Grassy Narrows and Muskrat Falls Dam: Hypothesis Testing and t-Tests

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Abstract

Methylmercury contamination within fish populations continues to be a modern day dilemma. The impacts of exceeding safe dietary methylmercury levels are tragically shown in Ontario, Canada where a First Nations community in Grassy Narrows are living with the consequences of methylmercury poisoning due to the dumping of mercury in their traditional waterways. More recently, there has been a highly publicized protest in Muskrat Falls in Labrador, Canada, where the Inuit people have raised direct concerns about the potential for a megadam project to increase mercury levels in fish in those waters, which are an integral part of their traditional diet. This module uses these real-world examples as a jumping-off point for exercises that will guide case-study driven discussion on mathematical, biological and ethical concerns around this issue.

The module is divided into parts and can be done in-class, online, or as homework. There are areas where additional resources are provided for students/courses that need instructional material not provided in the case study. In addition, there are opportunities to expand particular sections for classes that would like to emphasize certain content areas. Overall, students are introduced to concepts of hypothesis testing using elementary hypothesis tests, t-tests, p-value (and more) as they compare a given fish population for methylmercury levels (using real and hypothetical data) against real-world mercury standards in fish (in Canada). Additionally, students will be encouraged to research for themselves as well as have an open philosophical discussion at the close of the module.

The QUBES Team (Quantitative Undergraduate Biology Education and Synthesis) that developed this module includes mathematicians, biologists, industry professionals and professional educators who came together to produce this open sourced product for quantitative biology education.

Tags: Methylmercury, Bioaccumulation, Hypothesis testing, t-test, p-value, statistics, math, biology, traditional diets, mercury levels, fish population, Biogeochemical cycles, in-class, online, homework, mercury poisoning, native people, indigneous population, Canada, dam, hydroelectric

Learning Objectives:

At the end of this activity, students will be able to:

- Distinguish between a sample and a population.
- Distinguish between deterministic and stochastic problems.

- Recognize when it is appropriate to use sample data to infer information about the population.
- Assessing the way a sample is selected, identifying possible sources of sampling bias.
- Using a sampling distribution to estimate probability.
- Apply the concepts of estimation and testing to answer questions using real data.
- Explain the meaning of a level of significance and a p-value.
- Demonstrate an understanding of the concept of statistical significance versus biological significance.
- Evaluate a hypothesis using data.
- State the conclusion to a hypothesis test in context.
- Use probability rules or significance values to evaluate a course of action.

Teaching Notes: Grassy Narrows and Muskrat Falls Dam

This activity is divided into parts and can be done in-class, online, or as homework. There are areas where additional resources are provided for students/courses that need instructional material not provided in the case study. In addition, there are opportunities to expand particular sections for classes that would like to emphasize certain content areas.

Pre-Class Activity:

This activity provides students with context to the main issue in this case study. They are introduced to the area and people. By using news articles and documentary videos, students can learn the importance of the topic that will be discussed.

Students should read the news article and watch the videos. This can be done in class or assigned outside to be completed before class. Students should answer the questions and these can either be collected in class or allow for group/class discussion.

Part I: Are the fish in the rivers and lakes by Grassy Narrows First Nation safe to eat now?

The first part of the case study relates to mercury standards in fish in Canada. The focus is on hypothesis testing and using real and hypothetical data to test hypotheses about populations.

Students begin with a quick reminder about unit conversions as a way of understanding how deterministic problems differ from stochastic problems. Students should be paired up and presented with the problem. If your students are struggling with the method of how to properly convert units, here are some resources.

> "Mini-Mods: General Chemistry - Units" 2017 by Paul Cooper
> https://www.oercommons.org/authoring/14974-mini-mods-general-chemistry-units/2/view
>
> "Unit Conversion Worksheet - Barbara Gilbert at CNM" 2016 by Sonya Bennett-Brandt
> https://www.oercommons.org/authoring/15812-unit-conversion-worksheet-barbara-gilbert-at-cnm

Students now move into the question of the case study, “Is the average mercury concentration in the fish greater than 0.5 mg/kg, (Canadian standard for safety)?”. This question will help students understand whether the fish are actually safe for consumption. First, students need to understand how to define a population. Then, they are introduced to creating null and alternative hypotheses about all the fish in the lake. The questions presented evaluate the student's understanding of the material.

One of the fundamental elements of hypothesis testing is a sampling distribution. We have provided a table of a hypothetical population that conforms to the null hypothesis with a mean of 0.5 mg/kg. To help students understand a sampling distribution, this activity is done as a whole class. Students follow a random process that is provided by the instructor to select the data for the sampling distribution. One possible approach using cell phones is shown below. Every student will repeat this process enough times so the class generates at least 30 sample means.

- Have the students go to their list of contacts in your cell phone. Write down the last two numbers in the first 5 phone numbers. The second to last number will be the x coordinate, the last number will be the y coordinate. The data will be found at the intersection of the column for the x coordinate and the row for the y coordinate. Sample with replacement (the same number can be found more than one time).
- The provided powerpoint can also be used as an interactive method for students to use random numbers to simulate catching fish and getting data, when they don't see all the data in the other fish.
- A different strategy that could be employed is to have the numbers 0 to 9 written on papers, with students choosing one paper at a time and recording the number, then putting the paper back into the container (to make sure it can be picked again). Their first pick is X, the second pick is Y. Repeat this 5 times and fill in the table in a similar way as if phone numbers were used.

First 5 phone numbers	X	Y	Data
555-555-22**20**	2	0	0.75
-95**80**	8	0	0.55
-36**00**	0	0	0.84
-65**92**	9	2	0.41
-53**16**	1	6	0.43
		Mean:	2.98/5 =0.596

On the board, create a histogram of all the sample means from the students and create a histogram as a class (this is a sampling distribution). Students can draw this histogram in Figure 3 provided. If you have not covered graphical representations of data, here are some additional resources:

Basic instruction in making a histogram: https://www.youtube.com/watch?v=5IPJjUOqnoA

An Interactive explorable about histograms: http://tinlizzie.org/histograms/

This graph can be compared to Figure 4, which is a random histogram created from 500 samples. Students should complete Table 3 to demonstrate their understanding of the probabilities in the sampling distribution. A sample of Table 3 is shown here. Students should find the intervals on the horizontal axis and then add the numbers on top of the bars to find the probabilities.

Interval	Probability
[0.55,0.60)	0.16
[0.30,0.35)	0.04
[0.25,0.35)	0.05
Less than or equal to 0.35	0.05

There are four steps to deciding which hypothesis is supported by the data. Each step is presented individually, with questions to test student understanding.

In Step 4, questions under b provide an opportunity to engage in discussion and explanations.

These questions are posed to help students understand the Central Limit Theorem. Suppose that a sample is obtained containing many observations, each observation being randomly generated, with the arithmetic mean of the values computed. If this procedure is repeated many times, the central limit theorem says that the probability distribution of the average will closely approximate a normal distribution. A simple example of this is that if one flips a coin many times, the probability of getting a given number of heads will approach a normal distribution, with the mean equal to half the total number of flips. At the limit of an infinite number of flips, it will equal a normal distribution.

> Additional resource to help students understand Central Limit Theorem:
>
> "Central Limit theorem" 2017 by Salman Kahn
> https://www.khanacademy.org/math/ap-statistics/sampling-distribution-ap/sampling-distribution-mean/v/central-limit-theorem

Part II: Comparison of Fish Species Frequently Consumed

The second part of the case study has students compare fish populations and introduces them to t-tests. The formula for the t-test is provided so that students can attempt to use the formula first, so that they don't think that t values and p-values are produced magically from the technology. However, after using the formula once, then make use of the technology, if it is available to the students. Students will then apply the formula or technology to actual data.

Students are asked to use Excel for the t-test; however, if the instructor prefers, you can use any statistical package. If students are unfamiliar with entering formulas or using Excel for statistics, these resources can be helpful.

