hemoglobin than it is in distilled water (toxnet)

This problem is especially problematic for pregnant and breastfeeding women. Since the methylmercury can readily move across cell membranes it can makes its way through placentas and contaminate growing fetuses. Developing

nervous systems are at high risk from methylmercury due to its impairment of the developing nervous system.

  - Blood-brain barrier
  - Cognitive and neurological development

- What is Bioaccumulation?

Bioaccumulation is the phenomenon by which persistent contaminants accumulate within individuals and within food webs with potentially devastating effects on those in upper trophic positions, including humans (Fleming et al., 2006).

In aquatic ecosystems, bioaccumulation occurs primarily from consumption of prey (trophic transfer) (McIntyre & Beauchamp, 2007). The mechanism by which this occurs is complex; many factors, both physiological and environmental play a role in the process (McIntyre & Beauchamp, 2007). When humans consume food that contains methylmercury, that methylmercury can make its way through normal barriers to toxins and lodge in cells within the body. Once there, it can cross into the brain and blood and impact neurological systems and the kidneys (Government of Canada 2013).

  - Local example (Abir's college in Oregon re bird feathers)
  - Factors affecting bioaccumulation

- Aquatic ecosystems
  - Primary producers
  - Secondary producers
  - foodchain
- Daphnia as keystone species

  Daphnia is a common herbivorous zooplankton (Pickhardt et al., 2002) known to be a major food source for many planktivorous fish (Eagles-smith et al., 2008b; Mittelbach et al., 1995). Daphnia have even been called a keystone species in aquatic ecosystems (Mittelbach et al., 1995). Because of their ubiquitous presence in aquatic systems and because they, too, accumulate mercury and methylmercury, daphnia can play an important role in predicting the levels of methylmercury that can be found higher up the trophic system (Pickhardt et al., 2002).
- Biodilution theory
  - As seasons progress fewer daphnia so potentially increased mercury load?
- Seasonal changes in lakes
  - Stratification
  - Epilimnion/hypolimnion/thermocline
  - phytoplankton
  - Chlorophyll a production
- Seasonal variation in daphnia (maybe other zooplankton)
  - Life cycle and value to the system
  - Diurnal migration (migration across thermocline?)
  - Daphnia as integrated sample of lake (integrated mercury and integrated over time)

    - Daphnia feeding
- Lake geography/characteristics/ study sites
    - Lake similarities (Ozzette and Failor)
        - Wetland complexes
        - Geographic areas
        - Differences in mercury content
- Gaps in existing knowledge
    - Somewhat but not in this area
    - Drawing conclusions between zoops and fish
    - Use of fish tissue (I have access to archived fish tissue from Ozette and Failor so might be able to test mercury in those tissues as well and then compare them to zoops samples) only thing here is that fish samples are from bass which is a piscivorous fish and not a planktivorous fish so these trophic levels are one step removed.
    - Impacts on policy

- **Methods**
    - Zooplankton
        - Clean hand dirty hands protocol
        - Collection
        - Storage
            - Freezing
                - Hg analysis
            - Ethanol
                - Microscopy analysis
    - phytoplankton
        - collection
        - storage
    - Lake temperature profile
        - Collection
        - Analysis
            - Graphing
    - Chlorophyll a
        - Collection
        - Storage
            - Freezing
            - Thawing
            - Filtering
            - Spectrophotometry
    - Secchi depth
        - Collection

- **Results**
    - **Zooplankton**
        - Mercury analysis (concentrations)
        - taxonomy

- phytoplankton
  - (Still not sure if I will use)
- temperature profile
  - stratified or not/starting to change or all the way changed
- chlorophyll a
  - μg/l
- Secchi disk
  - Depth (m)

- **Discussion**
  - Changes in one lake (across all months)
    - Mercury
    - Taxonomy changes
    - Chlorophyll a
    - Secchi depth
    - Temperature profile
  - Comparisons between lakes (in each month)
    - Mercury
    - Taxonomy
    - Chlorophyll a
    - Secchi depth
    - Temperature profiles
  - Lake characteristics and activities near lakes such as logging
- **Conclusion**
  - Something happened
  - Future research
  - Call to action
- Appendix
- References

13) Provide a specific work plan and a timeline for each of the major tasks in the work plan. Be as realistic as you can, even though you will probably need to alter this schedule as you complete the tasks. Remember that faculty readers take time to return your drafts and that the final polishing and formatting of your thesis for binding will take longer than you ever imagined.

