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Analyzing the diet composition of Lake Trout (Salvelinus namaycush) in Upper Priest Lake

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Analyzing the Diet Composition of Lake Trout (*Salvelinus namaycush*) in Upper Priest Lake

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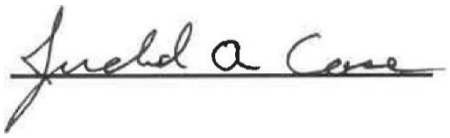

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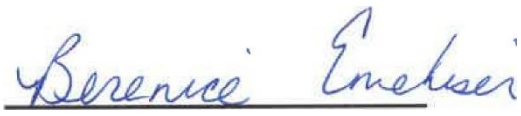

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Abstract

Lake Trout (*Salvelinus namaycush*) were intentionally introduced to the Priest Lake system in 1925 with the intentions of creating a recreational fishery. As the Lake Trout population increased within this system, the native Bull Trout (*Salvelinus confluentus*) population began to decline. Possible negative impacts of Lake Trout on Bull Trout include direct effects such as predation, or indirect effects, such as resource competition. In this study our objective was to estimate the frequency of piscivory of Lake Trout from Upper Priest Lake and document any possible Lake Trout predation upon Bull Trout in the Upper Priest Lake system. We obtained Lake Trout samples from this system during annual gill netting, which is performed to suppress Lake Trout. We then performed stomach dissections to identify incidents of piscivory. Although Mysis shrimp were predominant prey items, 61 of 133 examined stomachs contained partially digested fish tissue. We then extracted DNA from these tissues and used a species DNA barcode located in the cytochrome oxidase 1 gene of the mitochondrion to identify said fragments. Out of a total of 61 samples 63.4% were identified as Lake Trout; 19.0% were identified as Pygmy Whitefish (*Prosopium coulteri*); 14.2% were identified as Kokanee Salmon (*Onchohynchus nerka*); and 1.5% were identified as Yellow Perch (*Perca flavescens*). Therefore, we suggest that the effects of Lake Trout on Bull Trout are not direct effects, but rather indirect effects such as resource competiton.

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INTRODUCTION

Non-native species, here defined as a species introduced geographically beyond its native range purposefully via human activity (Seebens et al. 2018), can have severe negative impacts on native species in the surrounding ecosystem by forcing the native species to face a large amount of stressors to which they are not accustomed. For example, stressors such as predation (McClure et al. 2018), competition for resources (Corlett 2009), habitat loss (McClure et al. 2018), and trophic cascades (Britton 2015, Wieker 2016) can all negatively impact native species.

Although non-native species impact all ecosystems, they arguably have the most severe effects specifically within aquatic ecosystems (Jenkins 2003). Currently in the United States up to fifty three percent of all native freshwater fish species are influenced by stressors associated with non-native species (Wilcove 1998). This is alarming, as aquatic ecosystems play a large role in human culture. Many freshwater systems serve as gathering places as well as recreational areas that are common destinations for large proportions of the population. For example; activities such as water sports, fishing, and family gatherings can be commonly seen in many freshwater systems in the United States. This has caused a growing demand in freshwater resources which has led to a variety of negative impacts on a wide array of native species within such systems (Jenkins 2003).

Perhaps some of the most common non-native species found in freshwater systems are salmonids (Pascual et al. 2009; Jones and Closs 2015; Sahashi and Morita 2009). This is due to the appeal of salmonids as targets for sport fisheries (De Leaniz et al. 2010) as they are both popular and highly adaptable (Fausch 2007). Salmonids such as the Rainbow Trout (*Oncorhynchus mykiss*), the Brook Trout (*Salvelinus fontinalis*), and the Lake Trout (*Salvelinus namaycush*) were used to establish recreational fisheries in ecological systems in which they are non-native to throughout the United States (Martinez et al. 2009; Budy et al. 2012; Korman et al. 2017).