> Overview of Formulas in Excel by Microsoft Office
> https://support.microsoft.com/en-us/office/overview-of-formulas-in-excel-ecfdc708-9162-49e8-b993-c311f47ca173
>
> To find t and p values:
> https://ms-office.wonderhowto.com/how-to/find-p-value-with-excel-346366/

Here is additional information on native diets and which fish are consumed by Grassy Narrows. Fish species contributing to the MeHg intake included pickerel-walleye, pike, perch, bass and trout (Kinghorn et al. 2006 and Juric et al. 2017). Pickerel-walleye and pike account for 55% of fish intake and 75% of mercury exposure (Kinghorn 2007).

Part III: The Muskrat Falls Energy Project controversy

This section can be done alone or with the rest of the case study. This is a more biologically and ethically focused activity. As an alternative, this part can be done in the classroom or assigned as a project/homework.

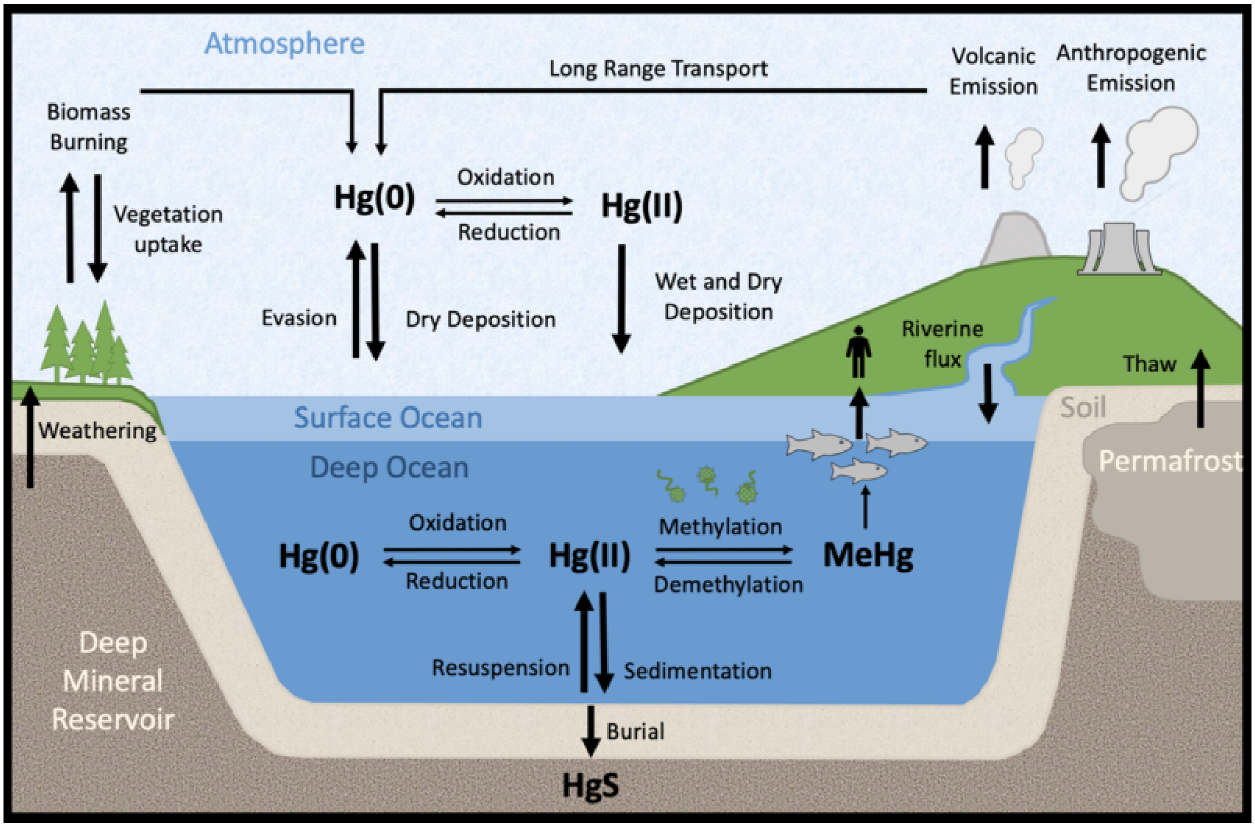
Students will need to read the news article to complete this section.

> CBC News. “What's the deal with Muskrat Falls? Answers to a few frequently asked questions.” May 2019. https://www.cbc.ca/news/canada/newfoundland-labrador/muskrat-falls-whats-the-deal-1.5083458

For students who are unfamiliar with biogeochemical cycles or instructors that want to explore the mercury cycle more in depth, you can use the mercury cycle image and explain the processes. Mercury has a biogeochemical cycle that is influenced by both natural processes and human influences. Mercury is transformed into multiple chemical forms and between environments. You can find mercury in the Earth’s crust and in various forms on the Earth’s surface. It can be elemental, inorganic, or organic. Mercury exists in three oxidation states: Hg(0) elemental mercury, Hg(I) mercurous mercury, and Hg(II) mercuric mercury (Figure 9). The primary source of mercury emission occurs by weathering of minerals and geothermal activity releases mercury from sedimentary rocks into the environment. Active volcanoes are another significant primary source of natural mercury. Human-influence emissions occur from gold mining, burning coal, and production of non-iron metals, such as copper or lead.

Elemental mercury is moved from the land to the ocean by atmospheric circulation. It is then returned by dry deposition (a major elemental sink of Hg(0)) or photo-oxidized to gaseous Hg(II) and then returned by wet or dry deposition.

Inorganic mercury is converted by bacteria and archaea into methylmercury $[CH_3Hg]^+$. The big problem with methylmercury is that it accumulates in aquatic species, such as tuna and wall-eye, who absorb more mercury than can be lost or eliminated and builds up further up the food chain. This is called bioaccumulation and biomagnification.



Students are instructed to research the status of the Muskrat Falls hydroelectric dam project. As of summer 2021, the project has only one working unit generating power.

Using the discussion questions, you can have students engage in a classroom discussion/debate or assign it as a written project. Here students can discuss traditional versus non-traditional diets and impact of methylmercury poisoning. It is interesting to note that one possible compromise to the dam situation is to clear land of vegetation before flooding, which will reduce the production of methylmercury.

<u>Answer Key:</u>

Pre-Class Activity

1. How would you know the fish in this population are contaminated? Answers may vary. Some responses may include soil testing and the testing of the fish themselves.

2. What are some of the impacts of the mercury contamination on the Grassy Narrows First Nation? Answers may vary. One impact was it limited the food sources and availability of clean water for the

residents. Some of the health effects of mercury poisoning on the residents included neurological symptoms of mercury poisoning include ataxia (a degenerative disease of the nervous system), numbness in the hands and feet, seizures, general muscle weakness, vision problems, and damage to hearing and speech.

3. Compare the level of importance for solving this problem if this problem was happening in your community and affecting you and your family with it happening to the Grassy Narrows community? Answers may vary. This question is meant to encourage students to think critically about how they make decisions.

Part I

1. Students should get the same answers and notice that they are just going forwards and backwards in the conversion.

2. Which of these hypotheses indicates the fish are safe to eat? The null hypothesis, H_0

3. Which of these hypotheses indicates the fish are not safe to eat? The alternative hypothesis, H_1

4. Do you want the average mercury concentration of the fish population to be at the maximal limit, or do we want to be more conservative and have it lower? How might this be affected by consumption levels? Answers May Vary.

5. What if we took some Bass samples and found the average concentration of mercury was 3 mg/kg, which hypothesis would be supported, and would it be wise to eat the fish? The alternative hypothesis, H_1. The fish would be indicated as not safe to eat. [Some data clearly supports the alternative hypothesis.]

6. What if we took some Bass samples and found the average concentration of mercury was 0.1 mg/kg, which hypothesis would be supported, and would it be wise to eat the fish? The null hypothesis, H_0. The fish would be indicated safe to eat. [Some data clearly supports the null hypothesis.]

