Northern Southeast Alaska Eulachon Population Monitoring

Annual Report 2023

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Summary

During 2023, the Chilkoot Indian Association (CIA) continued to partnered with the Skagway Traditional Council (STC), Takshanuk Watershed Council (TWC), and Oregon State University to conduct eulachon (*Thaleichthys pacificus*, aka Saak in Tlingit) population monitoring on 9 rivers within northern Southeast Alaska (Chilkoot, Chilkat, Ferebee, Katzehin, Taiya Skagway, Berners, Lace, and Antler Rivers) (Figure 1). This is an ongoing study that was initiated in 2010 through CIA Tribal Member desire to establish baseline population data for eulachon on the Chilkoot River. Prior to this monitoring effort there was only anecdotal data on run size, timing, and population trends. This study consists of conducting a mark-recapture population estimate at the Chilkoot River and conducting environmental DNA (eDNA) sampling at all rivers and estuary sites.

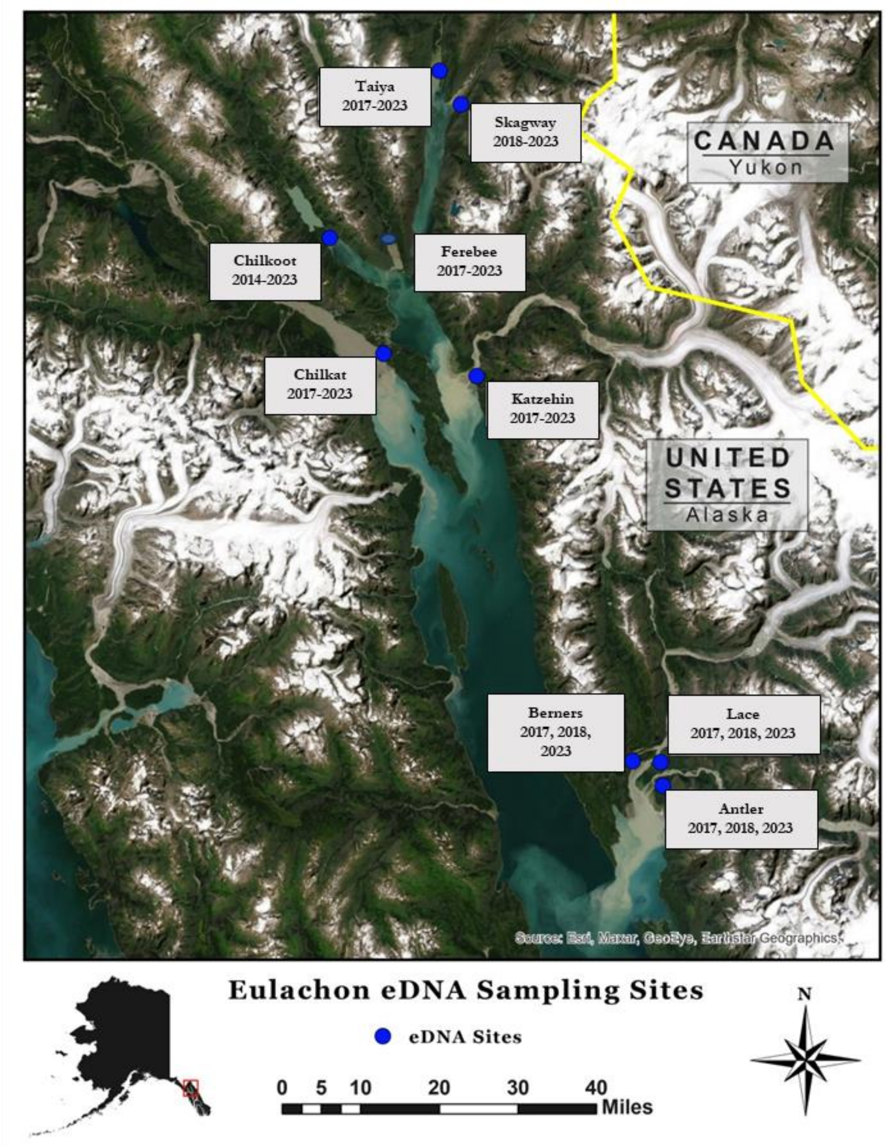

Figure 1: Location Map

Introduction

Eulachon are a lipid-rich, anadromous smelt of the family Osmeridae (Mecklenburg et al., 2002). Their historic range stretched from southern California to the Bering Sea in southwest Alaska (Hart, 1973). In Southeast Alaska, they are the first anadromous fish to return after the long winter, and as a result, are a key resource for indigenous people and for wildlife. The majority of eulachon populations have been declining since the 1990s (Hay & Mccarter, 2000a). In 2010, the National Marine Fisheries Service (NMFS) listed the southern distinct population segment in Washington, Oregon, and California as Threatened under the Endangered Species Act (NOAA, 2010). Because there is no commercial eulachon fishery in northern Southeast Alaska, there is no harvest regulation or management, agency oversight, or monitoring of population trends. While some eulachon population declines have been well documented (Hay & Mccarter, 2000b), most populations of eulachon are either unknown or anecdotal. In southeast Alaska, eulachon remain a key resource for indigenous people and wildlife, but little is known about their physiology or spatiotemporal dynamics (Betts, 1994; Olds, Moran, & Castellini, 2016).

A lack of eulachon population information and the cultural and subsistence value of the species led to the Chilkoot Indian Association (CIA) to initiate the first indigenous-led eulachon population monitoring program on the Chilkoot River, near Deishu (Haines), Alaska in 2010. From 2010-214, CIA's eulachon monitoring efforts were focused at the Chilkoot River conducting a mark-recapture population estimate of the annual eulachon spawning event. This dataset represents the first-ever eulachon spawning data ever collected on the Chilkoot River.

In 2014, CIA partnered with the Takshanuk Watershed Council (TWC) and Oregon State University (OSU) to investigate the ability to use the novel technology of environmental DNA (eDNA) as a means to gauge eulachon spawning abundance in addition to the mark-recapture population estimate at the Chilkoot River. At the time, eDNA, or the detection of DNA shed from an organism and sampled from the environment (i.e. water, air, soil) rather than sampling the organism directly, was a relatively new technology and most applications were using eDNA for presence/absence studies (Pilliod et al., 2013; Rees et al., 2014). Our goal was to quantify the abundance of eulachon DNA detected in eDNA water samples from the Chilkoot River and compare the eDNA quantity with the mark-recapture population estimate. We continued the eDNA and mark-recapture comparison at the Chilkoot River from 2014-2016 as a proof of concept if eDNA would be an effective method for quantifying eulachon spawning abundance. The initial results proved very promising and in 2017, CIA further expanded its eulachon population monitoring program to include eDNA sampling at the Chilkat, Ferebee, Katzehin, Berners, Lace, and Antler River, and partnered with the Skagway Traditional Council (STC) to include the Skagway and Taiya Rivers near Skagway, AK.

All of CIA's eulachon monitoring rivers previously had little to no data on eulachon spawning populations. Given the regional population structure of eulachon (Candy et al., 2015), CIA felt it was necessary to take a broad approach to eulachon population monitoring within northern Southeast Alaska. CIA has continued these regional eulachon monitoring efforts, with the partnering organizations, and now boasts one of the longest and broadest eulachon population datasets available for Alaska.

Methods

Mark Recapture

At the mouth of the Chilkoot River, eulachon are captured using a modified fyke net trap and dip nets. The initial captured eulachon (M group) are transferred in small groups to a plastic dish pan where they can be easily handled to clip off the adipose fin using retina scissors and returned to the river. To allow time for the marked fish to mix with the unmarked fish, the recapture group was captured approximately 0.75km upstream of the trap location (C and R groups) (Figure). Eulachon in the second group were collected by field crew wading through the river with dip nets making sure to sample all sections of the river and with the help of subsistence harvesters when their catch was available for counting. The captured fish were examined for a clipped adipose fin before releasing. To avoid repetitive sampling of the same fish, field crews started at a downstream point and worked their way upstream. A modified Lincoln-Peterson estimate equation was used $N = \frac{(M+1)(C+1)}{R+1} - 1$ where N = total population estimate, M = marked initially, C = total in second recapture, and R = marked recaptures. The standard error was calculated using the equation $SE = \frac{\sqrt{[(M+1)(C+1)(M-R)(C-R)]}}{[(R+1)^2(R+2)]}$ and the 95% confidence interval was calculated as $CI = N \pm (1.96)(SE)$

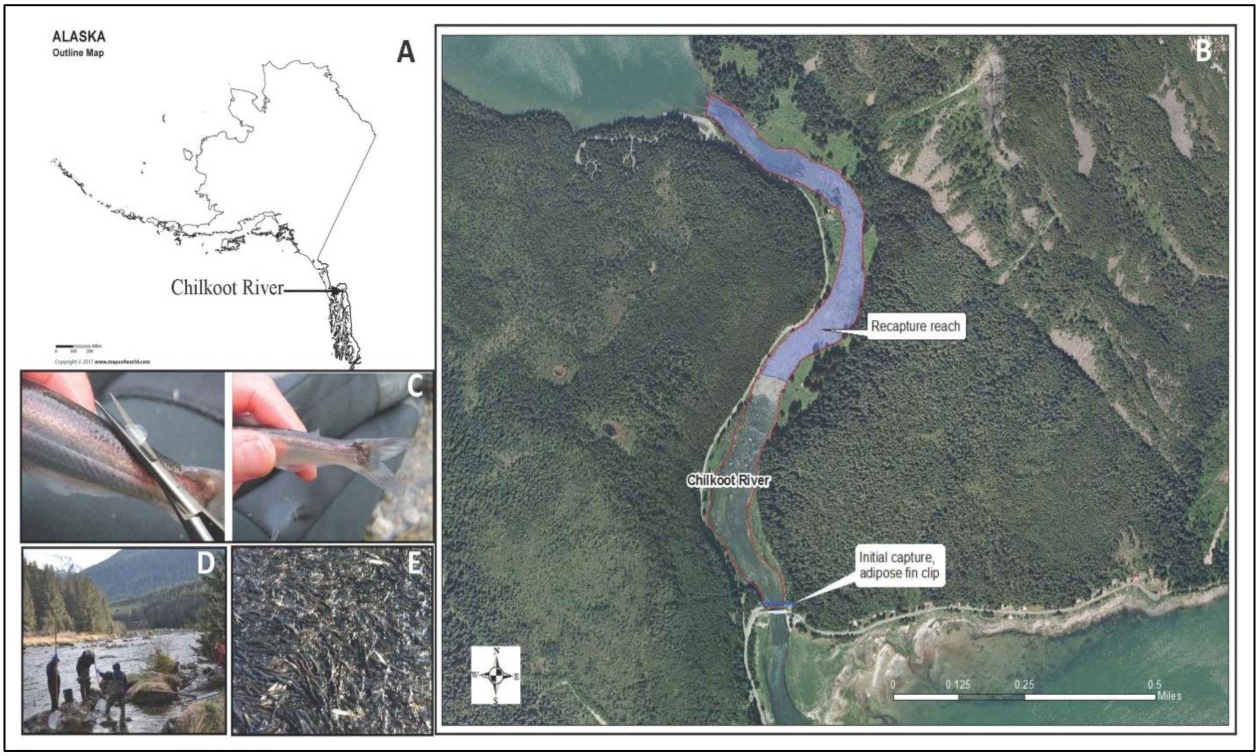

Figure 2: A: Location map of Chilkoot River; B: Layout of mark-recapture study at the Chilkoot River; C: fin clip used for marking; D: recapture crew within the recapture reach; E: Chilkoot River with eulachon.

<u>eDNA</u>

Initial eDNA samples were collected at the beginning of April before any eulachon were suspected to be in the rivers. This sample acted as the pre-run baseline of eulachon DNA present within the systems. As wildlife activity increased, indicating eulachon were moving into the estuary of each river, sample frequency increased to daily or every other day. Daily eDNA sampling commenced throughout the duration eulachon were observed within the rivers, if observations were possible, or based on wildlife congregations. Once eulachon were no longer observed within the rivers, eDNA sampling was scaled back to 2x per week for the following 2-3 weeks to ensure all eulachon spawner DNA could be captured with the eDNA sampling. In 2023, eDNA samples were collected at the Chilkoot River and estuary, Chilkat river and estuary, Taiya River and estuary, Skagway River and estuary, Ferebee River and estuary, Katzehin River and estuary, Berners River, Lace River, and Antler River.

All eDNA samples were collected as close to low tide as was feasible to avoid tidal influence of the eDNA concentration. Three replicate 1L water samples were collected at each site (river and estuary, if both sites were sampled) at each river during each sampling event. Samples were collected in 1L Nalgene sample bottles or sterile Whirl-paks. Prior to sample collection with the Nalgene bottles, the bottles were thoroughly decontaminated with a 10% bleach solution and DI water rinse. At the sample location the Nalgene sample bottles were rinsed three times with river water to thoroughly ensure all bleach residue was removed.

Each sample was transported from the field to the lab filtering location on ice in a cooler to ensure no thermal or sunlight degradation of DNA. Samples were filtered through a 47mm 0.45-micron cellulose nitrate filter using a vacuum pump with a three-sample manifold (**Error! Reference s ource not found.**, A). Filters were preserved in coin envelopes placed in plastic bags with silica beads (**Error! Reference source not found.**, B) and then stored in a freezer until all samples were s hipped to Dr. Taal Levi's lab at Oregon State University for extraction and ddPCR processing.