Lake Trout specifically have been shown to be harmful to many native species once introduced. A classic example is that of Lake Trout in Yellowstone Lake. Initially Yellowstone Lake had a fairly large population of Yellowstone Cutthroat Trout (*Oncorhynchus clarkii bouvieri*) (Koel et al. 2005). This population did not have any natural predators within this lake system, and as a result, it was severely impacted with the introduction of Lake Trout (Koel et al. 2005). Lake Trout become piscivorous as they increase in size (Eloranta et al. 2015). Within the Yellowstone Lake system individual Lake Trout have been estimated to consume approximately 41 Yellowstone Cutthroat Trout per year (Ruzycki et al. 2003). In addition to this, Lake Trout are not as beneficial to the surrounding ecosystem, as they reside almost exclusively within the lake, and do not enter the surrounding tributaries. This causes a disruption of the food web, as many predators within this system (bears for example) historically consumed Yellowstone Cutthroat Trout as they entered these tributaries (Varley and Schullery 1995).

In addition to Yellowstone Lake, the Flathead drainage in Western Montana has been impacted by Lake Trout. This drainage is home to a historically robust population of Bull Trout (*Salvelinus confluentus*). With the introduction of Lake Trout into the Flathead drainage a large decrease was observed in the Bull Trout population (Ferguson et al. 2012). This is primarily due to a large overlap in dietary items, as Bull Trout and Lake Trout have very similar diet compositions (Fraley and Shepard 1989). However, instances of Lake Trout consuming Bull Trout have been recorded (Hansen et al. 2016). This system is only one of many more that have seen Bull Trout population declines after the addition of Lake Trout (Martinez et al. 2009)

A similar instance to that of the Flathead lake system is the Priest Lake system in northwest Idaho. The Priest Lake system is composed of two water bodies, Upper Priest Lake and Priest Lake.

These water bodies are connected via a natural narrow channel named the Thorofare (see Figure 1). Historically the Priest Lake system housed many species such as the Bull Trout, West Slope Cutthroat Trout (*Oncorhynchus clarkii lewisi*), Mountain Whitefish (*Prosopium williamsoni)*, Pygmy Whitefish (*Prosopium coulterii),* and the Bridge lip sucker (*Castostomus macrochelius)* (Bjorn 1961). Originally Priest Lake housed a native Bull Trout population (Bjorn 1961) which was capable of sustaining relatively large harvests. For example, approximately 1600 Bull Trout were harvested during the year of a harvest census in 1956 (Bjorn 1961).

In 1925, this system was stocked with Lake Trout for the first time with the hope that a recreational fishery would be established (Bjorn 1961). Lake Trout established in this system initially, but the population remained relatively low and small until the introduction of Mysis Shrimp (*Mysidia diluviana*) into the system (Mauser et al. 1986). It was not until after this introduction that the Lake Trout population began to flourish (Reiman and Lukens 1979). In the 1970's Lake Trout catch rates and overall size saw a major increase (Reiman and Lukens 1979). But this would eventually come at a cost. It was at the same time that the Lake Trout population began to increase in this system that the native Bull Trout population began to display a major decline (Reiman and Lukens 1979). By 1984 the Bull Trout population became so low that the fishery was officially closed. Now Bull Trout are almost exclusively observed in the Upper Priest Lake system and are rarely found in Priest Lake (Entz 2017). Starting in 1997 the Idaho Department of Fish and Game (IDFG) began annual suppression efforts in Upper Priest Lake to suppress Lake Trout populations (Fredericks et al. 2013). Although a significant proportion of the Lake Trout population is removed from the Upper Lake each year Lake Trout remain established in the Upper Lake (IDFG 2013). To avoid native bicatch, gill nets are positioned in deep water every year.

Concerningly, Bull Trout typically out migrate between ages zero to three (Downs 2011). This puts out-migrating Bull Trout within a size range that Lake Trout are most likely to consume

(Ryan et al. 2014). Therefore, it would be ideal to elucidate the mechanism by which Lake Trout influence the Bull Trout population negatively. Within the Priest Lake system Lake Trout typically begin piscivorous diets once they reach a total length above 500 mm (Entz 2017). Lake Trout are known to consume fish up to one third of their total length (Clarke et al. 2005). Because of this, it is possible that Lake Trout are consuming sub-adult Bull Trout (Furgeson et al. 2012) This led us to believe that it is possible Lake Trout are predating upon Bull Trout within the Upper Priest Lake.

