Supporting Information for 'High-frequency and long-term observations of eDNA from imperiled salmonids in a coastal stream: temporal dynamics, relationships with environmental factors, and comparisons to conventional observations'

Table S1: Metadata from ESP deployments. Note some days of testing and control sampling are included. The names 'Waldo', 'Moe', and 'Gordon' each refer to distinct ESP deployed in Scott Creek.

Deployment	ESP	Start Date	End Date	Duration (days)	Gap (days)	N Samples
1	Waldo	3/25/19	5/2/19	39	-	114
3	Waldo	5/3/19	5/6/19	4	0	11
4	Moe	5/7/19	6/25/19	48	0	121
6	Moe	6/26/19	6/26/19	1	0	3
7	Gordon	6/27/19	8/22/19	56	0	62
8	Moe	8/25/19	11/21/19	89	2	100
10	Moe	11/22/19	11/25/19	4	0	11
12	Moe	11/26/19	12/2/19	7	0	13
13	Gordon	12/3/19	1/30/20	59	0	120
14	Waldo	2/4/20	2/5/20	2	4	4
15	Gordon	2/12/20	4/16/20	54	6	129

Table S2: Hatchery release counts of juvenile *O. kisutch* during the project period.

Release			Scott Creek watershed release site ID						
Date	Group No.	Life stage	SC	S0	S1	S2	S4	S5	BC
03/19/2019	1	Smolt (age-1)	---	1,608	---	304	232	216	---
03/29/2019	2	Smolt (age-1)	---	3,236	---	285	462	505	---
04/09/2019	3	Smolt (age-1)	---	3,441	---	351	359	343	---
04/19/2019	4	Smolt (age-1)	---	3,840	---	188	218	226	---
04/29/2019	5	Smolt (age-1)	---	3,560	---	209	212	307	---
05/09/2019	6	Smolt (age-1)	---	3,711	---	340	372	---	---
11/21/2019	Fall	Parr (age-0)	10,303	---	---	---	---	---	---
03/16/2020	1	Smolt (age-1)	---	3,675	---	206	---	248	---
04/02/2020	2	Smolt (age-1)	---	---	---	---	---	---	5,100

Site	Site ID	Latitude	Longitude
Lower Scott	S0	37.047039	-122.226319
Release Site 1	S1	37.080614	-122.246964
Release Site 2	S2	37.083081	-122.248275
Release Site 3	S3	37.095717	-122.251819
Release Site 4	S4	37.087364	-122.249156
Release Site 5	S5	37.099619	-122.252378
N/A	SC	---	---
Big Creek	BC	37.07457	-122.221611

Table S3: Summary of fish count data collected during the project period.

O. kisutch	**Count**			**Biomass (kg)**
	All Fish	Adult	Juvenile	
Total	3119	14	3105	98
Median	0	0	0	0
Maximum	297	2	297	7.6
Days Present	60/180			
O.mykiss	**Count**			**Biomass (kg)**
	All Fish	Adult	Juvenile	
Total	5506	258	5247	669
Median	1	0	0	0.5
Maximum	313	39	308	104
Days Present	96/180			

Table S4: Fish detection by monitoring method. Number of days are provided in each cell of the contingency table. McNemar's Tests were used to compare the fish detection rate between eDNA sampling and fish trapping

O. kisutch		
	Trap: Fish Present	Trap: Fish Absent
eDNA: Detected	60	118
eDNA: ND	0	2
McNemar's Test: $\chi 2 = 116.0$, $p < 0.001$		
O. mykiss		
	Trap: Fish Present	Trap: Fish Absent
eDNA: Detected	96	84
eDNA: ND	0	0
McNemar's Test: $\chi 2 = 82.0$, $p < 0.001$		