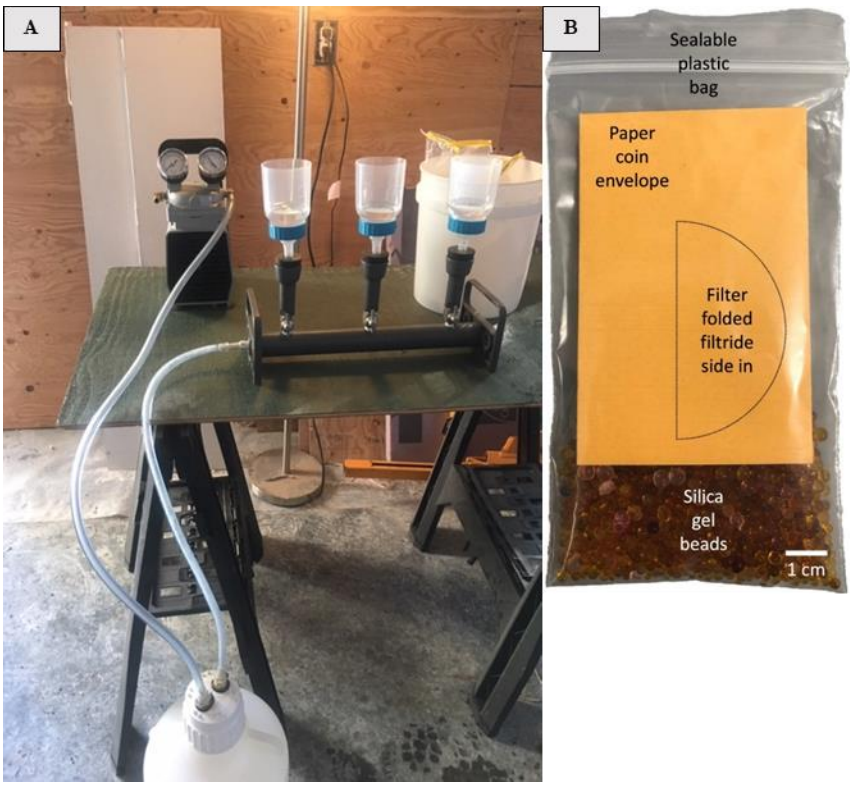

Figure 3: A: eDNA filtration set up. B: eDNA filter preservation with silica beads.

Samples were extracted using the Qiagen DNeasy Blood and Tissue kit and eluted in a total volume of 100ul. A Bio-Rad QX200 AutoDG Droplet Digital PCR system was used for ddPCR processing following the established protocol (Pochardt et al., 2020).

The ddPCR results received from Dr. Levi's lab are presented in eulachon eDNA concentration (copies/µL) for each sample replicate, for a total of 6 technical replicates for each sample (three field replicates and 2 ddPCR replicates). The average eDNA concentration and standard deviation of the technical replicates were then calculated for each sample. In order to evaluate the eDNA concentration as an index of eulachon abundance we calculated two main metrics – first, the maximum eDNA concentration (i.e. size of the peak), second, we used the area under the curve of the eDNA rate throughout the duration of the run. For the two rivers that had usable discharge data (Chilkoot and Taiya Rivers), we also calculated the flow-corrected eDNA rate by multiplying the average daily discharge (cubic feet/sec) with the eDNA concentration (copies/µL) to get an eDNA rate of copies/sec.

<u>Wildlife Observations</u>

During each sampling event at each river, wildlife observations were conducted. For some rivers there were multiple wildlife observation sites to ensure full coverage of the river and estuary areas. Observers would conduct the wildlife observations by identifying and counting all the marine mammals for 5 minutes and then identifying and counter all the birds within their viewshed for 5 minutes.

Results

Mark Recapture

The first sign of eulachon in the Chilkoot River was on April 25, 2023. As per cultural tradition, mark-recapture crews wait two tide cycles after eulachon are first observed in the river before beginning the mark-recapture process. Crews began clipping fins on April 26, 2023 and commenced with clipping and recapture on April 29, 2023. A total of 6,101 eulachon were clipped in the initial capture (M group), 10,821 were recaptured (C group) and of those recaptures 13 were clipped (R group). The overall population estimate for the 2023 Chilkoot River eulachon run was 4.7 million (95% CI: 2.3 – 7.2 million) (Figure 4). The overall average population estimate for all years where the mark-recapture method was conducted at the Chilkoot River was 8.1 million, indicating that the 2023 run was slightly below the overall average run size.

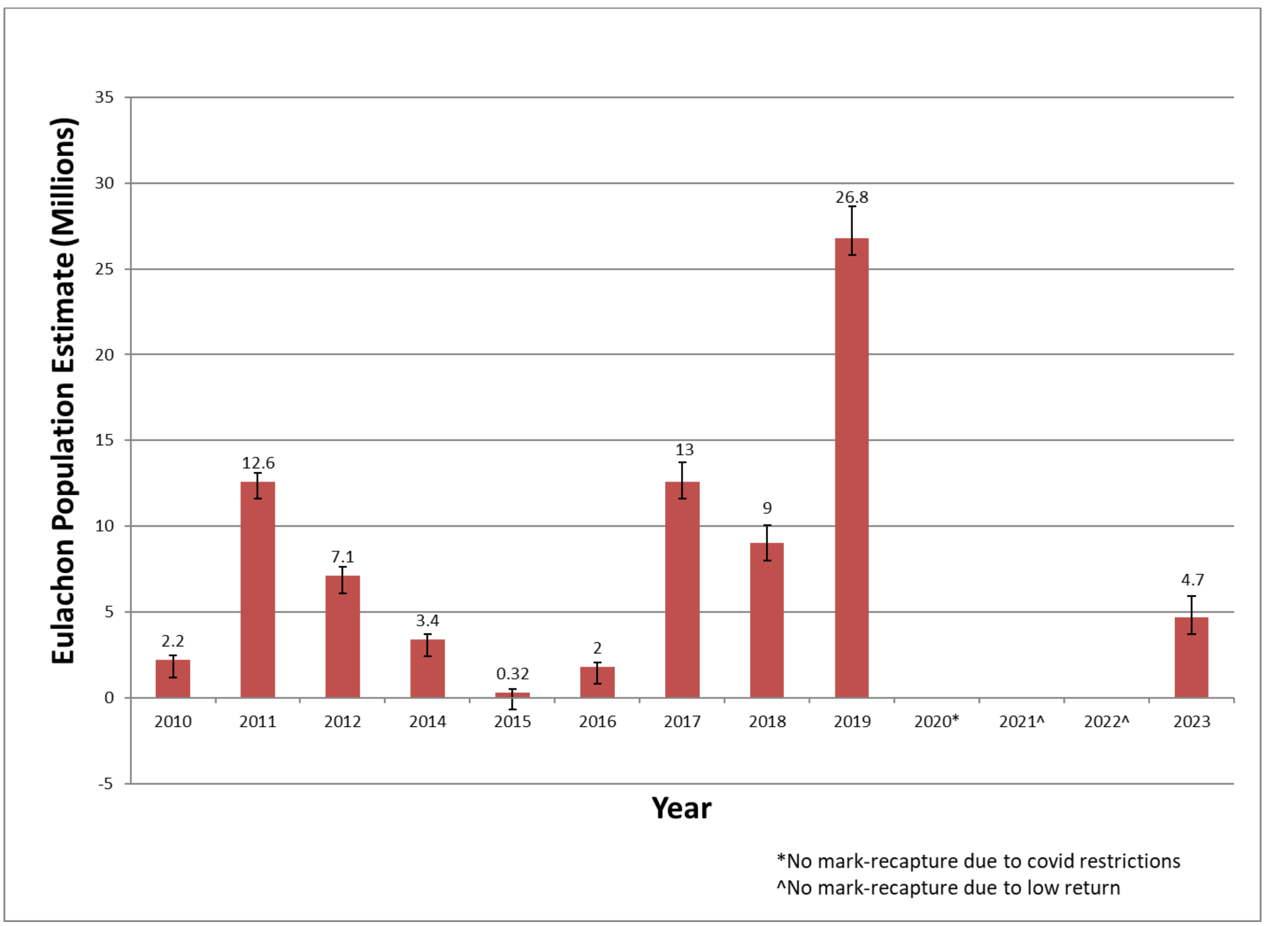

Figure 4: Chilkoot River mark-recapture population estimate results 2010-2023

eDNA:

The overall eDNA concentrations for 2023 were average – to slightly below average based on the timeseries we've monitored. Comparing the eDNA concentration between rivers without discharge correction is not advised since the eDNA concentration is a factor of the dilution of DNA within the river. In these instances where discharge data is not available; which accounts for all our rivers monitored except the Chilkoot and Tiaya Rivers, the eDNA concentration within the river from year to is used to evaluate the overall eulachon spawning population trends. For the rivers monitored without discharge data, it is assumed that the average discharge for each river during the eDNA sampling period is relatively the same from year to year, aside from any unprecedented storm events, and therefore it can be treated as a constant and the fluctuation in eDNA concentration can be assumed to correlate to the change in eulachon abundance. That said, given the regional population structure of eulachon it is often desired to track trends between rivers within the region and gauge general trends on abundance and what rivers are seeing high or low return annually. In examining nonflow-corrected eDNA concentration at the nine rivers monitored in 2023, it is shown that the Chilkoot and Chilkat Rivers had the overall highest eDNA concentrations, while the Ferebee, Katzehin, Skagway and Taiya Rivers all saw relatively low eDNA concentrations (Figure 5). See appendix for plots on annual trends at each river.

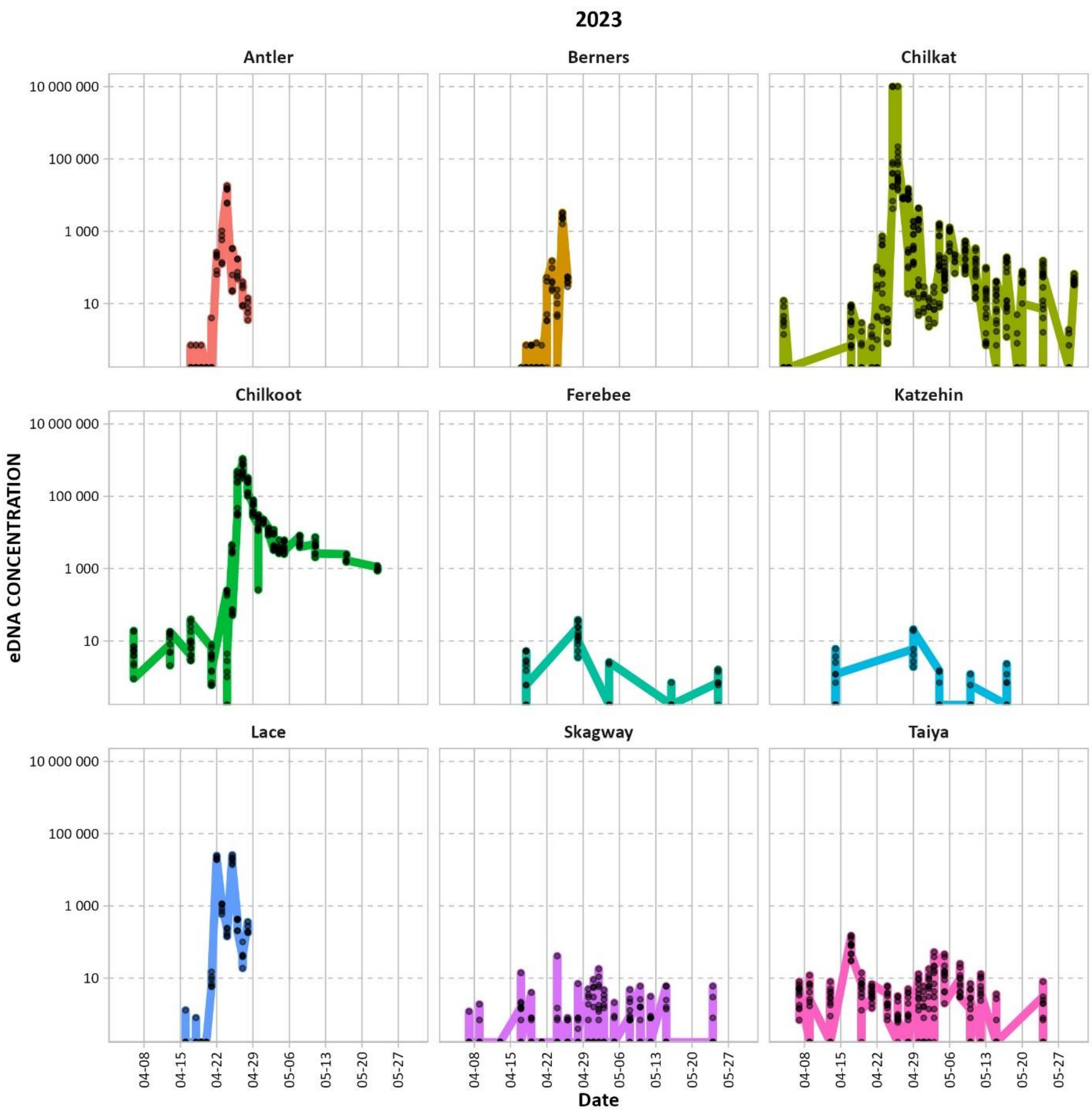

Figure 5: 2023 northern Southeast Alaska eulachon nonflow-corrected eDNA concentrations for nine rivers.

<u>Mark-Recapture & Chilkoot eDNA comparison</u>

The eDNA area under the curve (AUC) and size-of-peak (SOP) metrics are used to develop a regression between the mark-recapture population estimate. Since there was no mark-recapture in 2020 due to covid restrictions and no mark-recapture in 2021 and 2022 due to no return, the 2023 data is an opportunity to evaluate the correlation with mark-recapture and the eDNA concentration.

Both the AUC and SOP metrics remained statistically significant indicating that the eDNA concentration and mark-recapture population estimate are correlated. The AUC metric had a slightly more significant fit (p-value=0.018, R^2=0.7068) than the SOP metric (p-value=0.05, R^2=0.5714) (Figure 6).