7. What if we took some Bass samples and found the average concentration of mercury was 0.57 mg/kg, which hypothesis would be supported, and would it be wise to eat the fish? This is a critical question for the instructor to address. Students will almost certainly claim this supports the alternative hypothesis because they will compare the statistic of 0.57 to the hypothesis of 0.5. This is a good time to tell the class you don't agree with their conclusion. That will puzzle them, but give you the opportunity to explain that as a result of sampling, even if the null hypothesis is true, it is possible to get statistics that are greater than it, consequently when statistics appear to support the alternative, but are close to the hypothesized parameter, students should answer that it is too close to draw a conclusion. This will be the reason for learning about the methods that follow. See later conclusion for Northern Pike in Part II, question 6 where a statistic of 0.57 is shown to support the null hypothesis. [Some data appears to support the alternative hypothesis, but is too close to tell without hypothesis testing.]

8. Plot the number of observations for each class (bar) in Figure 3 with bars that are ~0.05 mg/kg wide (E.g. range from 0.2-0.249, 0.25-0.29). You are now looking at a sampling distribution that is based on the null being true. Answers will vary slightly, but since the class will generate at least 30 sample means the corresponding histogram will be approximately bell shaped.

Complete Table 3 below to demonstrate your understanding of the probabilities in the sampling distribution. Table 3: Probabilities in a sampling distribution (Figure 4)

Interval	Probability
[0.55,0.60)	0.16
[0.30,0.35)	0.04
[0.25,0.35)	0.05
Less than or equal to 0.35	0.05
[0.6,0.75)	0.13
Greater than or equal to 0.6	0.13
Greater than or equal to 0.75	0
Less than or equal to 0.25	0
Greater than 0.25	1

Part II

1. What is the effect on SE_x if the sample size increases? As the sample size increases, the standard error decreases.

2. What is the effect on *t* if the sample size increases? As the sample size increases, the t statistic increases.

3. How would the p-value differ if the sample size was 500 instead of 22 fish, but kept the same mean and standard deviation? What do you conclude about the effect of sample size? t-value = (0.53-0.50)/(0.3/) = 2.236, p = T.DIST.RT(2.236,499) = 0.013. The p-value decreased to 0.013 which is less than the level of significance of 0.05. In this case the data would support the alternative hypothesis. Larger sample sizes lead to smaller p-values.

4. How would the p-value differ if the standard deviation was 0.03 instead of 0.3, but kept the same mean and a sample size of 22? What do you conclude about the effect of variation in the sample as determined by standard deviation? t-value = (0.53-0.50)/(0.03/) = 4.690, p = T.DIST.RT(4.690,21) = 0.00006
The p-value decreased to 0.00006 which is less than the level of significance of 0.05. In this case the data would support the alternative hypothesis. Smaller standard deviation (less variation) lead to smaller p-values.

5. Would you expect the methylmercury levels to differ among these fish species and Large-mouth Bass? Why or why not? Yes, explanations may vary. Fish species that are higher up the food chain would be expected to have higher levels of methylmercury due to bioaccumulation.

6. Use what you have just learned above to test the hypotheses about each of the other fish species (Northern Pike, Walleye, and White Fish in Table 4), with the data in the table below provided for Separation Lake, write your conclusion for each fish and make a recommendation (safe or unsafe).

Northern Pike n = 10, 0.57, s = 0.32, t = 0.642 , p = 0.269

Walleye n= 12, 0.84, s = 0.43, t = 2.734 , p = 0.001

White Fish n = 15, 0.19, s = 0.05, t = -22.465 , p =1

Walleye would be unsafe to eat, Northern Pike and Whitefish would be safe.

Part III

1. In the study by Calder et al. (2016), a model was developed to predict how methylmercury concentrations are expected to change in locally harvested foods after flooding of the Muskrat Falls reservoir. The modeling results are presented in Figure 4 in the paper. Based on this figure, would you advise the local community to adjust their consumption of local foods to reduce their methylmercury exposure after flooding of the reservoir? Answers may vary. All fish species that are not commercially caught are modeled to increase in their methylmercury concentrations. Some species, such as lake trout, brook trout, and seals, see a much larger increase. As a result, the majority of methylmercury exposures will be due to the consumption of traditional foods.

2. Now consider that the consumption of traditional foods is known to have wide-ranging health and social benefits to indigenous communities, especially those in the Canadian North (Kuhnlein et al. 2007). Alternatives to traditional food sources are limited and expensive, and reducing reliance on traditional foods can lead to food security issues. Given these considerations, would you change your recommendations? Answers may vary.

3. How do you feel about the trade-off between building hydro power plants to mitigate climate change and creating health consequences for indigenous populations that have traditionally lived in the area and ate fish from these waters? Answers may vary.

4. The hydroelectric project at Muskrat Falls has gone ahead and methylmercury levels in and near the reservoir are being monitored. Time will tell if the modeling predictions by Calder et al. (2016) will be realized, and how the local Innu and Inuit communities will be impacted. Given that we won't know the implications of flooding the reservoir for decades, would you support the building of future dams in regions close to indigenous populations? Why or why not? Answers may vary.

Pre-Class Activity

Asubpeeschoseewagong First Nation (also known as Grassy Narrows First Nation or the Asabiinyashkosiwagong Nitam-Anishinaabeg in the Ojibwe language) is an Ojibwe First Nations band government who inhabit northern Kenora in Ontario, Canada. Their landbase is the 4,145 ha (10,240 acres) English River 21 Indian Reserve. It has a registered population of 1,595 as of October 2019, of which the on-reserve population was 971.

Between 1962 and 1970, Dryden Chemicals Ltd—a subsidiary of the British multinational, Reed International— discharged approximately nine or ten tons of mercury into the Wabigoon River, 100 kilometres (62 mi) upstream of Grassy Narrows, poisoning the fish which were the staple food of the Grassy Narrows First Nations. It is considered one of Canada's worst environmental disasters.

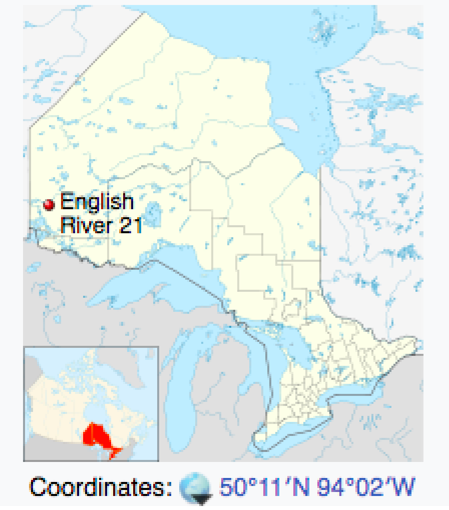


Figure 1: Map of Asubpeeschoseewagong First Nation in northern Kenora in Ontario, Canada.

News Article:

Mercury contamination in Grassy Narrows example of government ‘inaction’: UN human rights expert

By Kristy Kirkup The Canadian Press June 6, 2019

https://globalnews.ca/news/5362345/mercury-grassy-narrows-government-inaction-un/

OTTAWA – The case of a northern Ontario First Nation plagued by mercury contamination is “emblematic” of an overall pattern of inaction in the face of grave risks to the health of Indigenous Peoples, a United Nations human-rights expert said Thursday – the same day the community’s chief came to Ottawa to urge action.

Baskut Tuncak, the UN’s special rapporteur on human rights and hazardous substances and wastes, said he has heard during a two-week tour with various stops that members of Grassy Narrows First Nation are frustrated and they’re suffering from mercury contamination.

Tuncak released preliminary findings on Thursday about his tour, which involved visiting communities affected by toxins. He is to present his conclusions and recommendations to an upcoming session of the UN Human Rights Council. The need to address mercury contamination in Grassy Narrows dates back some 50 years, he said. Tuncak said the case should have been the “highest of priorities” and the government has failed to explain its “inaction.”

Tuncak, an American chemist and lawyer, also said he is left with a question about discrimination. A First Nation of about 650 people near Ontario's border with Manitoba, Grassy Narrows' water was contaminated by tonnes of mercury dumped into its water system by an upstream paper mill. One study estimated that 90 per cent of the population suffers from some degree of mercury poisoning, which can cause everything from cognitive impairments to hearing loss and emotional changes. The heavy metal can be passed from mothers to babies they carry, making it a problem that lasts generations.