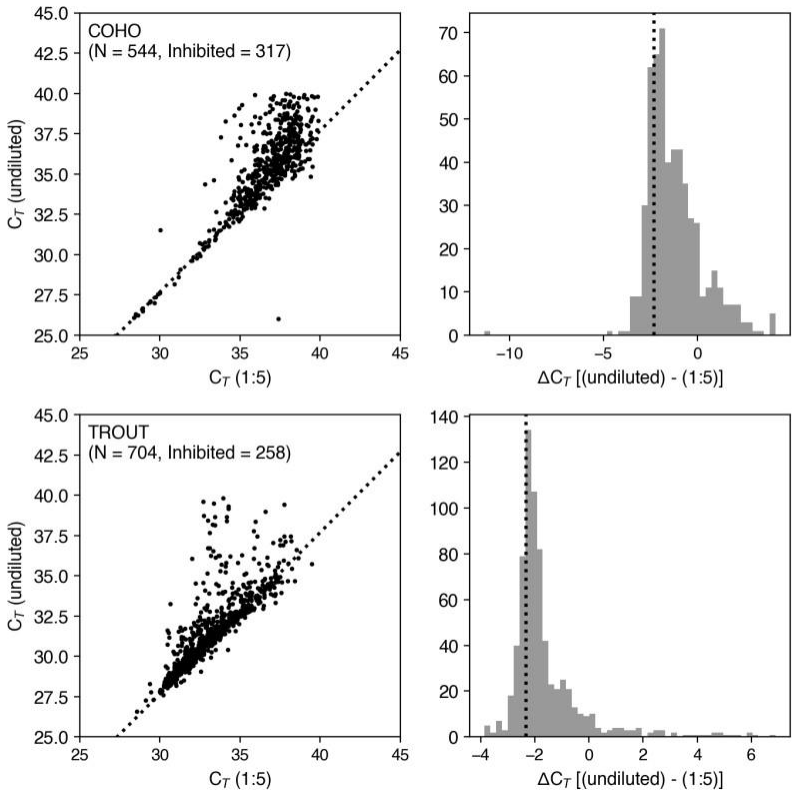

Figure S1: Inhibition assessment using sample dilutions. *O. kisutch* (COHO) results are presented in the top row while *O. mykiss* (TROUT) results are presented in the bottom row. Scatter plots comparing undiluted to diluted samples are presented in the first column. The dotted line represents a line with slope 1 and intercept of -2.3. Histograms of ΔC_T values are presented in the second column. Inhibition was detected in 58% and 37% of coho and trout samples where such an assessment could be made.

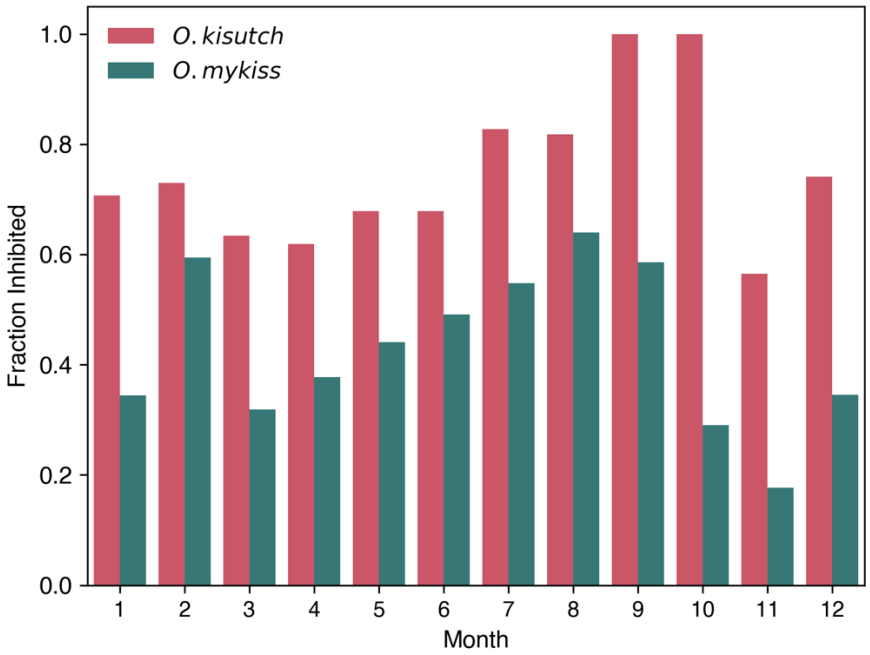

Figure S2: Fraction of samples with inhibition by month of collection. Only samples where amplification occurred in both dilutions for a given target are included.