Evaluating this correlation provides validation for the use of eDNA as a means of monitoring the annual eulachon spawning biomass at other locations where we cannot feasibly conduct a mark-recapture study.

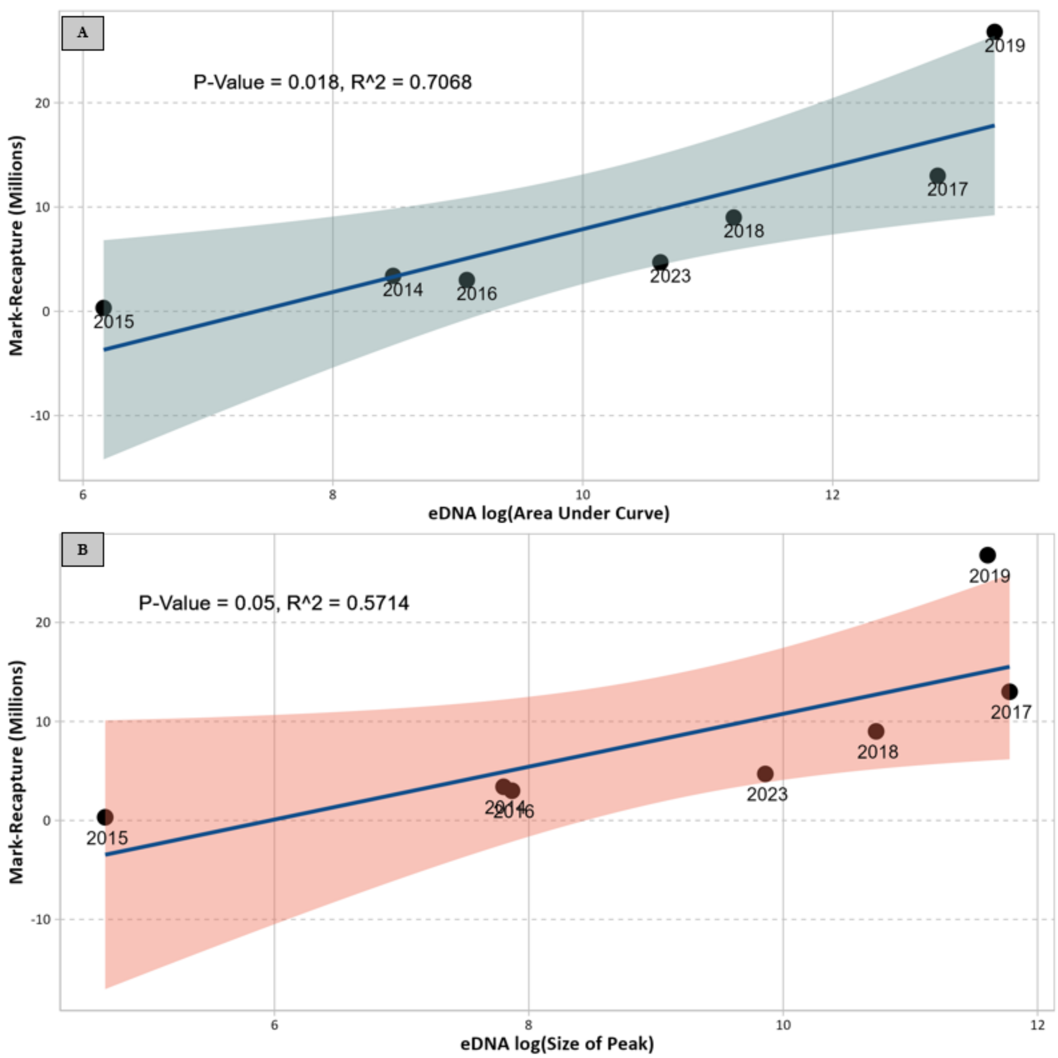

Figure 6: Linear regression comparing the mark-recapture population estimate and A: the eDNA area under the curve, and B: the eDNA size of peak concentration.

<u>Estuary Samples:</u>

The estuary samples often had higher eDNA concentrations earlier in the eulachon run as compared with the river eDNA concentration. This likely coincides with eulachon schooling behavior in the estuary before entering the river. The estuary samples also often had higher overall eDNA concentration and this could be due to DNA from the river washing down to the estuary. The river and estuary eDNA samples are both taken as close to low tide as possible, but changes in tide cycle and time of sampling could impact the estuary eDNA concentrations.

At the Chilkoot River in 2020 and 2023, the river and estuary eDNA concentrations became relatively similar, while the Chilkat River estuary eDNA concentrations continuously remain higher than the river eDNA concentration (Figure 7).

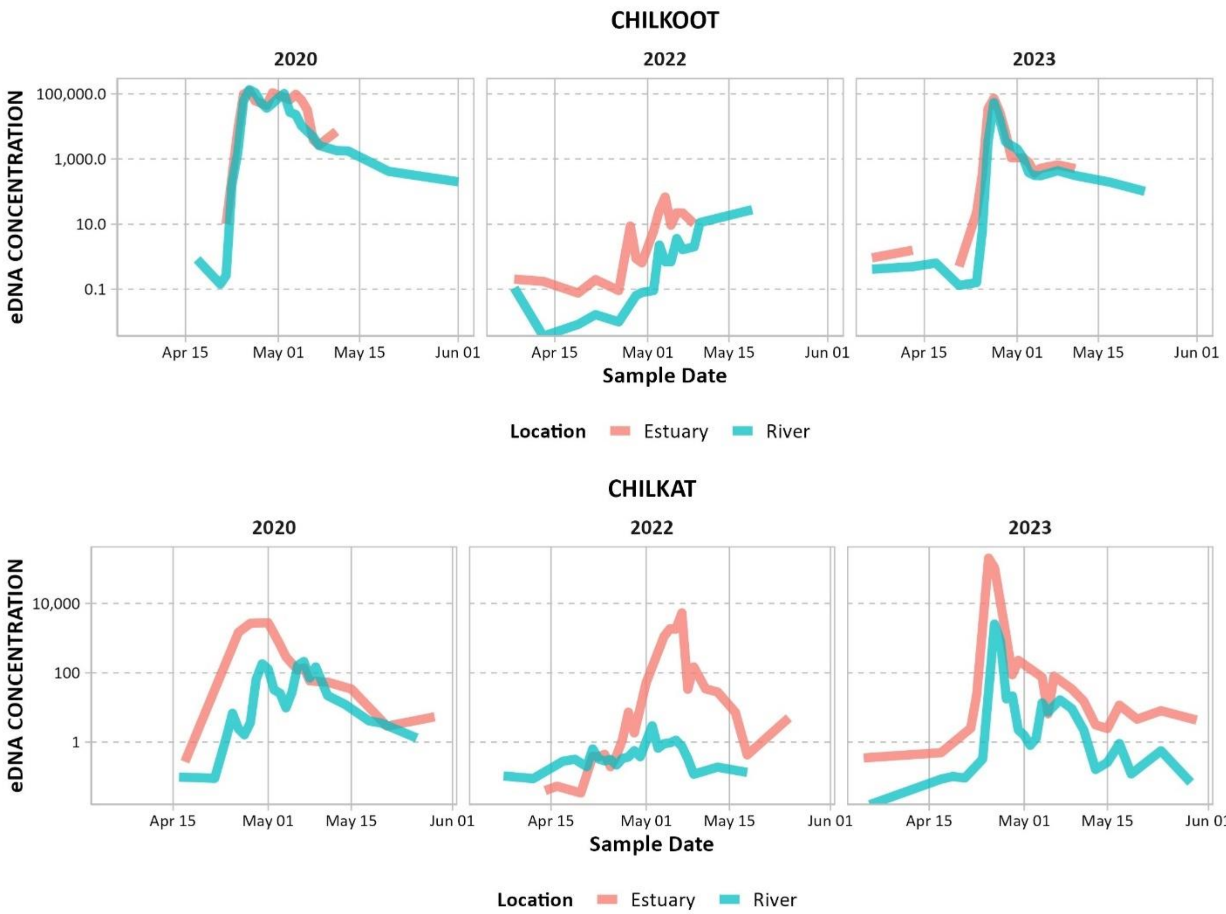

Figure 7: Chilkoot (top) and Chilkat (bottom) estuary and river eDNA concentrations.

Similarly, the Taiya River and estuary eDNA concentrations have been relatively similar in 2020, 2022 and 2023, while the Skagway River eDNA concentrations are often lower than the estuary eDNA concentrations (Figure 8). Estuary and river sampling locations can also influence this trend depending on how far apart these sampling locations are from each other.

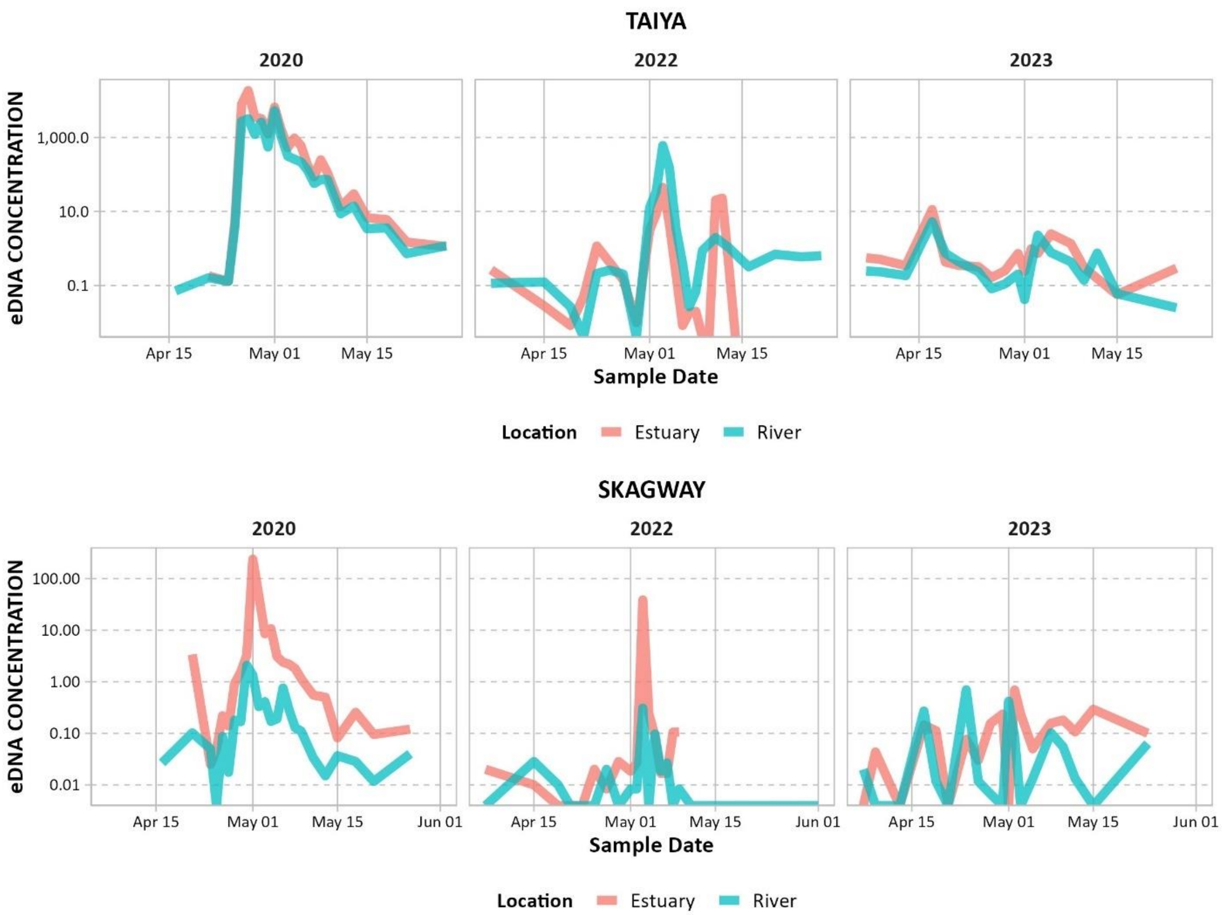

Figure 8: Taiya (top) and Skagway (bottom) estuary and river eDNA concentrations.

Run Timing

The arrival of eulachon to northern Southeast Alaska rivers varies on the order of a couple weeks, but typically occurs between late April and early May. At the Chilkoot River we've observed eulachon arrive as early as April 19th (2016) and as late as May 6th (2018) (Table 1). In 2023 eulachon were first observed in the Chilkoot River on April 25 and the run lasted approximately 4-5 days and ended around April 29.

The peak in eDNA concentration is also an indication of the peak in eulachon abundance and spawning activity. Generally, there is a latitudinal migration northward of eulachon, where we'll see the peak in eDNA concentration occur earlier at the more southern rivers (Berners, Lace, Antler), and a later peak at the more northern rivers (Table 2). This latitudinal migration in spawning is thought to allow for an elongated foraging period for marine mammals and seabirds that are taking advantage of the high-quality food source that is available while eulachon are spawning. With a staggered peak in eulachon concentration, foragers are able to follow their food source northward and increase the forging period and thereby the amount they can consume.

Year	Arrival Date	End of Mark-Recap
2010	4/23/2010	4/27/2010
2011	4/27/2011	5/8/2011
2012	5/2/2012	5/7/2012
2014	5/5/2014	5/9/2014
2015	4/26/2015	4/29/2015
2016	4/19/2016	4/24/2016
2017	4/27/2017	5/5/2027
2018	5/6/2018	5/11/2028
2019	4/25/2019	5/7/2019
2020	4/23/2020	5/8/2020
2021	-	-
2022	-	-
2023	4/25/2023	4/29/2023

Table 1: Chilkoot River eulachon arrival and duration of the mark-recapture population study.