Also in Ottawa on Thursday, Grassy Narrows First Nations Chief Rudy Turtle reiterated his call for the federal government to put $88.7 million, the estimated cost for a specialized health facility for residents affected by mercury contamination, into a trust fund. He argued that such a fund would ensure the project moves ahead no matter the results of the October federal election – a move supported by former Indigenous-services minister Jane Philpott, now an Independent MP.

Grassy Narrows should have the facility it's been seeking for some time, Philpott said in an interview Thursday, adding the federal government made a commitment to move ahead with it when she was minister. "It (the trust fund) is an extraordinary measure but I believe that under the circumstances, extraordinary measures are called for," she said. Canada has kept the issue on the back-burner for half a century, Turtle said, adding he believes the situation would have been addressed long ago if Grassy Narrows were a non-Indigenous community. "Even while I'm speaking today, there's people suffering in many communities," he said.

Indigenous Services Minister Seamus O'Regan said Thursday he will work with Grassy Narrows to sign an agreement on the fate of the facility even though the two sides could not reach a deal when he visited the First Nation last week. The government put out a press release ahead of his visit stating a signing ceremony would take place but after more than four hours of talks, no agreement was reached. "I am committed to working with Chief Turtle and council and committed to getting this right," O'Regan said in a statement.

Keith Conn, an assistant deputy minister for the First Nations and Inuit health branch of Indigenous Services, told a parliamentary committee on Thursday that creating a trust fund would take an "inordinate amount of time" and cause more delays. Instead, Conn told MPs the government wants to pursue a contractual agreement.

Another central issue is the federal government does not want to call the facility a "mercury treatment home" but rather an assisted-living facility, Turtle said Thursday. "I think the fact of the matter is the prime minister and Seamus O'Regan have refused to acknowledge that we have a mercury problem," he said, stressing terminology "definitely" matters to the community. "We are being poisoned by mercury and our people are suffering and I think that things should be called as they are."

Informational Videos:

Watch the videos to get a better understanding of the situation at Grassy Narrows.

- How mercury exposure has affected Grassy Narrows residents by The Toronto Star, 2018
  https://www.youtube.com/watch?v=jVNJlPBjhFQ

- Grassy Narrows - Forty years later by CBC News, 2011
  https://youtu.be/MzKza4lP-uo

Questions:
Using the news articles and the videos provided, answer the questions below.

1. How would you know the fish in this population are contaminated?

2. What are some of the impacts of the mercury contamination on the Grassy Narrows First Nation?

3. Compare the level of importance for solving this problem if this problem was happening in your community and affecting you and your family with it happening to the Grassy Narrows community?

Part I: Are the fish in the rivers and lakes by Grassy Narrows First Nation safe to eat now?

Nearly all fish and shellfish contain traces of mercury. But some contain high levels. Eating large amounts of these fish and shellfish can result in high levels of mercury in the human body. In a fetus or young child, this can damage the brain and nerves. Because of the mercury found in fish, Health Canada has set a standard (maximum limit) of 0.5 parts per million (ppm) total mercury in retail fish. Standards are enforced by the Canadian Food Inspection Agency (Health Canada, 2021).

Let’s conduct a hypothetical investigation of the fish in the lake. To determine if the fish are too contaminated to consume, we have to address a few problems.

- We cannot test all the fish, therefore we cannot know the absolute truth about the average contamination concentration.
- The concentration in the fish is heterogeneous – it varies.
- We want to base our decision on evidence, which means we need to take some fish samples.

There are two types of mathematical problems that you may be familiar with, deterministic and stochastic. Deterministic problems use math, such as arithmetic or algebra, and in which everyone should get the same answer.

1. Working in pairs, one partner should convert 0.5 ppm to mg/kg and the other convert 0.5 mg/kg to ppm. Compare your answers. Your answers should be the same.

Stochastic problems are ones in which the inputs or variables are described by probability distributions rather than a single value. For example, the mercury concentration in the fish varies between individuals. We can describe the fish population’s mercury concentration by using statistics, such as the mean and standard deviation. Statistics provide tools to understand variation in the world and make decisions when dealing with partial evidence. This is our current problem. How do we decide if the mercury concentration in the fish is too high for safe consumption when we can only get evidence from the fish we sample?

The process begins with asking an appropriate question such as: Is the average mercury concentration in the fish greater than 0.5 mg/kg, which is the Canadian standard for safety?

Next, we define the population: The population is all the Large-mouth Bass (*Micropterus salmoides*) in Separation Lake, which lies between two First Nation Communities (Grassy Narrows and Wabaseemong).

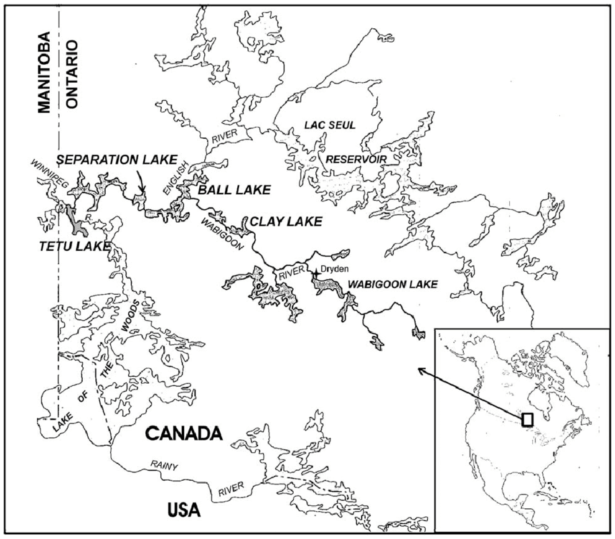


Figure 2: Map of Separation Lake in the Wabigoon River system (Neff et al. 2012)

For this population, we write hypotheses about the average we think we would find if we could actually test all the fish. This average is called a **parameter** (a number that summarizes a population). The symbol that we use for the average of a population is the Greek letter mu, **μ**. When we write hypotheses, we actually write two of them, the **null hypothesis** (H_0), which we will begin by assuming is true, and the **alternative hypothesis** (H_1), which we will accept if the evidence does not support the null. Our choice of parameter should be meaningful, leading to different courses of action if the evidence supports the null or alternative hypothesis. For this problem, the hypotheses would be:

$$H_0: \mu = 0.5 \text{ mg/kg}$$

$$H_1: \mu > 0.5 \text{ mg/kg}$$

2. Which of these hypotheses indicates the fish are safe to eat?
3. Which of these hypotheses indicates the fish are not safe to eat?
4. Do you want the average mercury concentration of the fish population to be at the maximal limit, or do we want to be more conservative and have it lower? How might this be affected by consumption levels?

Before exploring this problem with some evidence, it can be helpful to develop an intuitive sense of how partial evidence leads to drawing conclusions. We will do this using what-if questions.

5. What if we took some Bass samples and found the average concentration of mercury was 3 mg/kg, which hypothesis would be supported, and would it be wise to eat the fish?

6. What if we took some Bass samples and found the average concentration of mercury was 0.1 mg/kg, which hypothesis would be supported, and would it be wise to eat the fish?

7. What if we took some Bass samples and found the average concentration of mercury was 0.57 mg/kg, which hypothesis would be supported, and would it be wise to eat the fish?

Now consider that in the above questions, the average mercury concentration was an estimate based on the sample of fish that was caught, and may differ considerably from an estimate derived from another sample of fish. One of the fundamental elements of hypothesis testing is a sampling distribution. A sampling distribution is a collection of statistics, such as sample means, that could possibly be drawn from the population if we were to repeatedly draw samples from the population. We always start hypothesis testing with the assumption that the null hypothesis is true. The table below is an example of a population that conforms to the null hypothesis with a mean of 0.5 mg/kg.

Table 1: Mercury Concentration in a fictitious population of Bass in Separation Lake with a mean of 0.5 mg/kg.