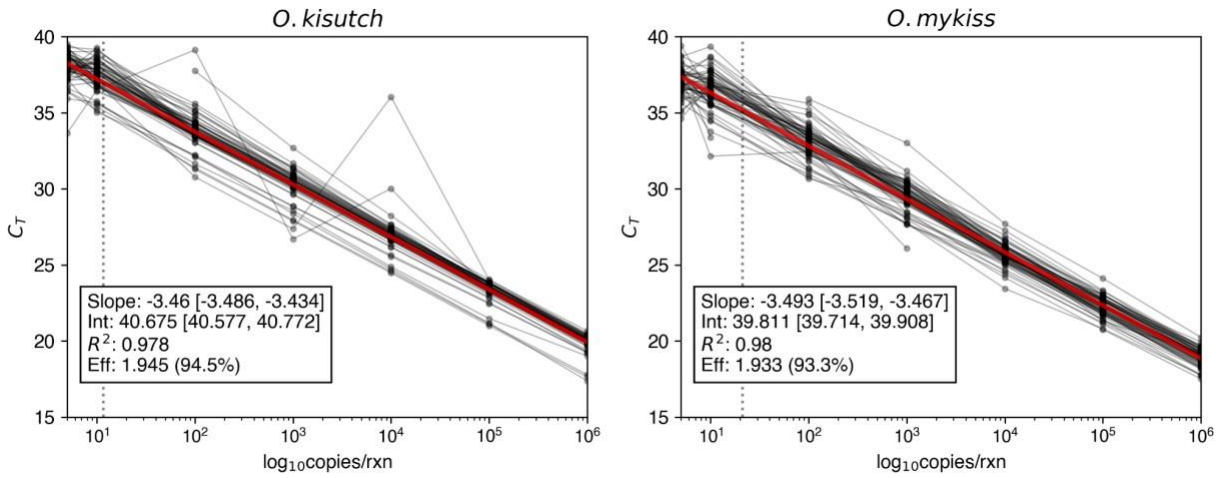

Figure S3: Master standard curves for each target species. Black solid lines represent individual standard curves for each qPCR plate and red lines represent the master standard curve for each target. The regression information corresponds to the master standard curve. Dotted vertical lines indicate the LOD/LOQ for each target.

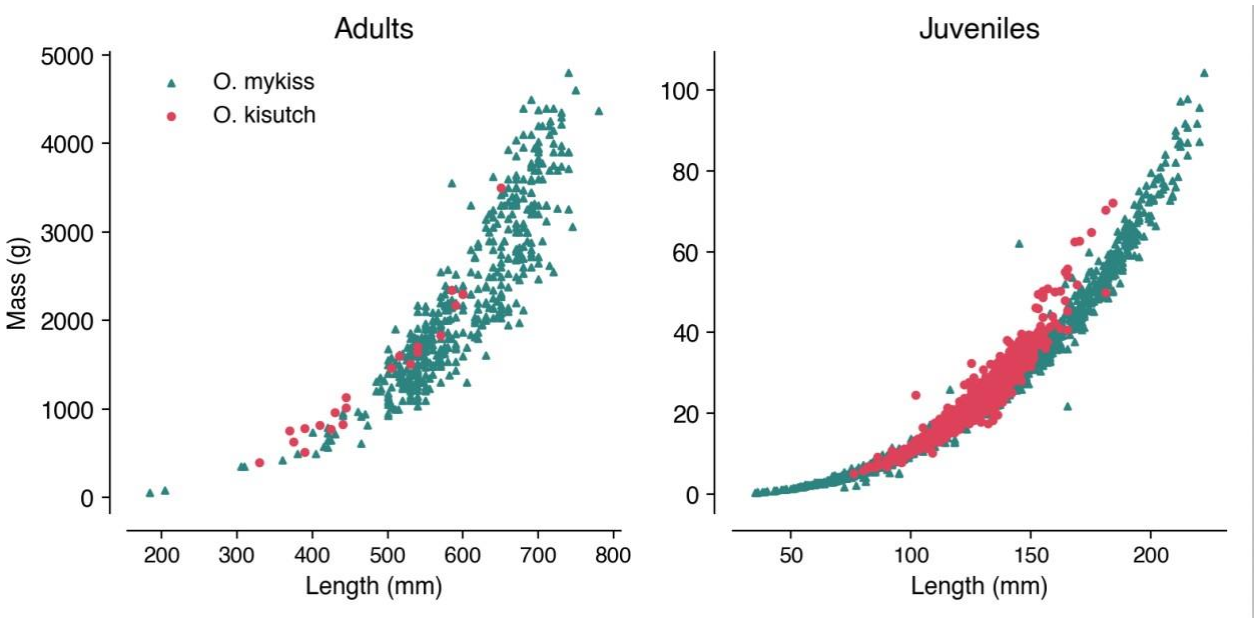

Figure S4: Fish biometric data used for regressions to estimate missing masses in the fish count data.