Year	Ant_TOP	Bern_TOP	Lace_TOP	Kaz_TOP	Fer_TOP	Kat_TOP	Skg_TOP	Taiya_TOP	Koot_TOP
2017	4/29	5/1	4/29	5/1	5/2	NA	NA	5/3	4/30
2018	5/2	5/4	5/2	5/9	5/9	NA	5/7	5/9	5/8
2019	NA	NA	NA	5/1	4/29	NA	5/4	5/1	4/29
2020	NA	NA	NA	5/1	5/1	NA	4/30	5/1	4/26
2021	NA	NA	NA	4/20	5/10	4/30	5/7	4/30	5/5
2022	NA	NA	NA	5/6	5/5	5/6	5/3	5/3	NA
2023	4/24	4/25	4/22	4/14	4/28	4/26	4/24	5/3	4/27

Table 2: Timing of peak (TOP) eDNA concentration at each river monitored from 2017-2023. NA indicates the river was not monitored that year, or there was no distinct peak.

Wildlife Observations:

The wildlife congregations coinciding with the eulachon run are often the most reliable indication of eulachon arrival into the surrounding estuaries and rivers. The peak in wildlife typically overlaps with the peak in eDNA concentration for each of the rivers. In 2023, wildlife observations were recorded each day that eDNA sampling occurred at most rivers monitored. The mew gull (*Larus canus*) is often the most abundant and reliable indication of eulachon presence. Three rivers that had the most reliable mew gull count coinciding with eDNA sampling in 2023 were the Taiya, Skagway and Chilkat Rivers (Figures 9-11). The peak in mew gull count was slightly ahead of the peak in eDNA concentration for the Taiya River, but the peak in eDNA concentration occurred just before the peak in mew gull count for the Skagway and Chilkat Rivers.

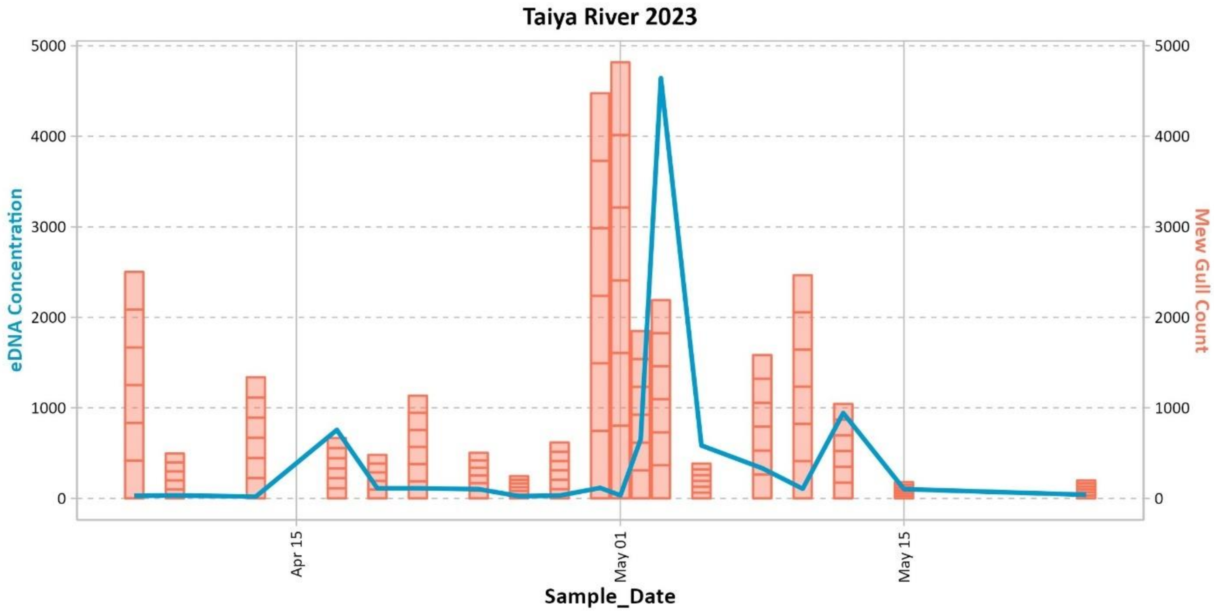

Figure 9: eDNA concentration (blue line) and mew gull count (orange bars) for the Taiya River 2023 eulachon run.

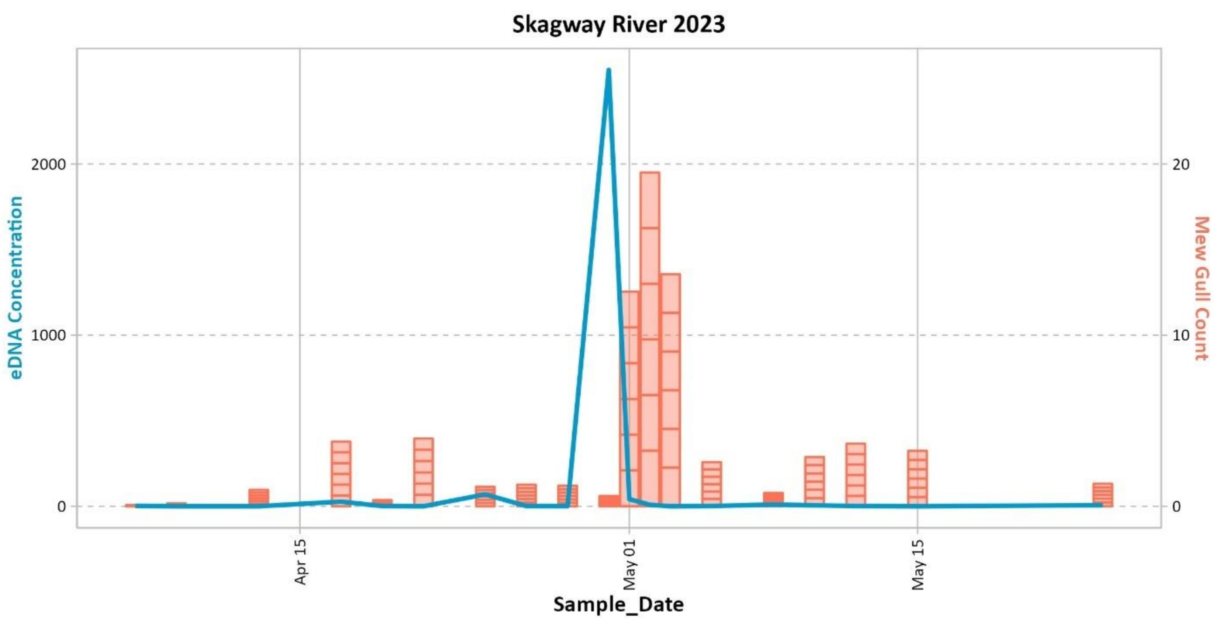

Figure 10: eDNA concentration (blue line) and mew gull count (divided by 100 for scaling) (orange bars) for the Skagway River 2023 eulachon run.

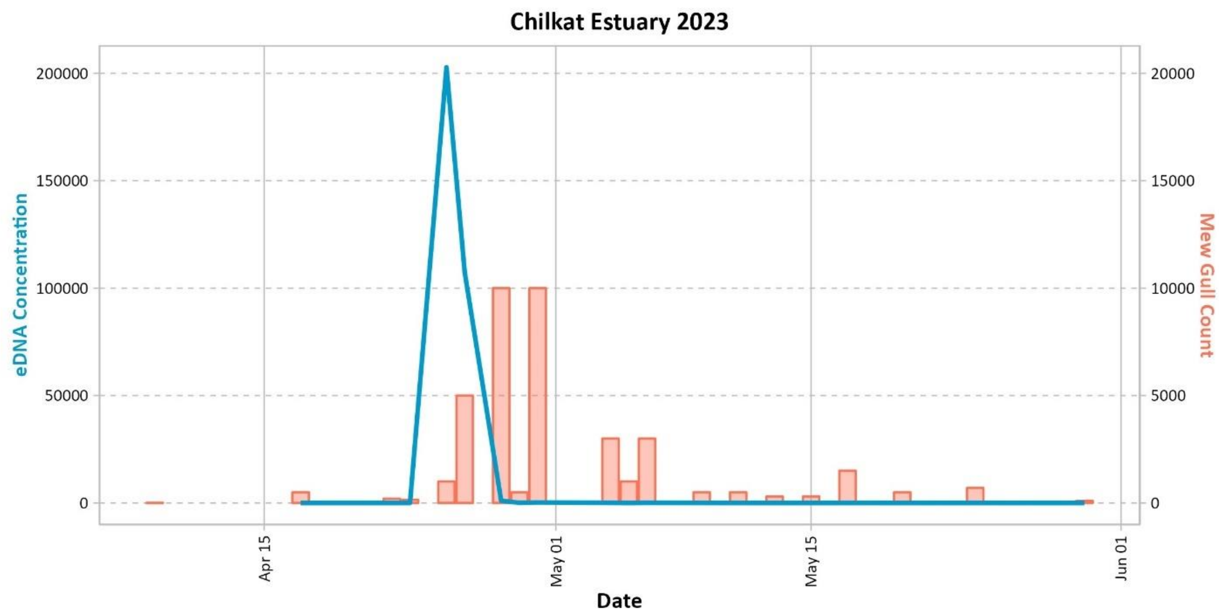

Figure 11: eDNA concentration (blue line) and mew gull count (orange bars) for the Chilkat River 2023 eulachon run.

Conclusion

Overall, the 2023 eulachon run in northern Southeast Alaska was average, with some rivers indicating a slightly below average return. The 2019 eulachon run still appears to be the biggest we've observed since monitoring began in 2010. The average to slightly below-average 2023 run comes after two years of almost nonexistent eulachon runs on most rivers monitored within the region (2021 & 2022). Harvesters, both subsistence and marine mammals and seabirds, appeared to be satiated and excited to see eulachon return to the rivers after the two-year hiatus.

The eDNA concentration and the Chilkoot River mark-recapture population estimate still reman significant, indicating that the use of eDNA as a means for monitoring the annual eulachon spawning population is an effective and efficient method to collect population data on rivers that otherwise would go unmonitored.

Eulachon population monitoring is also increasing beyond northern Southeast Alaska, with additional rivers being monitored by the Ketchikan Indian Community and Yakutat Tlingit Tribe. The addition of these regions to the overall eulachon monitoring network greatly expands our overall knowledge of eulachon population dynamics and overall population health.

Acknowledgements

The Chilkoot Indian Association would like to thank all the field technicians that put in many long days and hard work for this project. This important study would not be possible without the many field technicians that collect this valuable data. The Chilkoot Indian Association would also like to

thank our project partners, who also make this project possible. We look forward to continuing to work with you on this project in future years!