9	0.48	0.68	0.41	0.33	0.19	0.65	0.40	0.43	0.47	0.31
8	0.63	0.01	0.68	0.55	0.25	0.69	0.21	0.40	0.60	0.12
7	0.41	0.42	0.30	0.39	0.49	0.70	0.24	0.18	0.89	0.57
6	0.24	0.43	0.64	0.47	0.70	0.45	0.73	0.74	0.30	0.62
5	0.55	0.51	0.84	0.32	0.39	0.66	0.44	0.84	0.58	0.61
4	0.49	0.56	0.60	0.56	0.44	0.71	0.75	0.60	0.70	0.42
3	0.41	0.58	0.47	0.41	0.60	0.51	0.29	0.68	0.29	0.29
2	0.52	0.70	0.53	0.37	0.70	0.71	0.31	0.68	0.44	0.41
1	0.49	0.28	0.47	0.35	0.46	0.33	0.42	0.51	0.41	0.45
0	0.84	0.63	0.75	0.65	0.52	0.35	0.51	0.58	0.55	0.58
	0	1	2	3	4	5	6	7	8	9

To help you understand a sampling distribution, you will work with your classmates to generate a small one. You will need to use a random process to select the data for the sampling distribution. Follow the sampling process described by your instructor. Every student will repeat this process enough times so the class generates at least 30 sample means.

Table 2 shows randomly generated X and Y values. The data comes from Table 1. Make a similar table in your notes for the numbers you randomly selected.

Table 2: Random x and y values from Table 1.

X	Y	Data
2	0	0.75
8	0	0.55
0	0	0.84
9	2	0.41
1	6	0.43
	Mean:	2.98/5 =0.596

Draw a histogram of the class data for mercury contamination in Figure 3.

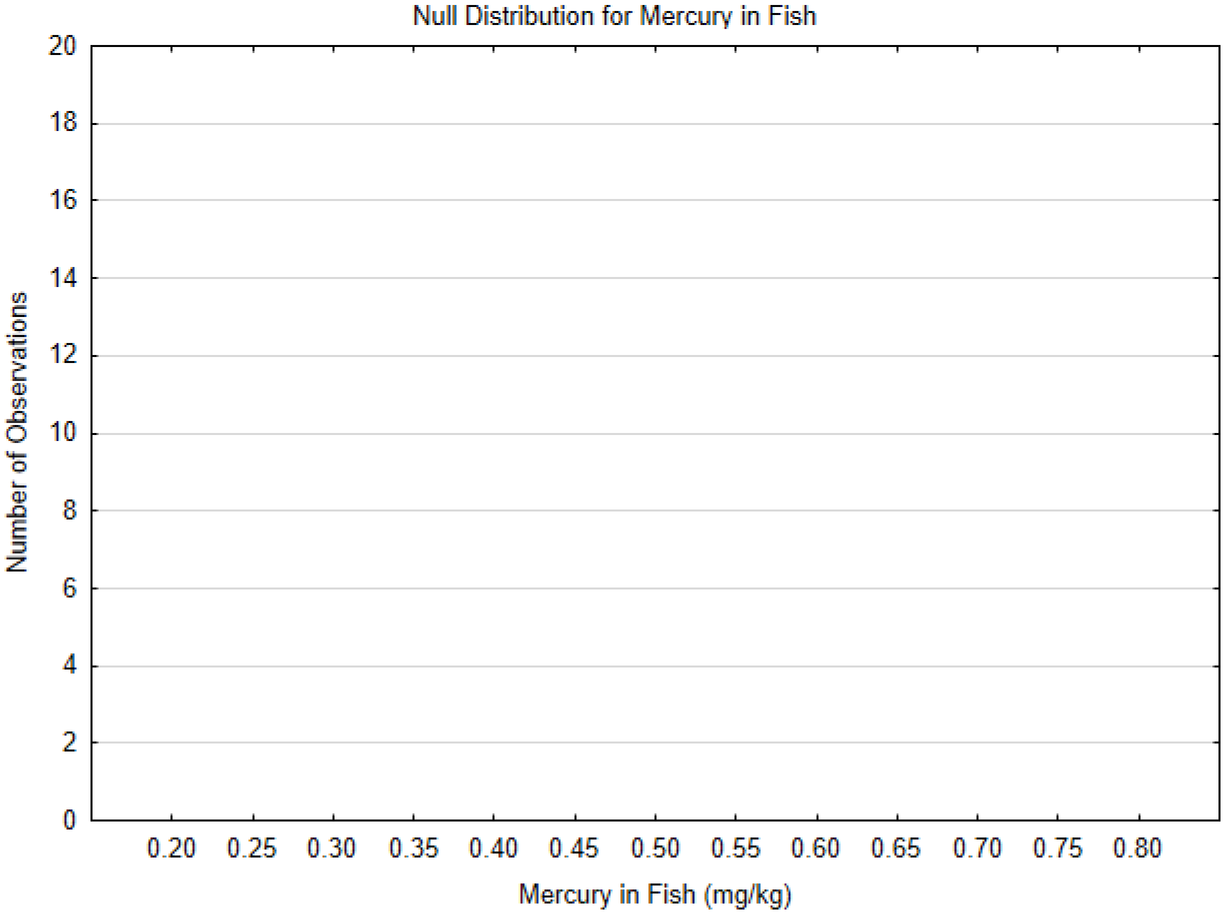


Figure 3: Class Data for Mercury Contamination in a fictitious population of fish in Separation Lake.

8. Plot the number of observations for each class (bar) in Figure 3 with bars that are ~0.05 mg/kg wide (E.g. range from 0.2-0.249, 0.25-0.299). You are now looking at a sampling distribution that is based on the null being true.

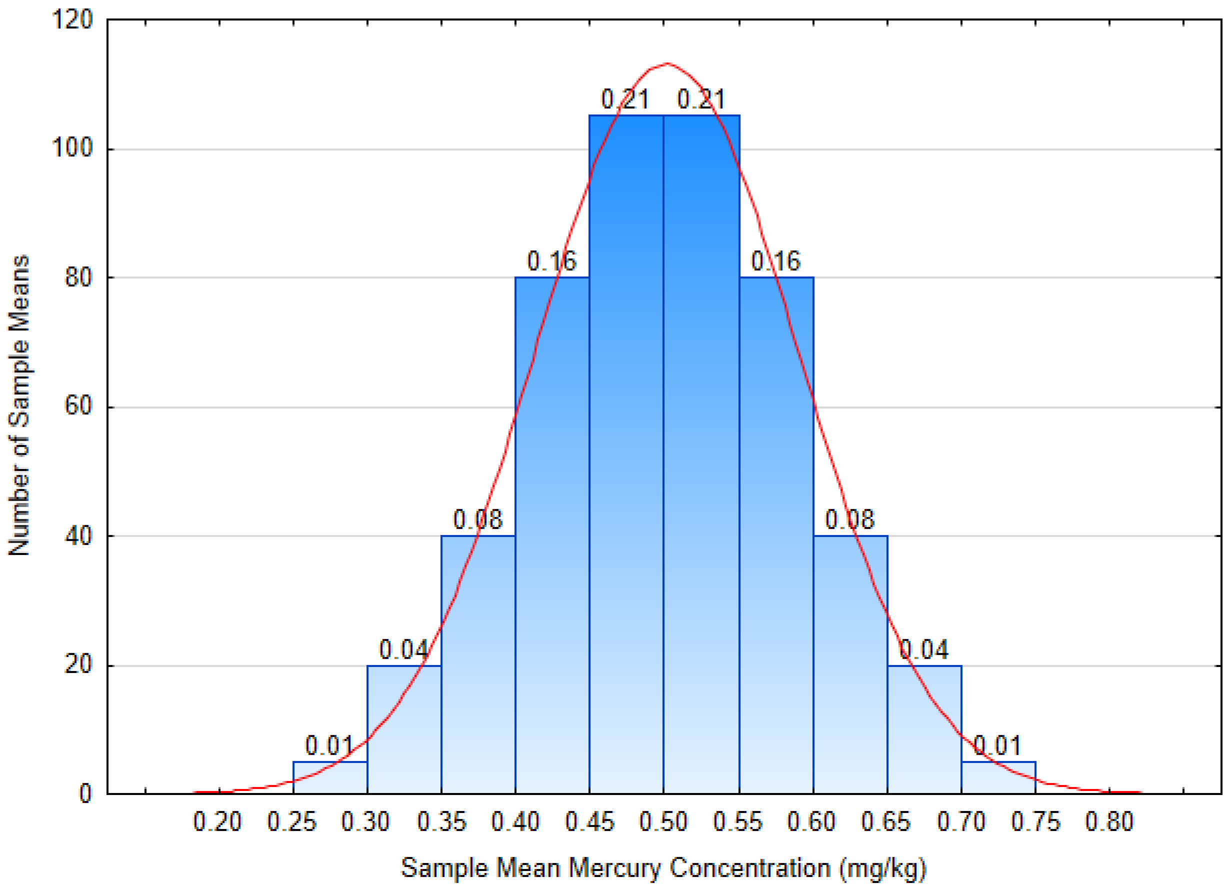


Figure 4: Sampling distribution of the mean mercury concentration in fish, assuming the null hypothesis is true.