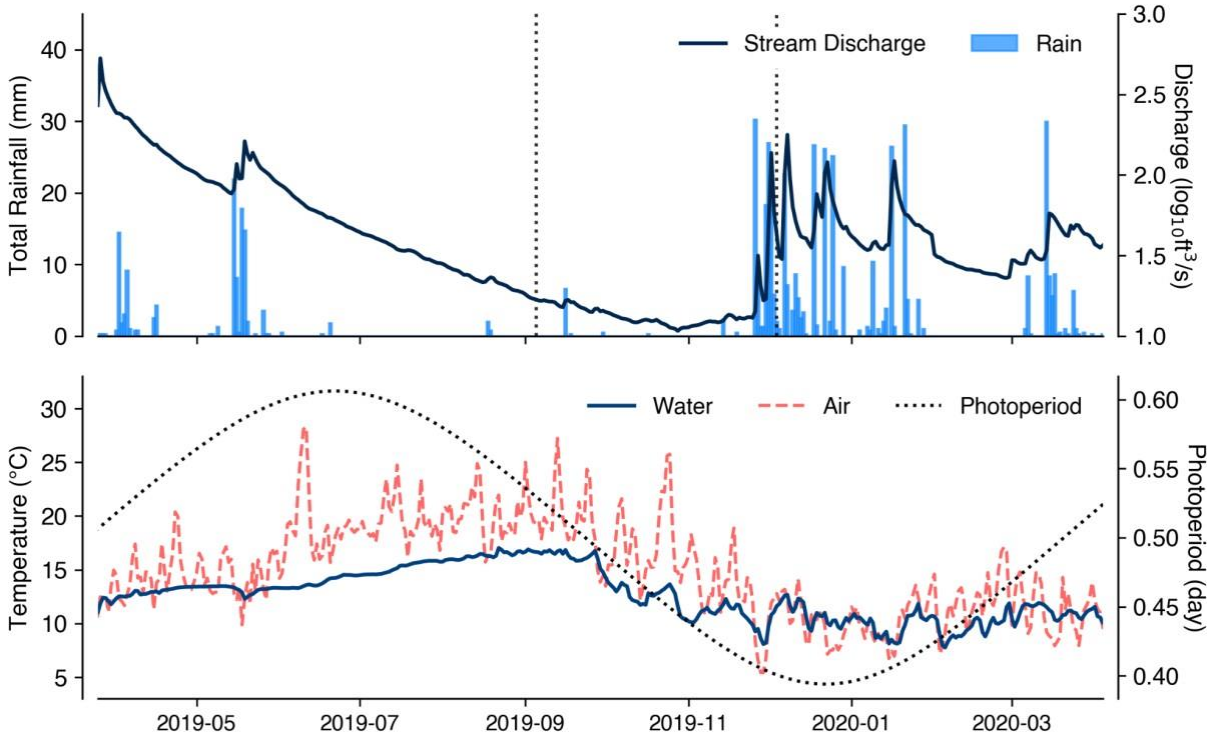

Figure S5: Environmental data time series. Vertical dotted lines in the top subplot indicate the dates when the mouth of Scott Creek was closed due to sandbar formation (5 September 2019) and when the mouth was open to the Pacific Ocean (4 December 2019).