The objective of this study is to evaluate the diet of Lake Trout in order to document any possible predation of Lake Trout on Bull Trout during the spring in Upper Priest Lake. This was done by obtaining Lake Trout samples from the annual IDFG gill netting attempts in the Upper Priest Lake. We dissected Lake Trout stomachs and recorded any consumed fish tissue. Tissue samples that were not visually identified to the level of species were genetically barcoded to discern the species of the digested tissue.

Methods

Fish Collection

Lake Trout samples were obtained using monofilament sinking gill nets over a 10-day period in the years 2015 and 2016 as a part of the IDFG annual gill netting suppression of Lake Trout in the Upper Priest Lake (Ryan et al. 2014). Lake trout stomachs were collected by dissection (n = 133) and stored in Whirl-Pak bags containing 70% ethanol and were then placed in a freezer to reduce decomposition of prey items.

Prey Item Identification

Stomachs were opened using a scalpel and had their contents emptied into petri dishes containing a 70% ethanol flush. Contents were then sorted by the categories of *Mysida diluviana*, unknown fish, Kokanee Salmon, *Prosopium*, and other invertebrates. Samples were then placed into individual containers. Lake Trout diets were quantified via a percent by composition of total weight and total number.

DNA extraction and quantification

Small clips of tissue from 61 samples were extracted after an initial rinse in distilled water. Tissue was taken from below the tissue surface in order to minimize damage due to digestion. These samples were then placed into a sterilized 1.5 mL microcentrifuge tube. All samples were then exposed to a cell lysis buffer containing 10 mM TrisHcl, 100 mM EDTA, 2% SDS, and 150 µL of solution containing .8 mg proteinase K. Samples were then incubated at 37 °C for 16 hours. After incubation 7.5 M ammonium acetate was added to each sample to precipitate any protein from solution (Siddiqui et al. 2011). Samples were centrifuged and the supernatant was extracted. One hundred percent isopropanol was then used to precipitate DNA which was then rinsed with 70% ethanol to remove impurities. Centrifugation steps were taken between treatments. After ethanol rinsing samples were allowed to dry for 15 minutes and were then placed into 50 µL of

low TE for re-suspension. Samples were stored overnight for re-suspension, at which point DNA quantity was measured via a Thermo Fischer Nanodrop Light.

DNA Amplification and sequencing:

DNA was amplified using primers for the cytochrome oxidase 1 gene of the mitochondria. This region of DNA is a species-conserved region and allowed for the determination of the identity of each sample. (Hubert et al. 2008). DNA sequencing was performed by the company Genewiz. Samples were examined using the NCBI BLAST database (Moran et al. 2015). Samples were evaluated based on percent identification and percent query (Moran et al. 2015). Samples at or above an 80 percent identification match to one species were accepted (Moran et al. 2015).

Microsatellite analysis:

To assess potential contamination by the tissue of predator Lake Trout, DNA was extracted and quantified from the tissue of ten different Lake Trout stomachs that contained a fish prey item identified as a Lake Trout based on sequencing data. Four different microsatellite loci were amplified using the primer pairs snaMSU05, snaMSU06, snaMSU08, snaMSU13 and conditions described by Rollins et al. (2009). This was done for ten different Lake Trout predator/prey tissue pairs. Reactions were consolidated into multiplexes containing all four loci for each sample for analytical processing. Fragment analysis was carried out by the company Genewiz to determine the size of each amplified allele. The genotype of each prey item and predator was analyzed for similarities. Presence of a different genotype in prey tissue relative to predator tissue resulted in confirmation that prey was a different individual than the predator.

Results

Diet Composition

With a sample size of 133 stomachs extracted, we visually determined the diet composition of Lake Trout in Upper Priest Lake (See Table 1). In this table we display the percentage of the total dietary biomass that each individual species made up. Mysis Shrimp were a large proportion of the dietary composition. Additionally, four Kokanee Salmon were observed in these stomachs. No Bull Trout were observed in the stomachs of this sample size.

DNA sequencing Analysis of Unidentified Tissues

Based on analysis using the CoxI mitochondrial gene, we observed 40 of 61 samples to be genetically identified as Lake Trout. Additionally, we observed 11 samples that were Pygmy Whitefish, 9 samples that were Kokanee Salmon, and one sample that was Yellow Perch (*Perca flavescens)*. No instances of Bull Trout predation were observed in our samples (see table 2 and Figure 2).