November 2019-

- 17th second draft of prospectus
- 24th final working draft lit review due

December 2019-

- Dec 13th - signed prospectus due
- 20th second draft of lit review/start methods
- spend time over holidays reading and talking with Jesse

January 2020

- 15th spectrophotometer work complete and microscope taxonomy during ILC
- 17th methods draft
- 22nd complete lyophilizing of all samples
- 31st complete mercury analysis

February 2020- start analysis of data.

- 7th complete taxonomy microscope work
- 7th organize mercury analysis results
- 14th run statistical analysis of mercury results (ANOVA)
- 21st have results draft done
- 28th have analysis/discussions draft done

March 2020

- 6th draft figures.
- 13th conclusion draft
- flex time

April 2020

- 10th first complete draft due
- 24th Submit request to present form (due may 1)

May 2020

- 19-21 and 26-28 thesis presentations
- 29th Final draft to reader

June 2020

- 5th final hard copies and electronic to MES office by June 5th

14) Who, beyond your MES faculty reader, will support your thesis? Indicate support both within and outside of Evergreen. Be specific about who they are and in what capacity they will support your thesis. If you are working with an outside agency or expert, be specific about their expectations for your data analysis or publication of results.

Other than my MES faculty reader I have worked closely with William Hobbs from the Washington Department of Ecology. He has been very involved from the start and has agreed to continue to be very involved for the duration of the process. He is available for laboratory assistance and fieldwork as well as assisting in the writing process. He has agreed to read drafts and comment on them. He is a technical expert/advisor on this project. I also have support from Abir Biswas who is an undergraduate professor at Evergreen. He is also acting as a technical expert on the Mercury analyzer machine and has offered to read some drafts of my thesis. I expect he will be very valuable for the methods and analysis section.

Additionally I have the limited support of Patrick Kockovsky with the USGS at the Lake Erie Biological station in Sandusky Ohio. He is a long time mentor and has offered to review some drafts.

15) List the 3-5 most important references you have used to a) identify the specific questions and context of your topic, b) help with issues of research design and analysis, and c) provide a basis for interpretation. For each reference, explain how your project specifically connects to the source by extending, challenging, or responding to the conclusions, methods, or implications.

McIntyre, J. K., & Beauchamp, D. A. (2007). Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. Science of the Total Environment, 372(2–3), 571–584. https://doi.org/10.1016/j.scitotenv.2006.10.035

This article identifies why assessment of mercury accumulation is important (effects on human health and other upper level trophic predators). This article looks at variation in mercury and organochlorine accumulation between key fish species and zooplankton specifically in Lake Washington in an effort to understand the ways in which bioaccumulation manifests as we move up the food chain/ trophic levels. This study found that age and trophic position were significant predictors of bioaccumulation. Trophic position was more important than age for predicting mercury but age was more important for predicting PCB (polychlorinated biphenyls (PCBs)) accumulation. There were also important findings indicating not just age but age/time in residence in the lake that played a significant role. Carbon and nitrogen isotopes were also used to assess the progression through the food web and identify the pathways from the bottom up. Perhaps the most significant finding was that lipid content was not correlated to contaminant load as has been indicated by many other studies in this field. This article will be valuable for my literature review because of its plethora of background information on mercury accumulation and factors other than season that affect accumulation in species up the food chain.

Pickhardt, P. C., Folt, C. L., Chen, C. Y., Klaue, B., & Blum, J. D. (2002). Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. Proceedings of the National Academy of Sciences of the United States of America, 99(7), 4419– 4423. https://doi.org/10.1073/pnas.072531099

Pickhardt et al. experimentally studied the theory of dilution bloom. They focused on algal blooms effect on mercury concentration on *Daphnia*. The purpose was to find out if algal blooms effectively reduce the amount of toxins in fish by reducing the density of the toxin in *Daphnia*, which is just below fish in the food chain. This is important as it introduces the problems of mercury, the important and central role that *Daphnia* plays in an aquatic system, as well as exploring the way that algae, zooplankton and fish interact with one another. This is an older

paper and it will be important to find newer articles that corroborate this or potentially disprove it.

Slotton, D. G., Reuter, J. E., & Goldman, C. R. (1995). Mercury uptake patterns of biotain a seasonally anoxic northern California Reservoir. Water, Air, & Soil Pollution,80(1–4), 841–850. https://doi.org/10.1007/BF01189735

This source is valuable to my literature review as it discusses the effect of thermal stratification on the bioavailability of mercury. This is a central theme of my research questions so knowing other researchers findings is key. Slotton et al. (1995) found a pulse of increased mercury in the time right after thermal destratification. They hypothesize that the mixing of anoxic waters increased methylation temporarily allowing for more availability and therefore greater loads in in both the fish and the zooplankton. This article includes "a simple and effective, syringe-based cold vapor atomic absorption method." I will most likely not be using this method for my analysis but it is good to know about methods used by other researchers.