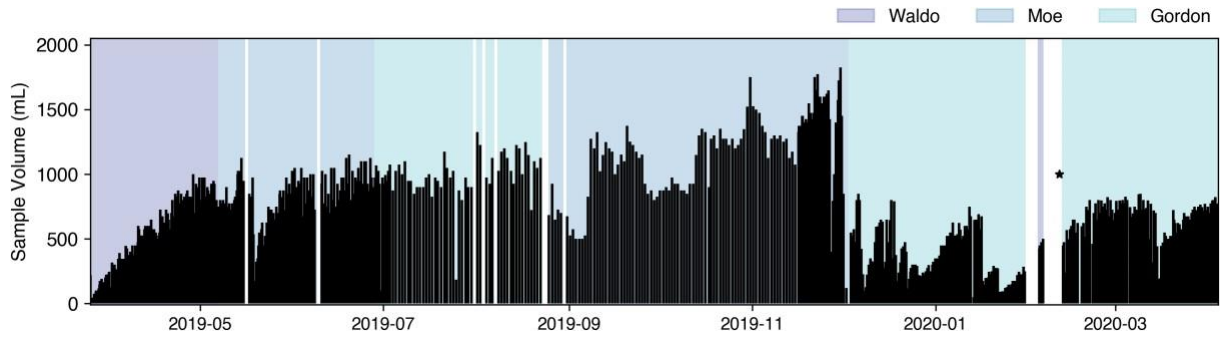

Figure S6: Volumes of water samples collected during the study. The color of the background corresponds to the specific ESP used to collect the sample. A white background indicates that ESPs were offline. The star indicates the volume of the hand-collected sample on 11 February 2020.

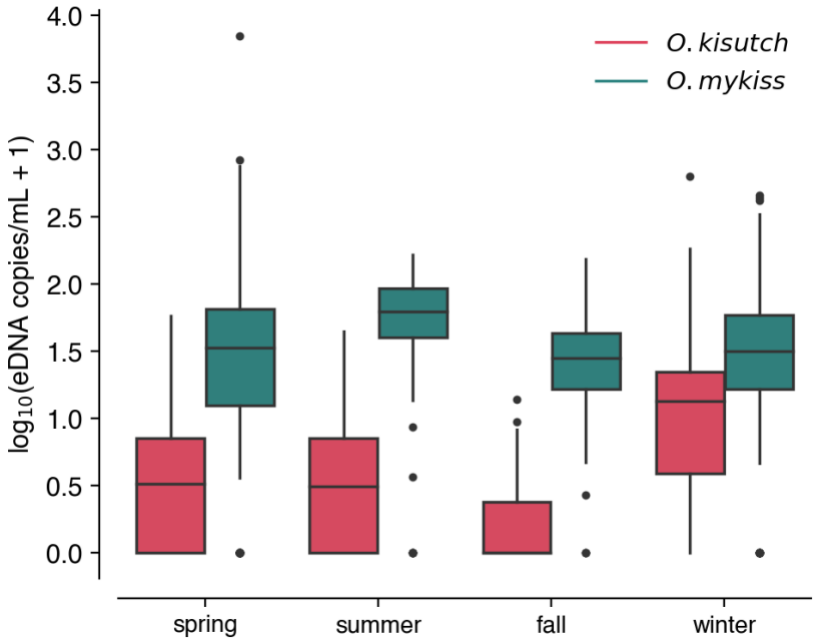

Figure S7: eDNA concentration distributions by season. Seasons were defined according to the month a sample was collected: spring (March–May), summer (June–August), autumn (September–November), and winter (December–February).