Figure 4 is a sampling distribution constructed from this data but with 500 sample means. The numbers at the top of the bars indicate the proportion of the sample means in that class (bar). They also indicate the probability that a sample mean would fall in that class. Notice the shape of the distribution and that 0.5 is in the center.

Complete Table 3 below to demonstrate your understanding of the probabilities in the sampling distribution.

Table 3: Probabilities in a sampling distribution (Figure 4)

Interval	Probability
[0.55,0.60)	0.16
[0.30,0.35)	
[0.25,0.35)	
Less than or equal to 0.35	
[0.6,0.75)	

Greater than or equal to 0.6	
Greater than or equal to 0.75	
Less than or equal to 0.25	
Greater than 0.25	

There are four steps to deciding which hypothesis is supported by the data.

Step 1: Determine the direction of extreme, the direction (right or left) on the number line where you find values that support the alternative hypothesis.

a. For our hypotheses: H_0: $\mu = 0.5$ H_1: $\mu > 0.5$, what is the direction of extreme, left or right?

Step 2: Determine the probability of making a Type I error, which is when we think the evidence supports the alternative hypothesis, but the null hypothesis is really true. The probability is called the level of significance and represented with the Greek letter alpha, α. The researcher determines the probability *a priori*. For this example, the probability will be 0.05.

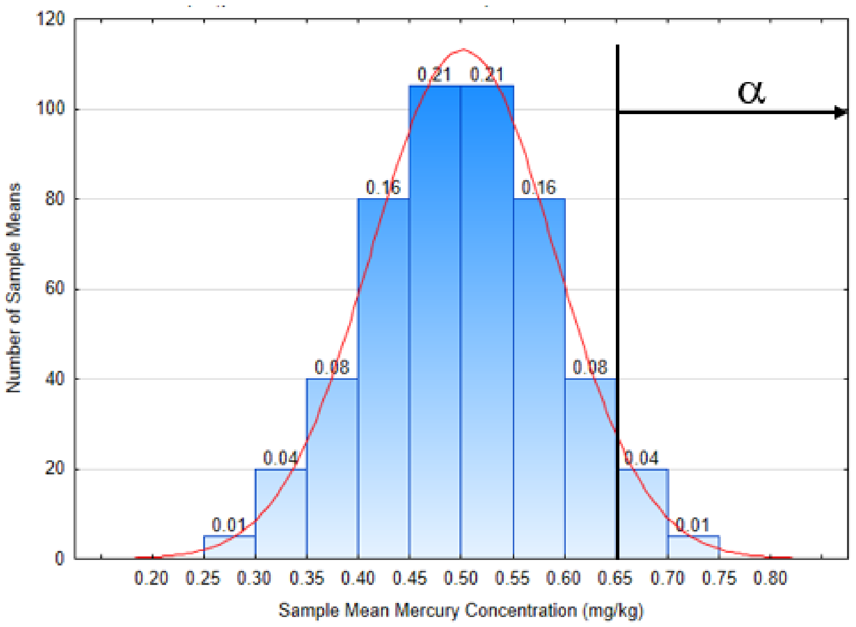


Figure 5: Sampling distribution of the mean mercury concentration assuming the null hypothesis is true, with the level of significance indicated (the sum of the probabilities on the two right bars = 0.05). In our example, the *direction of extreme* is to the right (because large values will make us think the fish are not safe to eat).

Step 3: Determine the probability of getting our statistic (sample mean) or more extreme values, assuming the null hypothesis is true. This probability is called a p-value.

a. If the sample mean was 0.70, the p-value would be 0.01.
b. What would the p-value be if the sample mean was 3 mg/kg?
c. What would the p-value be if the sample mean was 0.1 mg/kg?
d. What would the p-value be if the sample mean was 0.6 mg/kg?

Step 4: Compare the p-value to α.

a. If the p-value ≤ α, the data are significant and support the alternative hypothesis.
b. If the p-value > α, the data are not significant and support the null hypothesis.
    i. If the sample mean was 0.70 mg/kg, the data are significant because the p-value of 0.01 is less than α (0.05).
    ii. If the sample mean was 3 mg/kg, are the data significant or not? Why? Would it be wise to eat the fish?
    iii. If the sample mean was 0.1 mg/kg, are the data significant or not? Why? Would it be wise to eat the fish?
    iv. If the sample mean was 0.60 mg/kg, are the data significant or not? Why? Would it be wise to eat the fish?

Now let's consider actual data. In 2003, a project was initiated by the Grand Council Treaty #3 in collaboration with researchers from the Centre for Indigenous Peoples' Nutrition and Environment at McGill University in Montreal, to study the levels of methyl-mercury in fish in the English-Wabigoon river system. The aim of the study was to provide members of Grassy Narrows and Wabaseemong First Nations communities data required to make informed choices on fish consumption from their traditional waterways (Kinghorn et al. 2007). As part of the study, 22 Large-Mouthed Bass from Separation Lake were sampled and the mean mercury concentration was found to be 0.53 (± 0.3 SD) mg/kg.

- How comfortable would you be if you were to consume a Bass caught from Separation Lake in 2003?
- Would you recommend that members of Grassy Narrows and Wabaseemong First Nations consume Bass from Separation Lake?
- What factors should you consider when making your recommendation?
- What impacts will your recommendation have on the members of the First Nations communities?

Part II: Comparison of Fish Species Frequently Consumed

In the real world, no one actually has complete sampling distributions. Instead, the sampling distributions for quantitative data are modeled using t distributions. These distributions vary slightly based on degrees of freedom, which is one less than the sample size. P-values are typically found using either tables or technology. For this lesson, we will use Excel, but you may have access to other software or graphing calculators, to find the p-value.

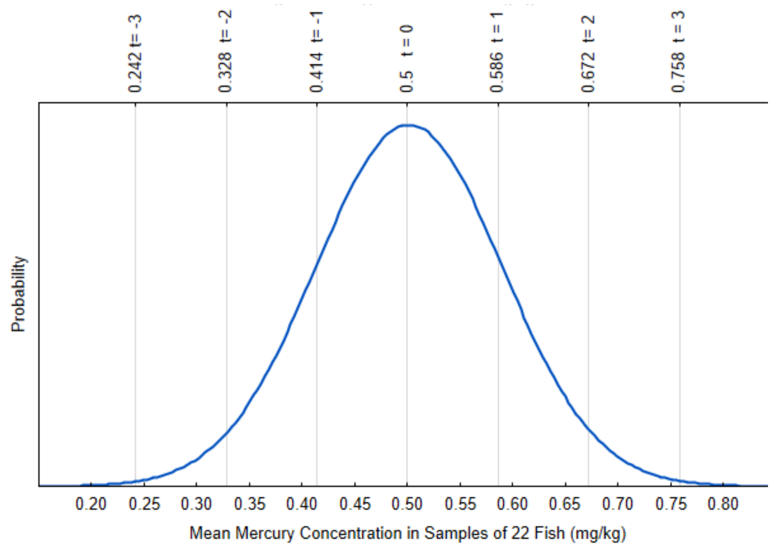


Figure 6: T-distribution for mercury in fish assuming the null hypothesis of 0.50 mg/kg is true

The center of the t distribution is equal to the **mean** of the population as stated in the null hypothesis, which is 0.5 mg/kg for our fish population (Figure 6). The amount the distribution is spread out is based on the standard error. The **standard error** is the standard deviation of the statistics (e.g. sample means) assuming we could find every possible sample mean that could be drawn from a population.