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[1] You are not locked into this title; its purpose is to help you identify the main point or topic of your thesis at an early stage.

[2] You might discuss selection of case studies, sampling methods, experimental
[2] You might discuss selection of case studies, sampling methods, experimental
design, and/or specific hypotheses you will test. You should also address any specialized knowledge or skills that are necessary to complete the research.

[3] If you are planning to use existing data, explain the specific source, contact information, arrangement with collaborating agencies, and expectations about use of data and final products of your research. If you are planning to gather new data, describe specific methods, time, place, and equipment that will be required.

[4] Your *positionality as a researcher* refers to the fact that one's "…beliefs, values systems, and moral stances are as fundamentally present and inseparable from the research process as [one]'s physical, virtual, or metaphorical presence when facilitating, participating and/or leading the research project…" (The Weingarten Blog 2017).

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MES Thesis Fund Application
Itemized Budget: Applicant, Emilia Omerberg

Travel

Location	Distance (mi)	Round trip (mi)	Times traveled	Total miles	Cost (.59/mile)
Ozette	204	408	3	1,224	$722.16
Failor	70	140	3	420	$247.80
Black lake	13	26	3	78	$46.02
				Subtotal	$1,015.98

Equipment

Item	Price	Times used	Cost
Kayak	$40	3	$120
		Subtotal	$120

Total Cost
$1,135.98

MES Thesis Fund Application
Budget Justification: Applicant, Emilia Omerberg

Travel

I am requesting mileage reimbursement for:

- Three round trips to Lake Ozette (408 miles each)
- Three round trips to Failor Lake (140 miles each)
- Three round trips to Black Lake (26 miles each)

My home in Lacey is both start and end point for these calculations as it is the most central location for reaching each of the sites.

Why are you requesting funding for the specific item and how will it contribute to the success of your thesis research:

I am requesting funds to drive to and from my three study sites because it is not possible to collect my samples remotely. I tried to consolidate excursions to Lake Ozette and Failor Lake however the time it took to drive to each site did not allow for enough daylight to safely collect all the data necessary at each location.

This funding will allow me to collect novel data that is essential to my thesis research. My study focuses on Lake Ozette and Failor Lake because of their high mercury levels in fish and Black lake because of its proximity to Olympia. Zooplankton research in all three sites is novel and could open doors for future research.

Equipment

I am requesting funding to reimburse myself for the rental of a kayak to aid in data collection.

Why are you requesting funding for the specific item and how will it contribute to the success of your thesis research:

I am requesting funding to rent a kayak from The Outdoor Program (TOP) at Evergreen. TOP has discounted student rates and this substantially less expensive than purchasing a new or used kayak. A kayak was also the most appropriate vessel to collect data from because it did not require boating licenses or special access at the study sites. It was also the least invasive choice.

I rented all other scientific monitoring equipment from the Science Support Center at Evergreen or from The Department of Ecology in order to reduce the cost of my research.

Other

I am no longer in need of $117 to pay for the application fee and permit fee for a scientific collection permit for Washington Fish and Wildlife because I have been added to an existing permit listed under the Department of Ecology. Additionally the permit needed to collect samples in Olympic National Park did not require a fee.

Statement of funding

I do not have any other pending funding requests that would apply to the items listed here.

evergreen

Master of Environmental Studies

MES Thesis Fund Application Checklist for (*student name*): Emilia Omerberg

Please check **any and all** boxes that are applicable to your intended thesis research, and complete any required subsequent information.

☐ **My thesis research involves working with human subjects.** I submitted my Human Subjects Review Application to the Academic Deans Office on the following date: -

☒ **My thesis research will involve the use of Evergreen's Science Support Center and laboratory spaces.** I have completed the required forms to begin using these spaces and have been assigned a Scientific Instructional Technician (SIT). **My assigned SIT is** (if you do not currently have an assigned SIT, please explain):
Sina Hill

☒ **My thesis research requires that I have obtained special permits and/or permissions in order to collect data.** The required permits/permissions I need, and the status of my requests and approvals is detailed below. Please attach electronic copies of any permits already obtained.
- National Park Service Permit attached
- WDFW permit held by Department of Ecology

☐ **I am requesting reimbursement for the purchase of software and/or licensing** for technology needed to complete my research and analysis. I have spoken with the following staff or faculty member(s) at Evergreen and am assured this is not available on campus: -

evergreen

Master of Environmental Studies

☐ **I have requested funding for my thesis from other sources.** If checked, please list the source of funding (e.g., Evergreen Clean Energy Committee), and the status of your request (funded/not funded/in review).

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☐ **None of the above apply to my research plans**