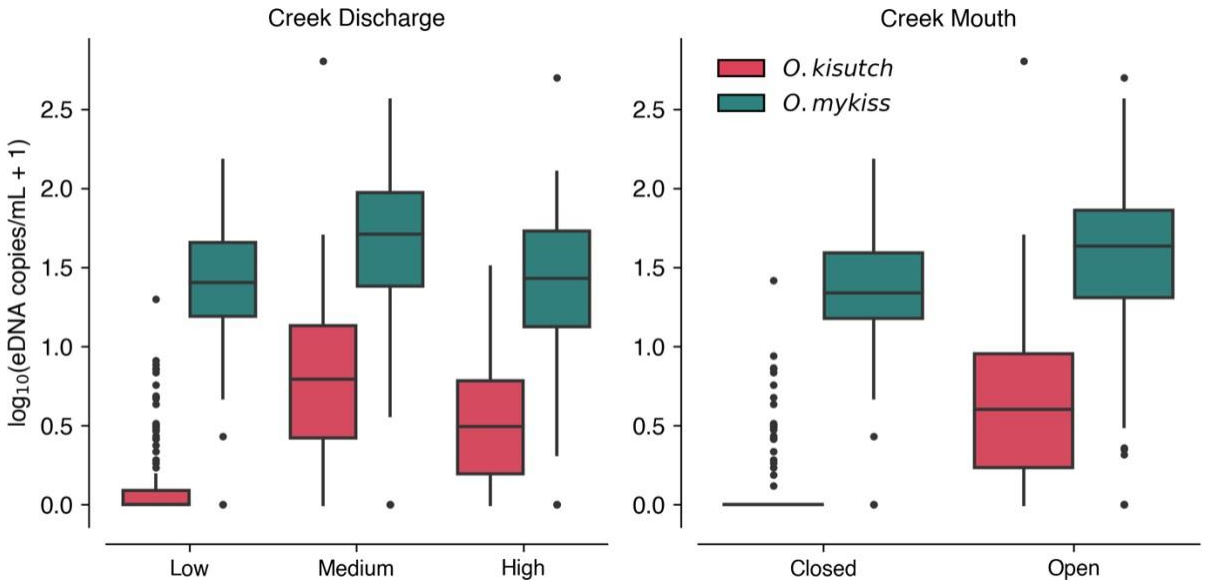

Figure S8: eDNA concentration by environmental condition during sample collection. Left: concentrations grouped by low (discharge < 0.65 m^3/s), medium (0.65 m^3/s < discharge < 1.47 m^3/s), and high (≥ 1.47 m^3/s) creek discharge regimes; Right: concentrations grouped by the condition of the Scott Creek mouth (i.e. closed or open). The middle line in the box plots represents the median concentration; the upper and lower edges of the boxes represent the 75^{th} and 25^{th} quantiles, respectively. The whiskers extend to 1.5 times the interquartile range (75^{th} quartile–25^{th} quartile). The remaining points represent values outside of that range.

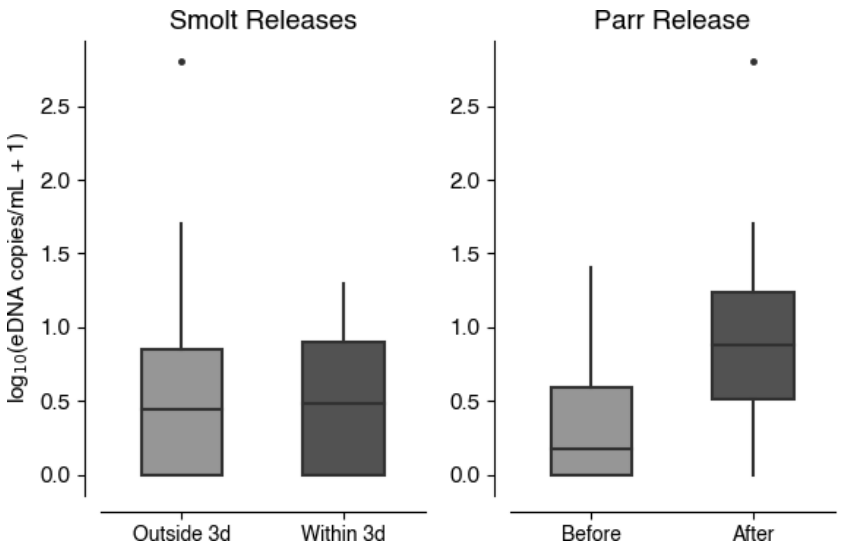

Figure S9: *O. kisutch* eDNA concentration by hatchery variable. Left: concentrations grouped by if a release of hatchery-origin smolts had occurred within the previous 3 days; Right: concentrations grouped by if the 21 November 2019 release of 10,000 hatchery-origin parr had occurred. The middle line in the box plots represents the median concentration; the upper and lower edges of the boxes represent the 75th and 25th quantiles, respectively. The whiskers extend to 1.5 times the interquartile range (75th quartile−25th quartile). The remaining points represent values outside of that range.