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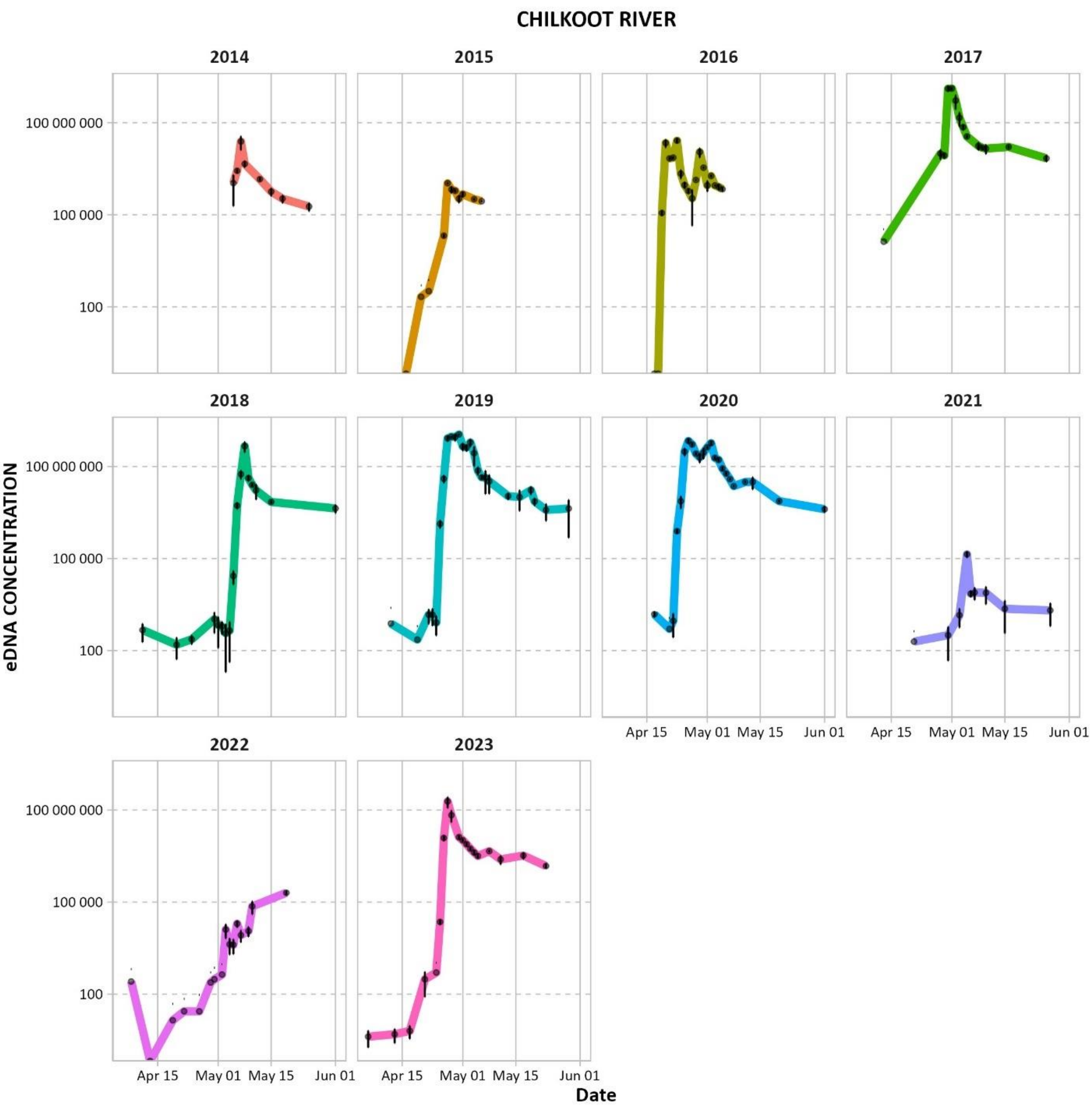
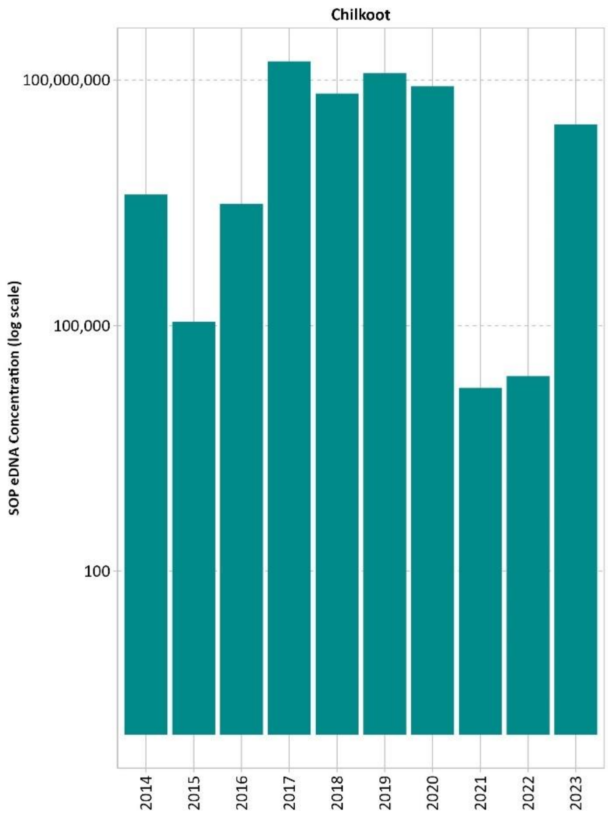
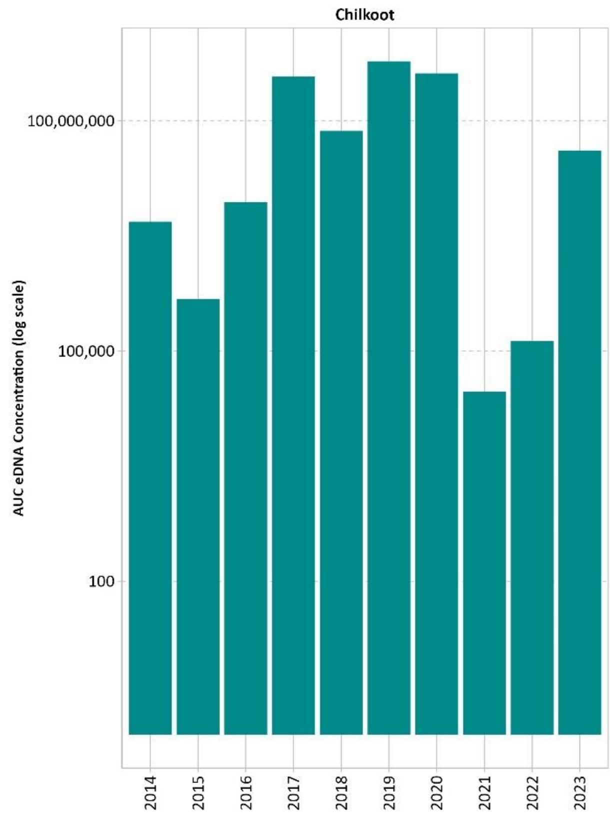
APPENDIX

Chilkoot River:




Chilkoot
SOP eDNA Concentration (log scale)
100,000,000
100,000
100
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023


Chilkoot
AUC eDNA Concentration (log scale)
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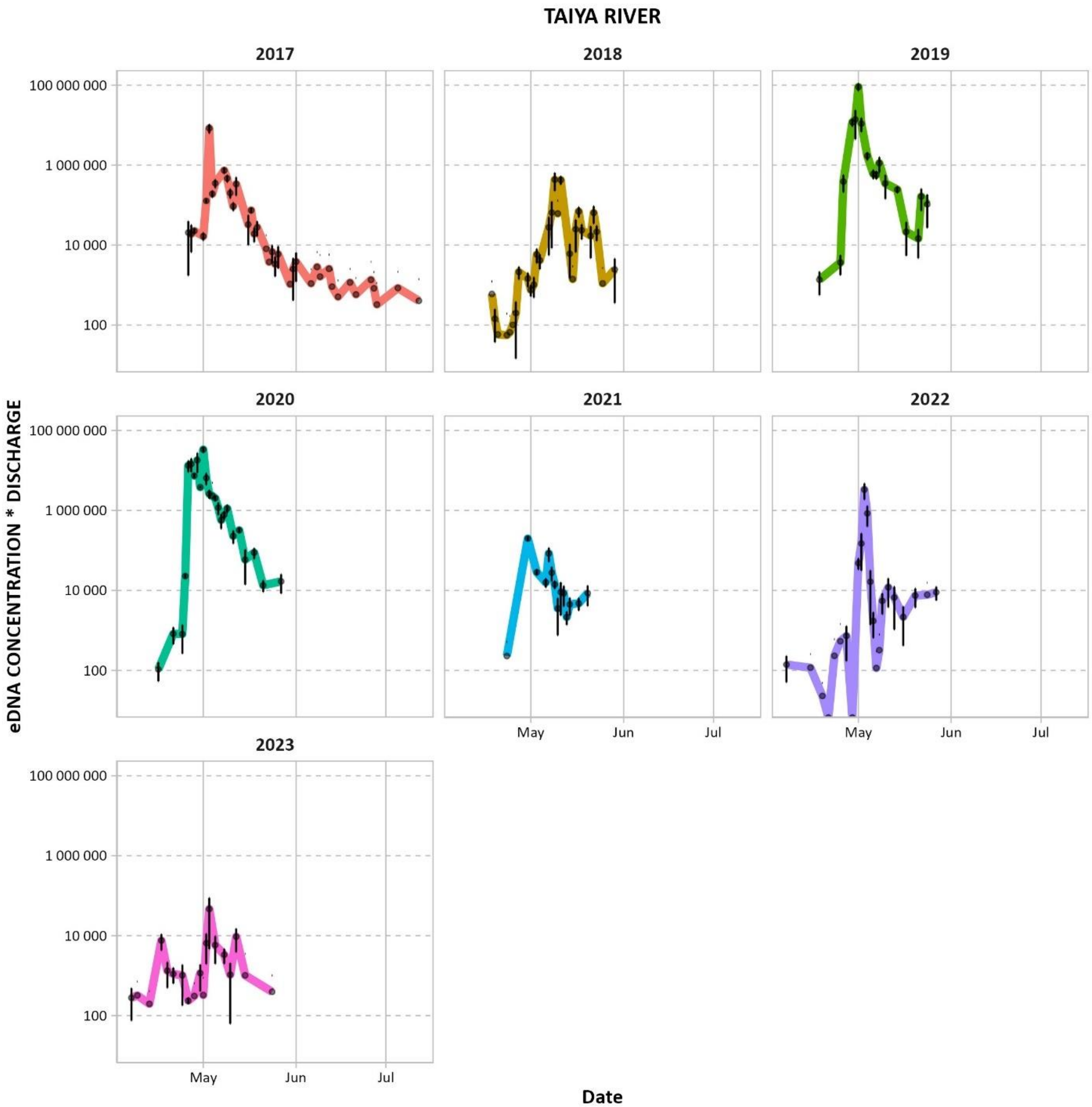
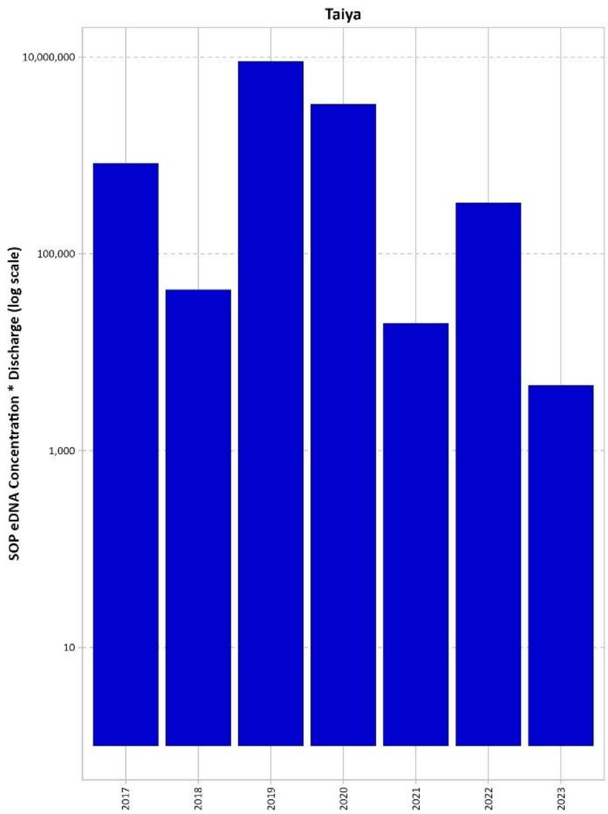
Taiya River:



TAIYA RIVER
2017
2018
2019
2020
2021
2022
2023
100 000 000
1 000 000
10 000
100
eDNA CONCENTRATION * DISCHARGE
May
Jun
Jul
Date