Microsatellite Analysis

Microsatellite analysis showed prey individuals to be distinctively different from predator individuals. Out of ten sets of tissues analyzed we observed a distinctive difference at one or more loci for all ten samples (see table 3). This confirms that prey individuals were different from the predators that consumed them.

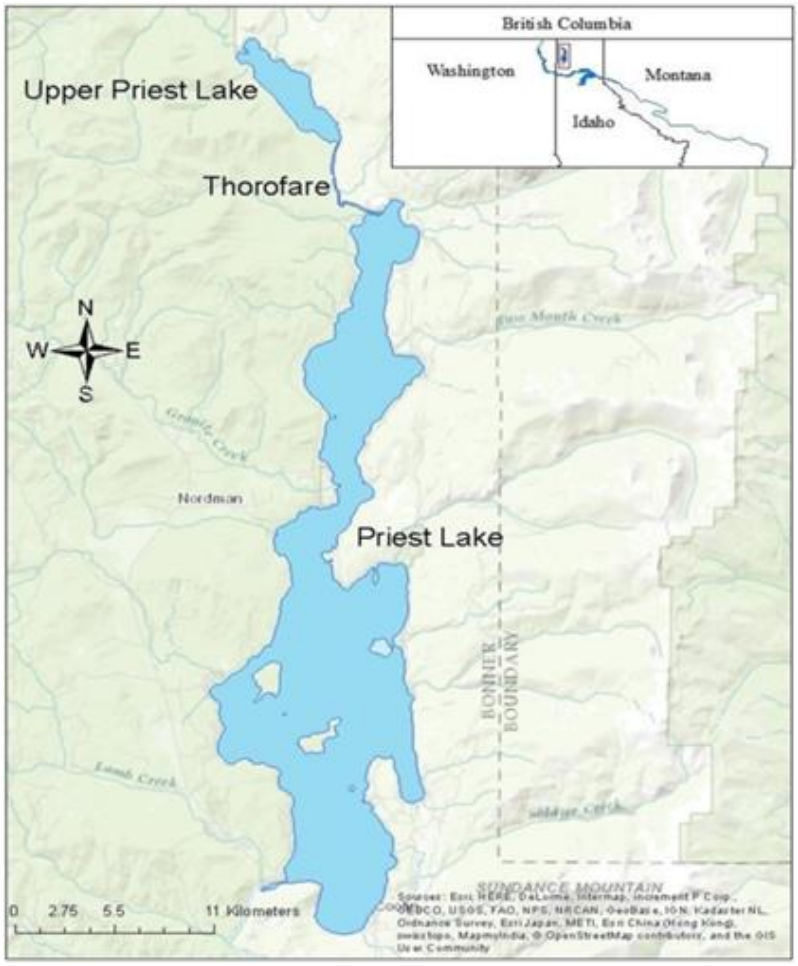

Figure 1

Map of Priest Lake, Upper Priest Lake, and the Thorofare that connects them. (Figure from Entz 2017, Used with permission.)