Since this can't be done, we use the formula

$$SE_{\bar{x}} = \frac{s}{\sqrt{n}}$$

, where **SE**$_x$ is the estimated standard error, **s** is the standard deviation of the sample, and **n** is the sample size.

1. What is the effect on SE$_x$ if the sample size increases?

To find the p-value, it is necessary to determine the number of standard errors our data is from the hypothesized mean. Doing so will give us a *t*-value, which we will use to find a p-value.

The *t* formula is:

$$t = \frac{\bar{x} - \mu}{\frac{s}{\sqrt{n}}}$$

2. What is the effect on *t* if the sample size increases?

The numerator of this formula finds the distance between the sample mean and the hypothesized mean of the population. Dividing by the standard error results in *t* being the number of standard errors that the sample mean is from the hypothesized mean of the population.

We will use the Separation Lake data to demonstrate how to test the hypothesis using the t-test.

H_0: $\mu = 0.5$, H_1: $\mu > 0.5$, Level of Significance: 0.05

Using the actual data: Bass in Separation Lake

Sample Mean: $\bar{x} = 0.53$

Sample Standard Deviation: s = 0.3,

Sample size: n = 22 Degrees of Freedom (df = n-1) = 21

t-value $$t = \frac{\bar{x}-\mu}{\frac{s}{\sqrt{n}}} = \frac{0.53-0.5}{\frac{0.3}{\sqrt{22}}} = 0.469$$

Since the direction of extreme is to the right, we can use the following formula in Excel to find the probability a t-value would be greater than 0.469, assuming the null hypothesis was true.

Excel Formula for finding the p-value if the direction of extreme is to the right: =T.DIST.RT(t value,df)

Excel Formula for finding the p-value if the direction of extreme is to the left: =T.DIST(t value,df)

For this problem, =T.DIST.RT(0.469,21) = 0.322

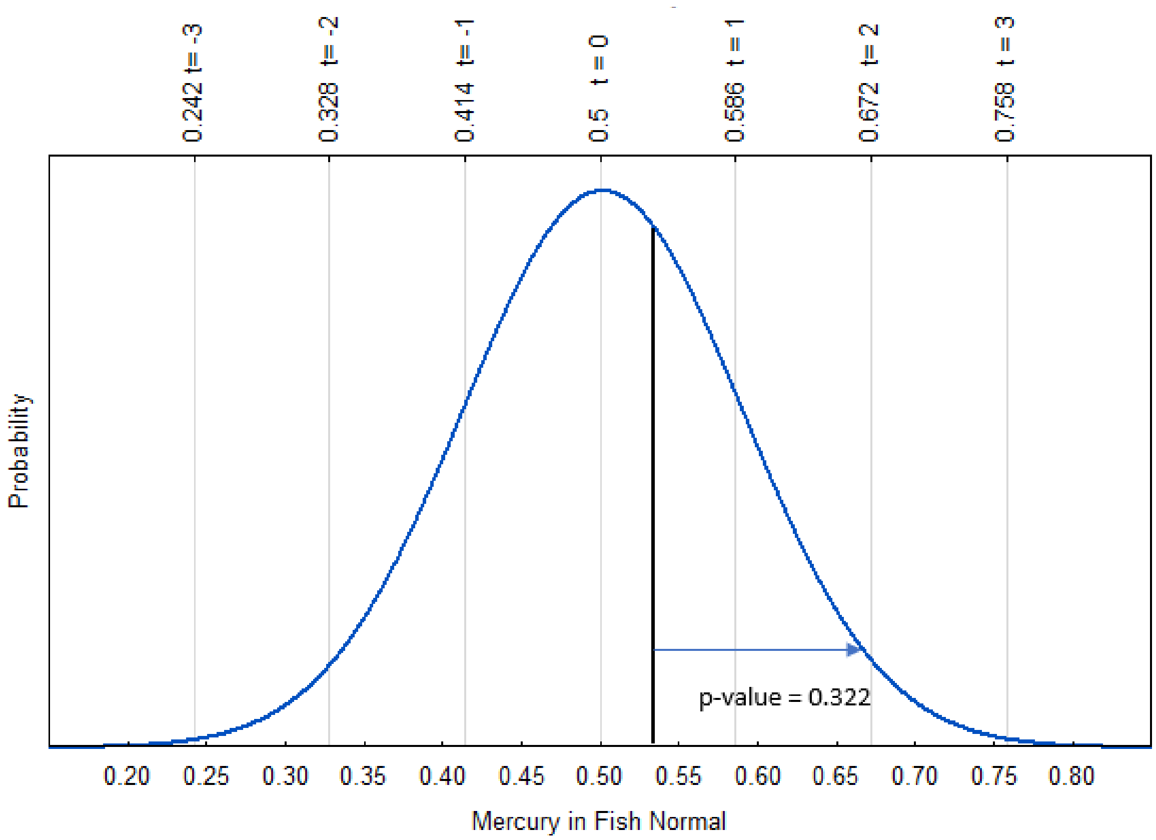


Figure 7: T-distribution for mercury in bass in Separation Lake

The p-value of 0.322 is greater than the level of significance of 0.05 (Figure 7). Therefore, the data supports the null hypothesis, so we would conclude that the average mercury concentration in the population of fish is not significantly greater than 0.5 mg/kg (t = 0.469, p = 0.322, n = 22).

3. How would the p-value differ if the sample size was 500 instead of 22 fish, but kept the same mean and standard deviation? What do you conclude about the effect of sample size?

4. How would the p-value differ if the standard deviation was 0.03 instead of 0.3, but kept the same mean and a sample size of 22? What do you conclude about the effect of variation in the sample as determined by standard deviation?

Large-mouth Bass is one of the fish species most frequently consumed by Grassy Narrows and Wabaseemong First Nation community members. Walleye (*Stizostedion vitreum vitreum*), Northern Pike (*Esox lucuis*) and Whitefish (*Coregonus clupeaformis*) are three other commonly harvested fish species in the English-Wabigoon river system.

5. Would you expect the methylmercury levels to differ among these fish species and Large-mouth Bass? Why or why not?

6. Use what you have just learned above to test the hypotheses about each of the other fish species (Northern Pike, Walleye, and White Fish in Table 4), with the data in the table below provided for Separation Lake, write your conclusion for each fish and make a recommendation (safe or unsafe).

Table 4: Amount of mercury (mg per kg of fish tissue) in fish collected in the English–Wabigoon river system in Ontario, Canada (Kinghorn et al., 2007).

Northern Pike	Walleye	White Fish
0.15	0.63	0.23
0.76	1.40	0.28
0.76	0.65	0.19
1.20	0.46	0.13
0.49	1.20	0.18
0.39	1.30	0.16
0.57	0.04	0.23
0.11	0.69	0.20
0.72	1.10	0.19
0.50	0.54	0.17
	0.67	0.15
	1.40	0.13
		0.27
		0.12
		0.27

7. Did the methylmercury levels differ among the fish species? Why might this be?

8. Now combine all of the methylmercury data for the fish species of Separation Lake and propose a series of fish consumption recommendations for the First Nations community members. Consider the provided scientific data, as well as other factors that you have identified.

Discussion Questions:

- It will be expensive to remediate this situation. Should the cost of remediation be the responsibility of Dryden Chemicals Ltd, which profited during the creation of this situation, or the taxpayers, who may have been using products produced here?

- If there are 60 more years before the mercury levels are reduced sufficiently, what actions should be taken by all the players in this situation, the Grassy Narrows community, the company, the government, the public?