Taiya
SOP eDNA Concentration * Discharge (log scale)
10,000,000
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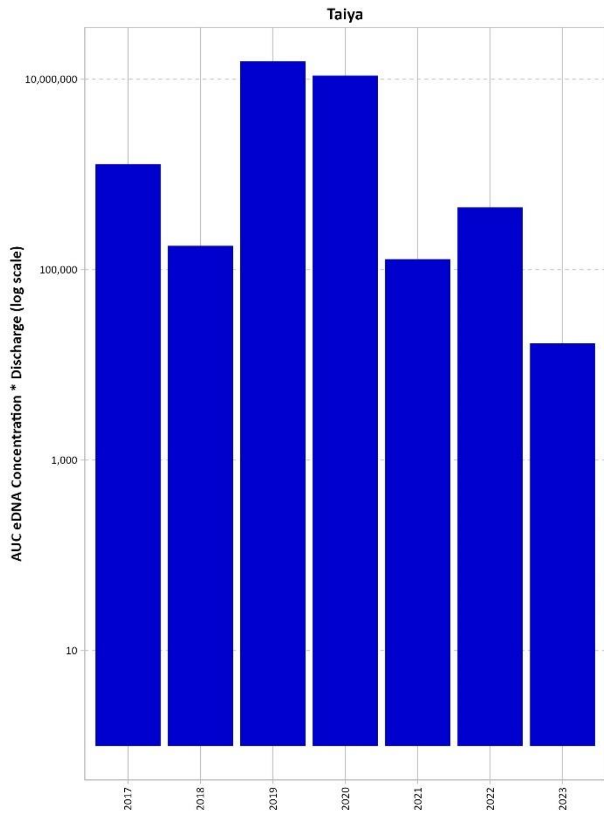
Taiya
AUC eDNA Concentration * Discharge (log scale)
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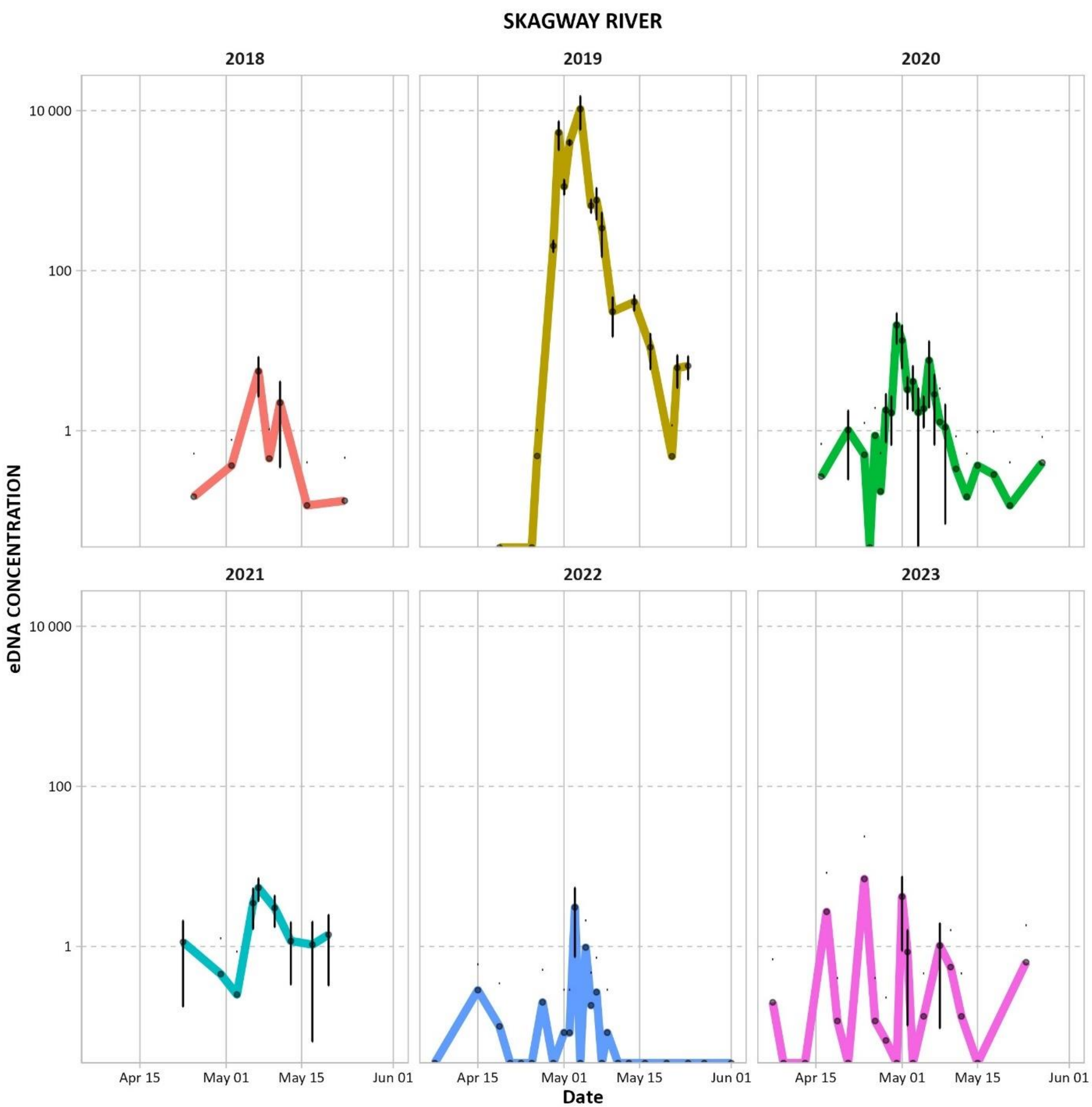
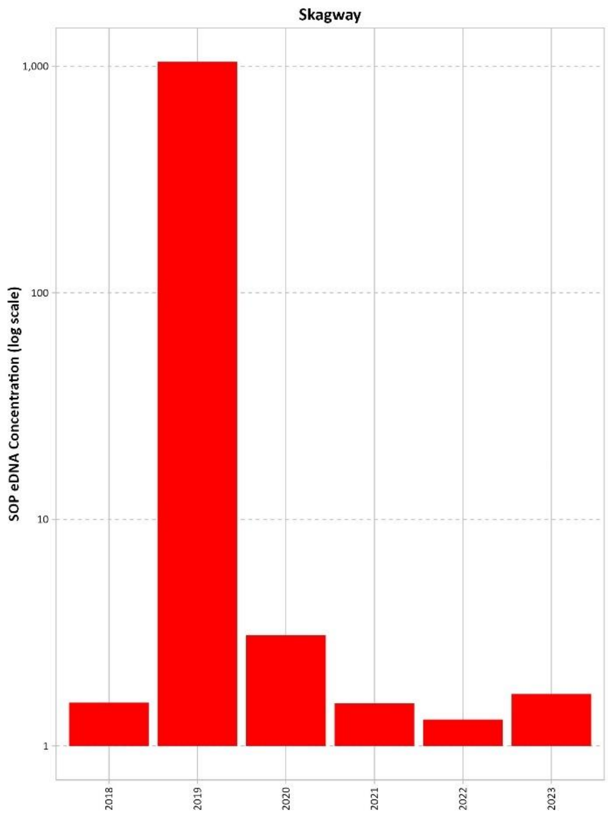
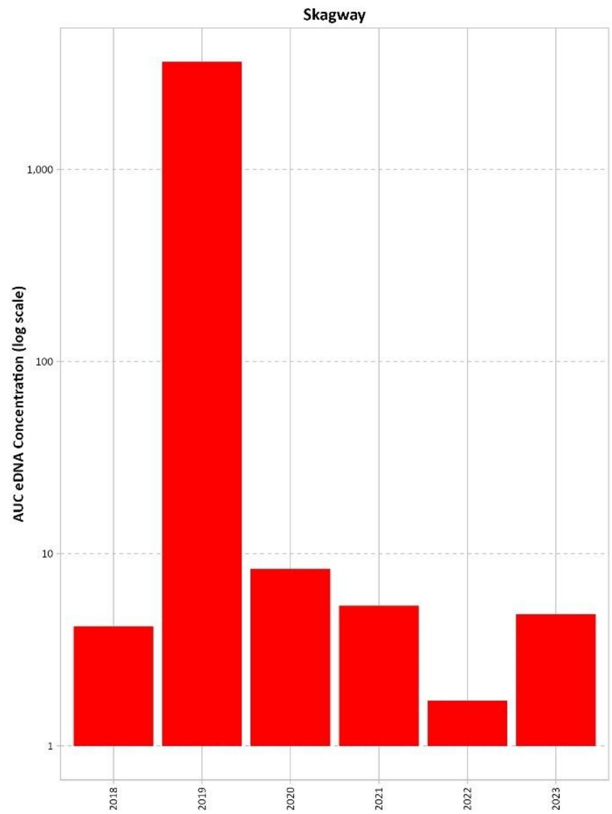
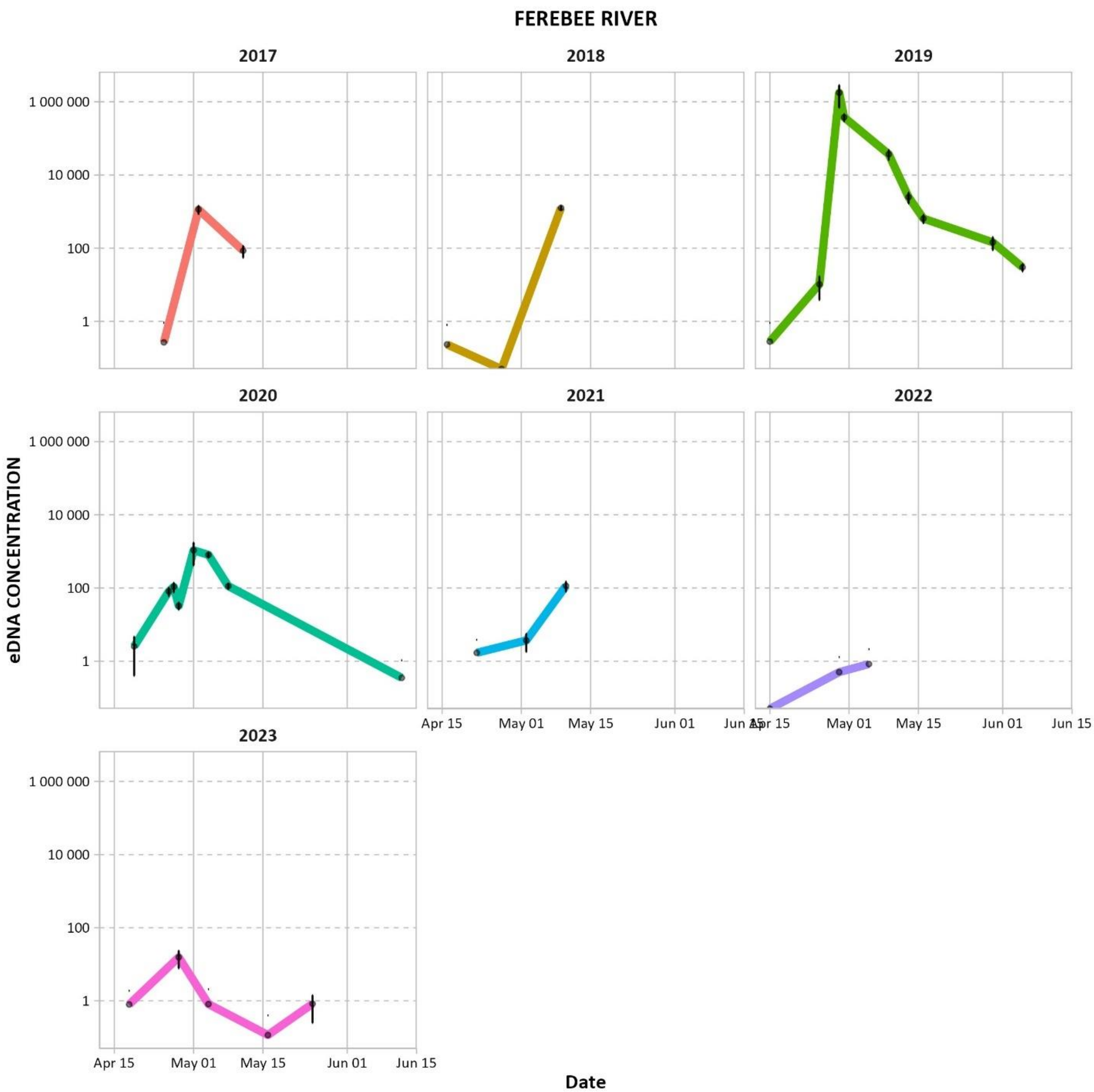
Skagway River:

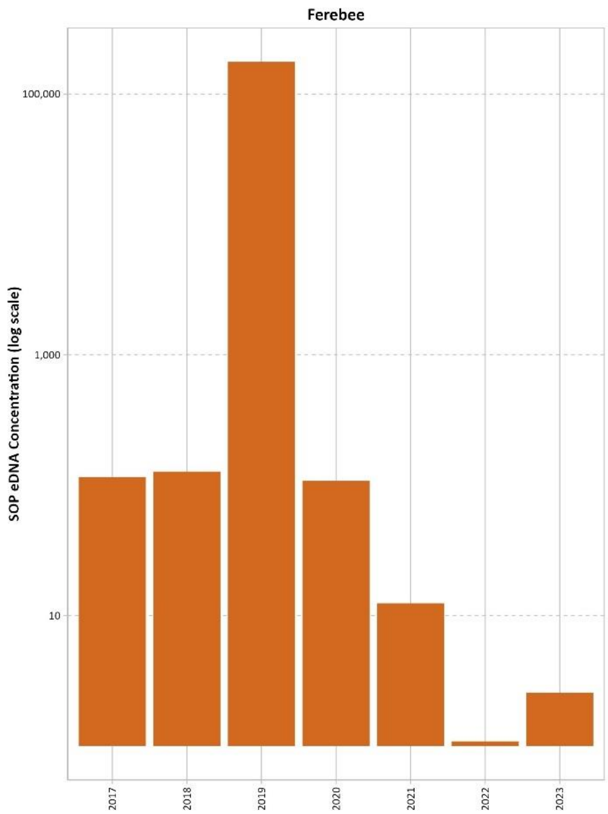


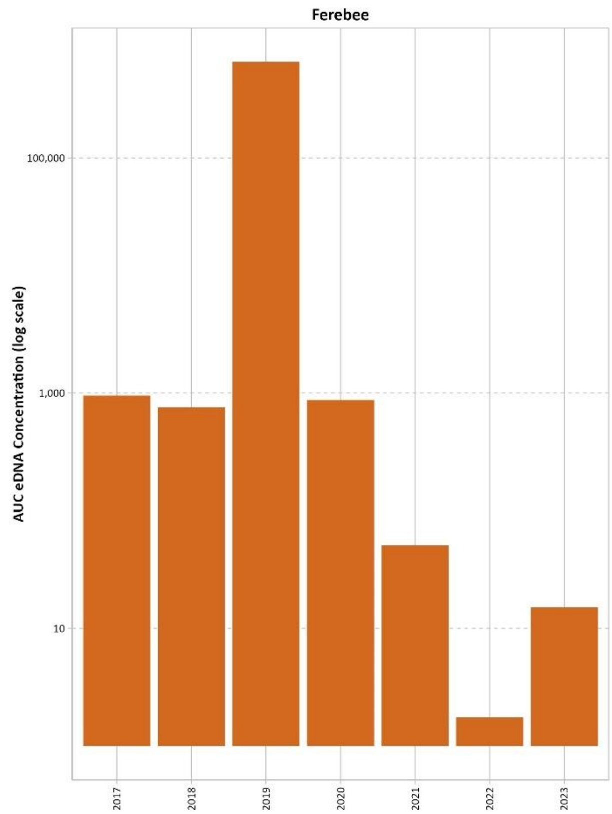

Skagway
SOP eDNA Concentration (log scale)
1,000
100
10
1
2018
2019
2020
2021
2022
2023


Skagway
AUC eDNA Concentration (log scale)
1,000
100
10
1
2018
2019
2020
2021
2022
2023