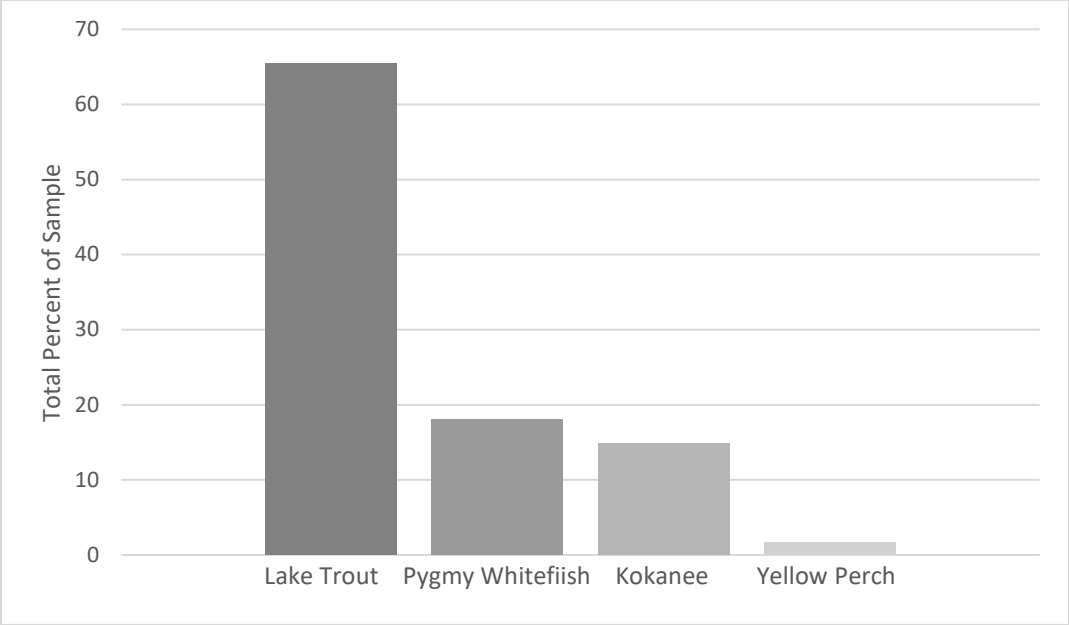

Figure 2

Total percentage of DNA sequence-based identity of prey items recovered from the stomachs of Lake Trout from Upper Priest Lake.

Table 1

Percentage of each category contained of the total biomass and total number of all biological matter extracted from Lake Trout stomachs.

Taxon	Percent total biomass	Percent Total Number
Mysida diluviana	33.38	98.51
Unknown Fish	42.10	.77
Kokanee Salmon	17.00	.04
Prosopium	.42	.01
Other Invertebrates	.11	.64

Table 2

Genetic identification based on sequence similarity of the cytochrome oxidase one gene found in the mitochondria. Common name, query cover, and identification percentage are displayed. Query cover represents the percent alignment of the total amplified sequencing to the database sequence. Individuals found within the same stomach have been labeled with the letter S followed by a number.

Common Name	Query Cover	Percent Match
Pygmy Whitefish	88%	99%
Pygmy Whitefish	89%	99%
Lake Trout (S1)	91%	100%
Lake Trout (S1)	89%	100%
Pygmy Whitefish (S2)	90%	99%
Lake Trout (S2)	91%	89%
Lake Trout (S3)	83%	82%
Lake Trout (S3)	91%	99%
Lake Trout	92%	95%
Lake Trout (S4)	91%	100%
Lake Trout (S4)	91%	100%
Lake Trout	91%	100%
Lake Trout	91%	95%
Pygmy Whitefish	88%	93%
Lake Trout (S5)	92%	99%
Lake Trout (S5)	92%	98%
Lake Trout (S5)	91%	100%
Lake Trout (S5)	92%	84%
Lake Trout	91%	96%
Lake Trout	93%	99%
Pygmy Whitefish	89%	96%
Lake Trout (S6)	90%	99%
Kokanee Salmon (S6)	91%	99%
Lake Trout	93%	100%
Lake Trout	91%	100%
Lake Trout (S7)	89%	98%
Lake Trout (S7)	91%	100%
Lake Trout (S7)	91%	99%
Lake Trout (S8)	92%	95%
Lake Trout (S8)	91%	100%
Pygmy Whitefish	90%	100%
Kokanee Salmon	91%	100%
Lake Trout	91%	99%
Pygmy Whitefish	92%	99%
Kokanee Salmon	91%	99%
Kokanee Salmon	93%	97%
Kokanee Salmon (S9)	93%	99%
Kokanee Salmon (S9)	93%	93%
Kokanee Salmon (S9)	93%	92%
Lake Trout (S10)	90%	100%
Lake Trout (S10)	95%	95%
Pygmy Whitefish	92%	99%
Lake Trout	91%	98%
Pygmy Whitefish	93%	99%
Lake Trout	94%	99%

Table 2 [Cont.]

Lake Trout	**91%**	**94%**
Lake Trout	87%	95%
Kokanee Salmon	90%	97%
Kokanee Salmon	93%	97%
Yellow Perch	93%	98%
Lake Trout (S11)	91%	100%
Lake Trout (S11)	92%	100%
Lake Trout	91%	100%
Pygmy Whitefish	91%	99%
Lake Trout	91%	98%
Lake Trout	92%	99%
Lake Trout	91%	93%
Lake Trout (S12)	83%	99%
Lake Trout (S12)	91%	98%
Lake Trout	92%	99%
Pygmy Whitefish	89%	99%

Table 3

Genotypes of each predator/prey pair. Values represent the size of each peak observed in base pairs.