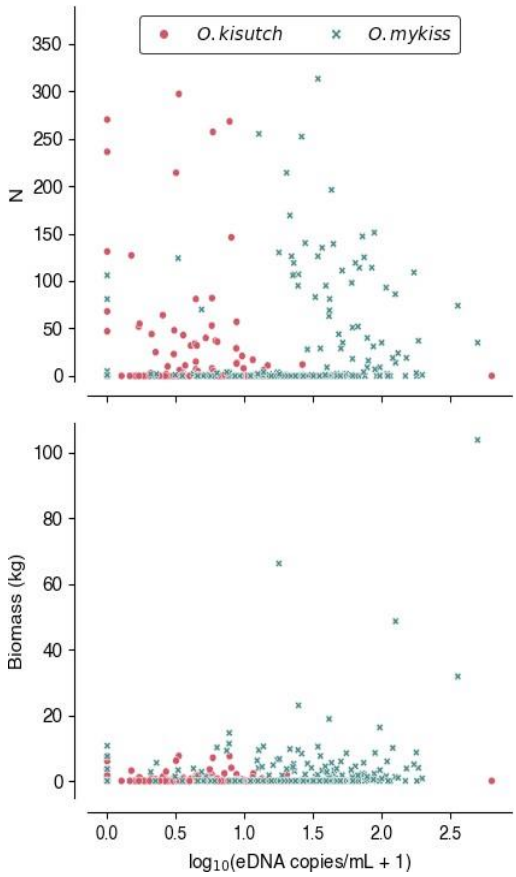

Figure S10: Mean daily eDNA concentration vs. fish abundance. Total daily fish count (top) and biomass (bottom) were assessed from the adult (weir) and juvenile (smolt) traps. eDNA samples measured below the LOQ are valued 0 in this figure.

Environmental Microbiology Minimum Information Checklist

Study Description

Study:
Date:
Completed by:

Environmental Sampling	Sample Treatment	Sample Reduction	Nucleic Acid Extraction	Reverse Transcription	PCR Detection	Analysis
	☐ Performed	☐ Performed		☐ Performed	☐ qPCR ☐ dPCR	

Control Checklist

	Environmental Sampling	Sample Treatment	Sample Reduction	Nucleic Acid Extraction	Reverse Transcription	PCR Detection	
Step performed	☐	☐	☐	☐	☐	☐	
Step has control info	☐	☐	☐	☐	☐	☐	**Negative Controls**
# control SFQMJDBUFT							
Control result reported	☐	☐	☐	☐	☐	☐	
%BUB Iandling reported	☐	☐	☐	☐	☐	☐	
Control introduced Internal/External Independent/Parallel	☐	☐	☐	☐	☐	☐	**Positive Controls**
Step has control info	☐	☐	☐	☐	☐	☐	
# control SFQMJDBUFT							
Control result reported	☐	☐	☐	☐	☐	☐	
%BUB)BOEMJOH reported	☐	☐	☐	☐	☐	☐	

Process Checklist

Environmental Sampling

- ☐ Sampling Procedure
- ☐ Number of samples
- ☐ Sample amount, mean, range
- ☐ Sampling locations, dates, times

Sample Treatment

- ☐ Performed
- ☐ Treatment procedure
- ☐ Reagents

Sample Reduction

- ☐ Performed
- ☐ Reduction procedure
- ☐ Reagents
- ☐ Concentration Factor

Nucleic Acid Extraction

- ☐ Extraction procedure
- ☐ Amount extracted, amount obtained
- ☐ Extract storage conditions

qPCR or dPCR

- ☐ Target gene name, amplicon length
- ☐ Thermocycling temperatures and times
- ☐ Master mix: composition, vendors, concentrations
- ☐ Additives: vendors, concentrations
- ☐ Template amount added, pre-treatment (if any)
- ☐ PrimerT: sequences, concentrations, vendorT, referenceT
- ☐ Amplicon confirmation method (probe, melt curve, etc)
- ☐ Probe sequence, concentration, vendor, reference
- ☐ Instrumentation
- ☐ Equivalent volume of sample analyzed by PCR
- ☐ Inhibition assessment procedure
- ☐ Inhibition control description (if used)
- ☐ Number samples tested and found inhibited

Reverse Transcription

- ☐ Performed
- ☐ One or two step
- ☐ cDNA storage conditions (if two step)
- ☐ Reaction temperatures and times
- ☐ Reaction reagents and concentrations
- ☐ Priming method
- ☐ Reaction volume, added template amount
- ☐ Inhibition assessment procedure
- ☐ Inhibition control description (if used)
- ☐ Number samples tested and found inhibited

Analysis – dPCR

- ☐ Threshold settings
- ☐ Technical replicates, number, well merging
- ☐ Partitions measured, number, mean, variance
- ☐ Partition volume
- ☐ Target copies per partition, mean, variance
- ☐ Program used for dPCR analysis
- ☐ Explanation of control results, example plots

Analysis – qPCR

- ☐ Method for handling failed negative controls
- ☐ Technical replicates, number, calculations
- ☐ Calibration standards: description and source
- ☐ Method of quantifying standards
- ☐ Calibration curve slope
- ☐ Calibration curve R2
- ☐ Lowest standard measured or 95% LOD
- ☐ Cq value determination method