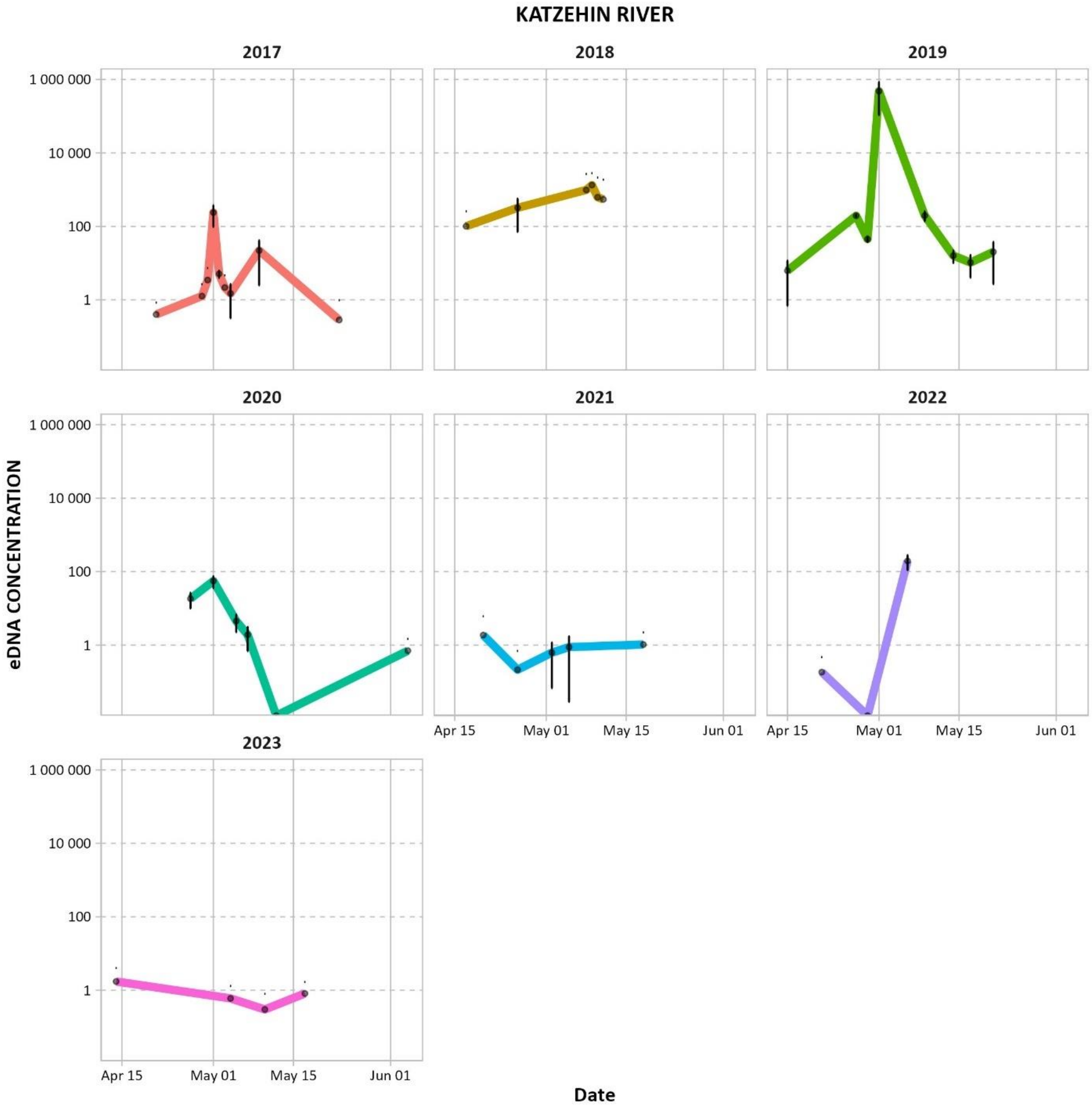
Ferebee River:

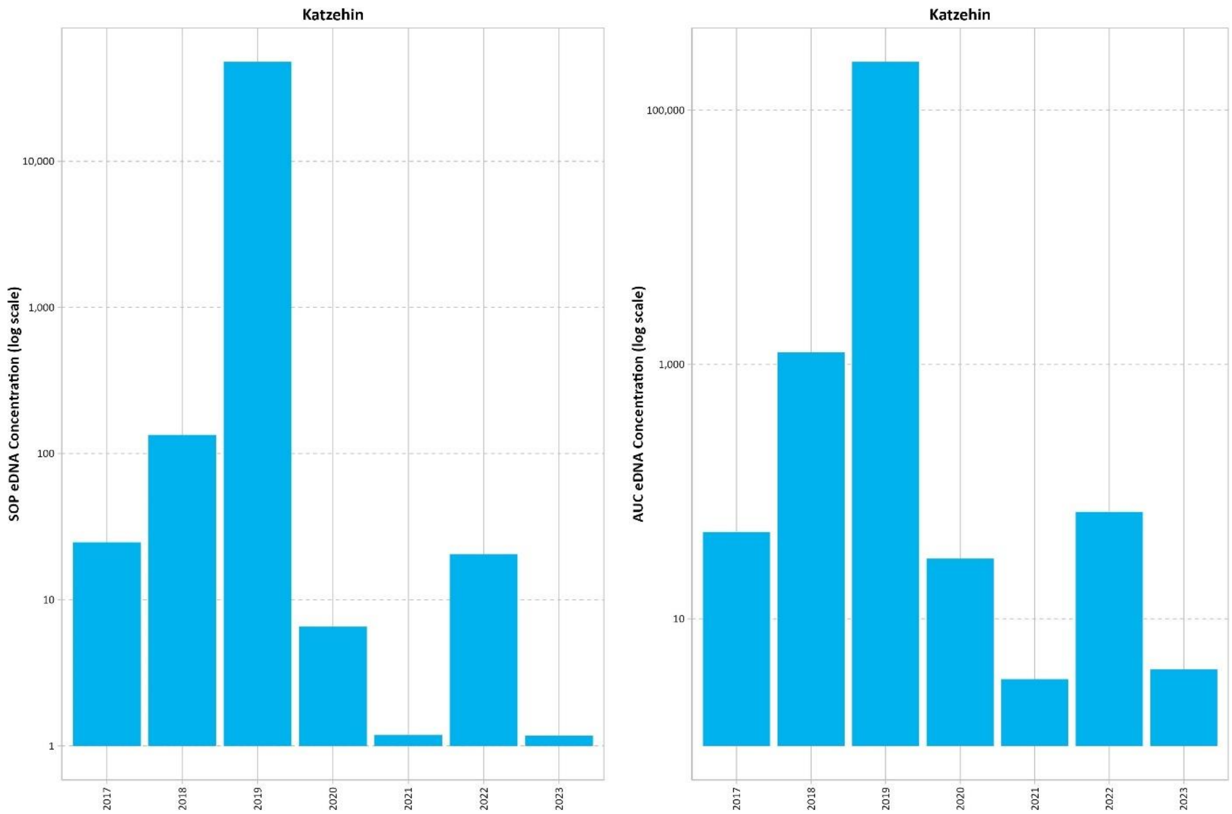



Ferebee
SOP eDNA Concentration (log scale)
100,000
1,000
10
2017
2018
2019
2020
2021
2022
2023

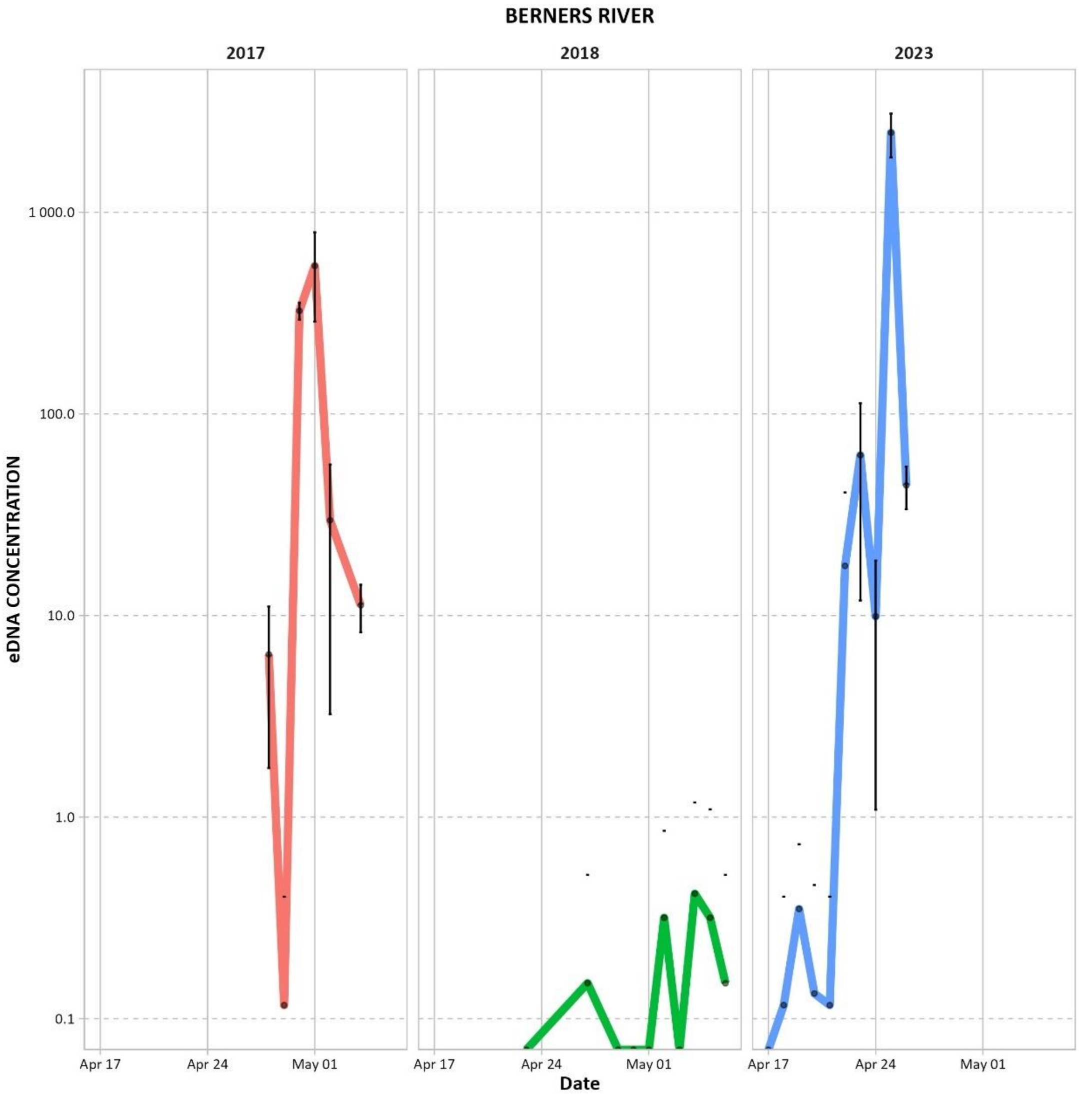

Ferebee
AUC eDNA Concentration (log scale)
100,000
1,000
10
2017
2018
2019
2020
2021
2022
2023

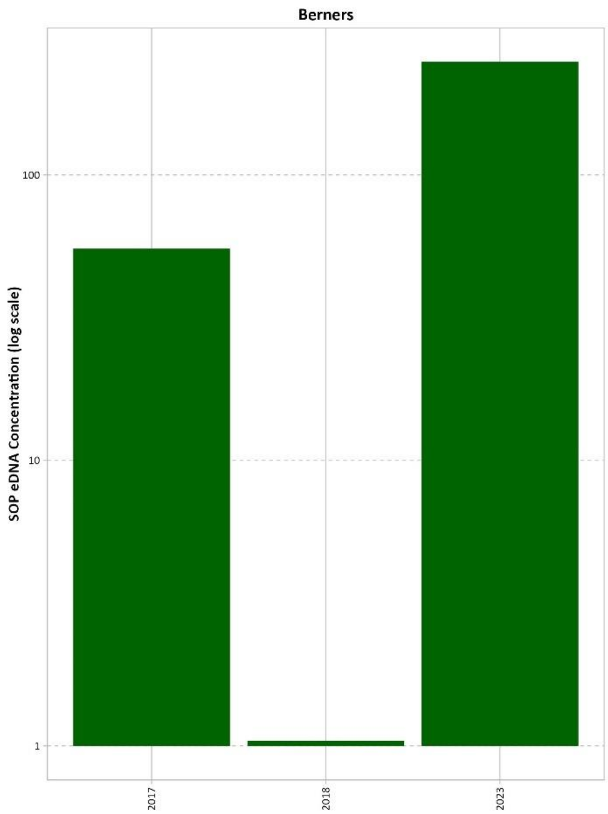
Katzehin River

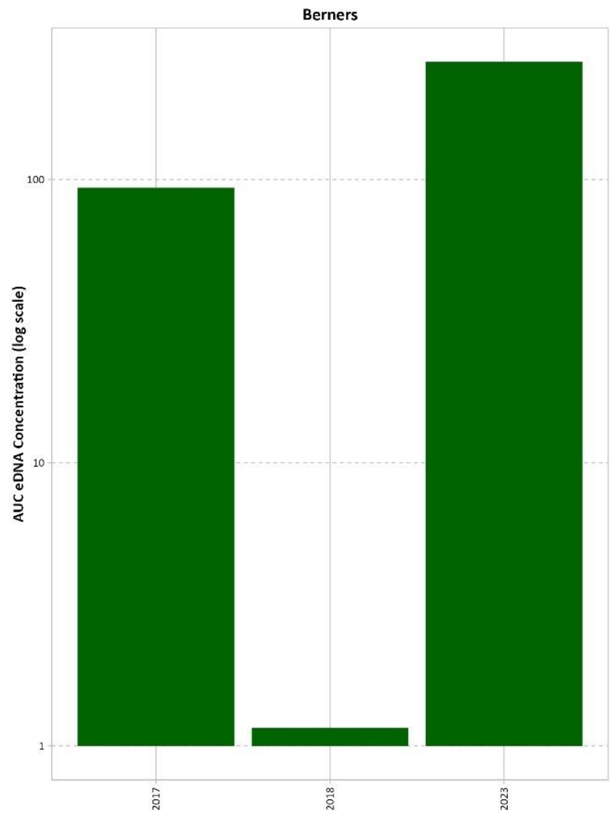



Katzehin
SOP eDNA Concentration (log scale)
10,000
1,000
100
10
1
2017
2018
2019
2020
2021
2022
2023
Katzehin
AUC eDNA Concentration (log scale)
100,000
1,000
10
2017
2018
2019
2020
2021
2022
2023