Sample	Loci 1	Loci 2	Loci 3	Loci 4
Predator 1	183/183	261/281	172/172	104/104
Prey 1	184/184	162/162	172/172	195/199
Predator 2	267/267	180/220	172/172	214/214
Prey 2	N/A	220/220	172/172	214/218
Predator 3	180/180	269/269	172/172	214/214
Prey 3	180/220	162/162	172/172	195/199
Predator 4	N/A	N/A	N/A	N/A
Prey 4	180/180	N/A	172/172	214/214
Predator 5	220/220	269/269	172/172	214/222
Prey 5	183/183	271/271	172/172	214/222
Predator 6	N/A	N/A	N/A	N/A
Prey 6	180/220	180/210	172/172	180/224
Predator 7	N/A	281/281	172/172	206/226
Prey 7	162/162	162/162	172/172	191/195
Predator 8	182/182	N/A	172/172	214/214
Prey 8	180/180	162/162	172/172	191/195
Predator 9	184/184	265/265	162/172	206/256
Prey 9	162/162	162/162	172/172	195/199
Predator 10	160/232	268/285	N/A	225/256
Prey 10	182/182	162/162	172/172	195/195

Discussion

We confirmed that within the samples we obtained there were no evidences of predation upon Bull Trout. Lake Trout are Mysis Shrimp consumers (Chavarie et al. 2016) so it is not surprising to see large instances of Mysis consumption by Lake Trout within this system. Lake Trout that were actually found to be piscivorous appeared to be primarily cannibalistic. This is not unexpected, as Lake Trout are known to be cannibalistic (Hansen et al. 2016). However, these instances of cannibalism have not been previously documented in Upper Priest Lake and may be more common than we would have estimated.

These results support previous suggestions in Fergeson et al. 2012 that negative interactions between Lake Trout and Bull trout are primarily of a competitive nature rather than predator/prey interactions. Previous work has found that Bull Trout heavily consume Mysis Shrimp in systems where the two are present (Fraley and Shepard 1989). It would make sense within this system that Bull Trout are competing with Lake Trout over Mysis Shrimp as a food source in Upper Priest Lake. Mysis Shrimp are typically located in the depths at the center of this lake, which is also typically where Lake Trout are found. Therefore, future studies to observe the diet of Bull Trout in this system could help determine if there is a spacial overlap causing a competitive barrier that results in Bull Trout not having access to Mysis Shrimp as a primary food resource in this system.

Although these are encouraging results with regards to the native Bull Trout population it is worthy to note that these findings are from a brief period of time of several days within the springs of 2015 and 2016. Salmonids take approximately two days to completely digest the tissues of other fish (He and Wurtsbaugh 1993). Therefore, the data observed in this study show that over a period of about two days in the springs of 2015 and 2016 there was no predation between Lake Trout and Bull Trout observed.

Additionally, the samples collected were only from regions where gill nets were placed during the annual gill netting efforts in the spring by IDFG. As mentioned previously, these gill nets are placed in regions of the lake that avoid bicatch of native species. Because of this the samples obtained from these gill netting efforts may not be from regions of the system in which we would expect to see Lake Trout predating upon Bull Trout. It would be beneficial to obtain samples from the mouth of the Upper Priest River, as well as the margins of the lake. Lake Trout possibly use the lake margins during periods of gill netting (Entz 2017), and Bull Trout use the Upper Priest River as a means to spawn and rear (IDFG 2002). Due to the adfluvial life history of Bull Trout there would be a large outmigration of juvenile Bull Trout when the hydrograph is at its highest (Downs 2011). Therefore, a more wholistic approach for future studies would be to obtain samples from these regions, as they are most likely the regions that Lake Trout would predate upon Bull Trout. Additionally, it would be beneficial to obtain Lake Trout stomachs throughout multiple time points for each season to confirm that Lake Trout diets do not shift throughout each season.

To conclude, we have shown that there are no instances of Lake Trout predation upon Bull Trout during IDFG spring gill netting. Although these data are promising, it is a brief window in time of what occurs throughout this system. Before we can fully disregard the possibility that Lake Trout are consuming Bull Trout more data need to be collected from more regions of this system during additional times of the year.

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