Part III: The Muskrat Falls Energy Project controversy

Background

Drastic reductions in carbon emissions are required to curtail climate change, which requires a major change in how we produce and consume energy. Switching from fossil fuels to hydroelectric power is one strategy to develop carbon neutral energy sources. The complexity of resolving the climate change problem, however, is evident when we consider the impact of alternate sources of generating electricity, such as hydroelectric power, on people, in particular, on indigenous populations. According to research, originally published in C&EN (Chemical & Engineering News), in an effort to reduce greenhouse gas emissions, Canada is contemplating the construction of 22 hydropower dams, all of which are within 100 km of indigenous populations. The problem is that mercury, which occurs naturally in soil and which is deposited from the air as a result of fossil fuel emissions (especially coal), can be converted to methylmercury by soil microbes when land is flooded, such as when dams are built for hydroelectric projects. As so tragically apparent among Grassy Narrows First Nations, methylmercury is a potent neurotoxin, and as we have seen in Separation Lake, it enters the food web and bioaccumulates in fish. It has been known since the late 1970’s that fish populations in many newly flooded areas have higher levels of methylmercury than fish in nearby lakes, and that methylmercury production increased for 1-3 decades after flooding.

Concerns over potential methylmercury contamination of fish and traditional foods as a result of hydroelectric dam development, led Innuit and Innu who lived near a megadam project, in Central Labrador, Canada to engage in a long-standing protest. A multi-billion-dollar hydroelectric dam was slated to be built at Muskrat Falls on the lower Churchill River, about 30 kilometres west of Happy Valley-Goose Bay (Figure 8). The project was hailed as a vital way to transition away from an oil-based economy while supplying clean, renewable power across Newfoundland and Labrador. However, a modeling study predicted that methylmercury would increase 10-fold in the impacted river and estuary (Calder et al. 2016). Similar controversies will occur as more dams are proposed and built in an effort to offset climate change.

Read the news article “What's the deal with Muskrat Falls? Answers to a few frequently asked questions.“ published and updated by CBC News in 2019. Local Inuit communities were concerned that flooding the reservoir will elevate methylmercury in the Churchill River and downstream in Lake Melville, where many Inuit people live.

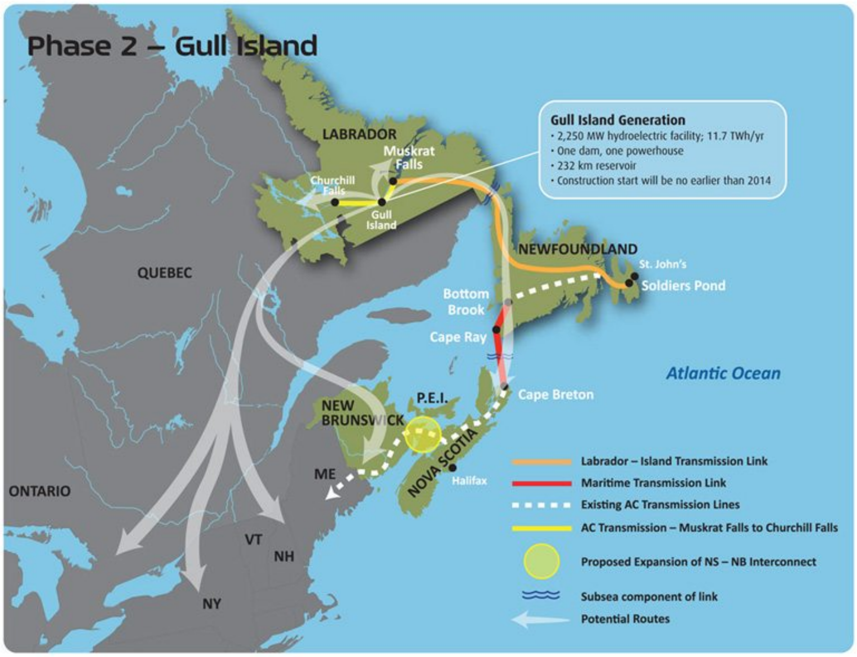

Phase 2 – Gull Island
Gull Island Generation
• 2,250 MW hydroelectric facility; 11.7 TWh/yr
• One dam, one powerhouse
• 232 km reservoir
• Construction start will be no earlier than 2014
LABRADOR
Muskrat Falls
Churchill Falls
Gull Island
QUEBEC
NEWFOUNDLAND
St. John's
Soldiers Pond
Bottom Brook
Cape Ray
Atlantic Ocean
Cape Breton
NEW BRUNSWICK
P.E.I.
NOVA SCOTIA
Halifax
ME
ONTARIO
VT
NH
NY
Labrador – Island Transmission Link
Maritime Transmission Link
Existing AC Transmission Lines
AC Transmission – Muskrat Falls to Churchill Falls
Proposed Expansion of NS – NB Interconnect
Subsea component of link
Potential Routes

Figure 8: Map of Phase 1: Labrador Island Transmission Link and Maritime Link and Phase 2: Gull Island (Source: Nalcor Energy)

Discussion Questions

1. In the study by Calder et al. (2016), a model was developed to predict how methylmercury concentrations are expected to change in locally harvested foods after flooding of the Muskrat Falls reservoir. The modeling results are presented in Figure 4A in that paper. Based on this figure, would you advise the local community to adjust their consumption of local foods to reduce their methylmercury exposure after flooding of the reservoir?

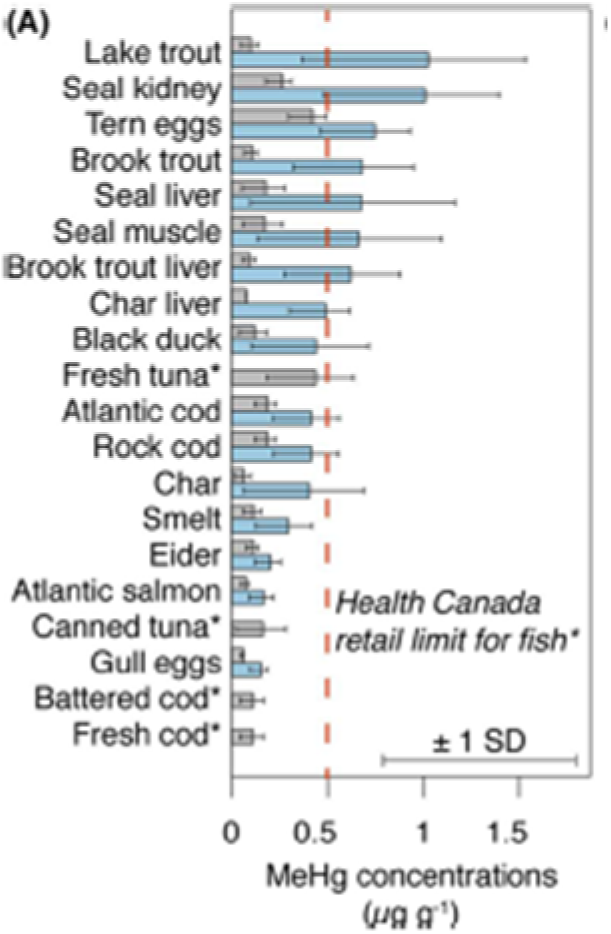


Figure 9: Top 20 methylmercury exposures for Inuit downstream of the Muskrat Falls hydroelectric reservoir measured before flooding in 2014 (grey bars), and modeled peak concentrations after flooding (blue bars). Commercial species unaffected by local conditions are denoted by "*". Error bars indicate +/- 1 standard deviation for baseline and post-flooding (simulated) means. Figure and Legend from Calder et al. (2016).

2. Now consider that the consumption of traditional foods is known to have wide-ranging health and social benefits to indigenous communities, especially those in the Canadian North (Kuhnlein et al. 2007). Alternatives to traditional food sources are limited and expensive, and reducing reliance on traditional foods can lead to food security issues. Given these considerations, would you change your recommendations?

3. How do you feel about the trade-off between building hydro power plants to mitigate climate change and creating health consequences for indigenous populations that have traditionally lived in the area and ate fish from these waters?

4. The hydroelectric project at Muskrat Falls has gone ahead and methylmercury levels in and near the reservoir are being monitored. Time will tell if the modeling predictions by Calder et al. (2016) will be realized, and how the local Innu and Inuit communities will be impacted. Given that we won't know the implications of flooding the reservoir for decades, would you support the building of future dams in regions close to indigenous populations? Why or why not?

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