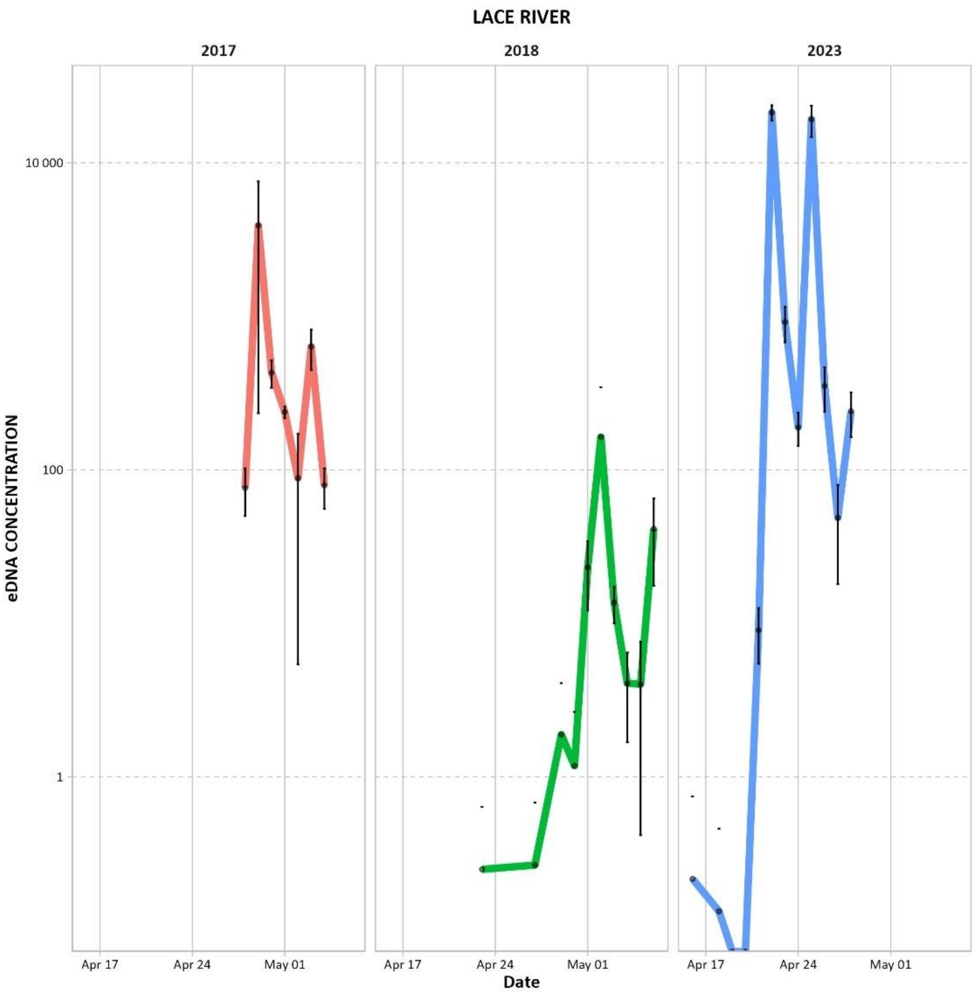
Berners River:



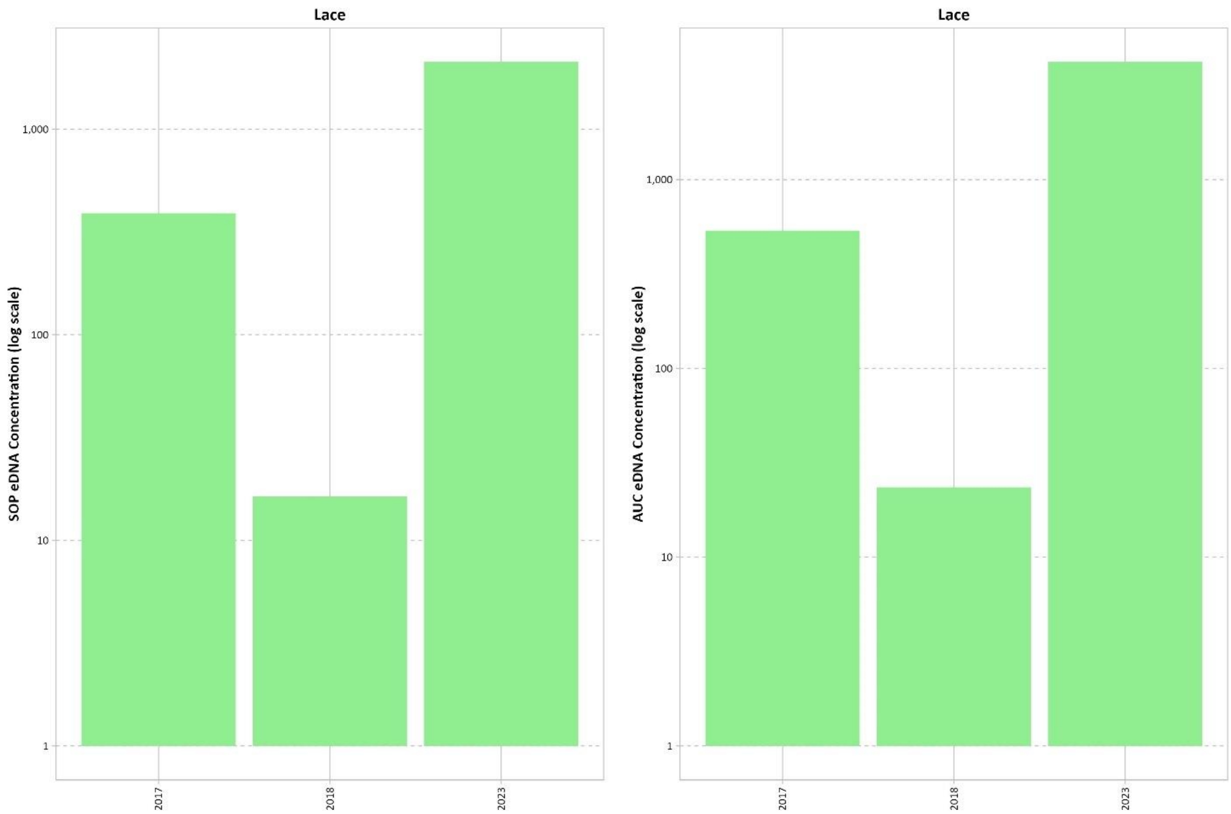

Berners
SOP eDNA Concentration (log scale)
100
10
1
2017
2018
2023


Berners
AUC eDNA Concentration (log scale)
100
10
1
2017
2018
2023

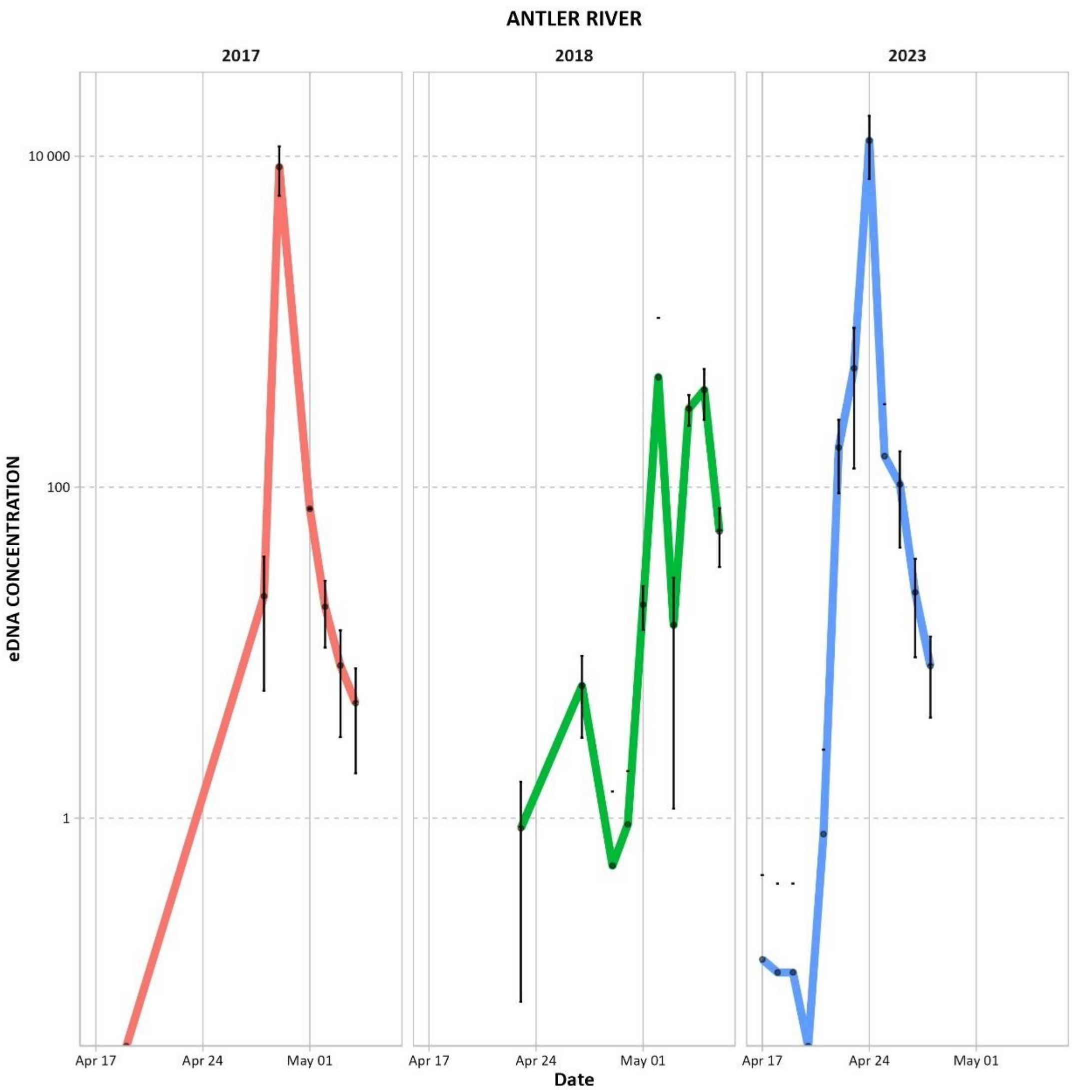
Lace River:

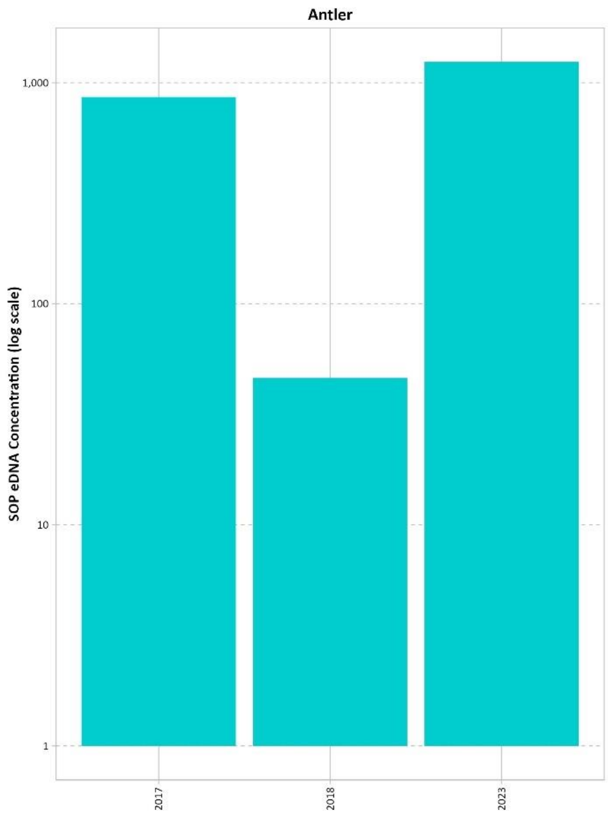


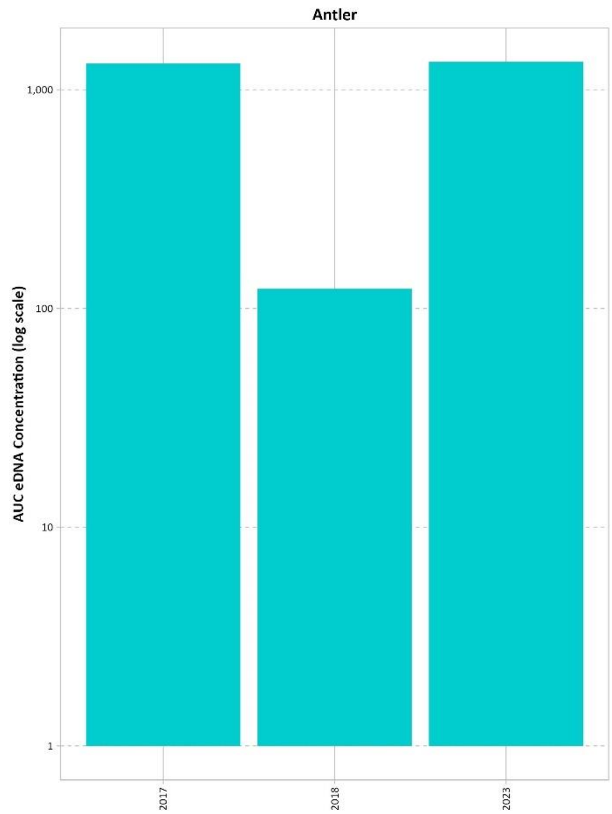
LACE RIVER
2017
2018
2023
10 000
100
1
eDNA CONCENTRATION
Apr 17
Apr 24
May 01
Apr 17
Apr 24
May 01
Apr 17
Apr 24
May 01
Date


Lace
SOP eDNA Concentration (log scale)
1,000
100
10
1
2017
2018
2023
Lace
AUC eDNA Concentration (log scale)
1,000
100
10
1
2017
2018
2023

Antler River:




Antler
SOP eDNA Concentration (log scale)
1,000
100
10
1
2017
2018
2023


Antler
AUC eDNA Concentration (log scale)
1,000
100
10
1
2017
2018
2023