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MEMORANDUM

Date: January 8th, 2025

To: Amy van Riessen, Watershed Restoration Manager, North Clackamas Watersheds Council

From: Andy Lara, Research Associate I, Cramer Fish Sciences
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Subject: Results of environmental DNA analysis in Kellogg Creek: 2024 to 2025

This memorandum summarizes results from extended baseline environmental DNA (eDNA) monitoring conducted in the Kellogg Creek–Mt. Scott Creek watershed during 2024–2025, building on baseline surveys completed in 2022–2023. Together, these data characterize seasonal and spatial patterns of fish presence prior to the planned removal of Kellogg Dam using a consistent before–after–control–impact (BACI) study design. Results indicate that detections of anadromous salmonids were generally infrequent and low in magnitude across seasons, while a ubiquitous resident species, Coastal Cutthroat Trout, exhibited consistently higher eDNA concentrations and broader spatial occupancy. These findings provide additional context on relative fish presence across seasons and strengthen the pre-removal dataset that will inform future post-removal evaluation and resource management decisions.

Contents

Executive Summary

Cramer Fish Sciences (CFS) and the North Clackamas Watersheds Council (NCWC) conducted extended baseline environmental DNA (eDNA) surveys in the Kellogg Creek–Mt. Scott Creek watershed during the fall, winter, spring, and summer of 2024–2025 to describe the occurrence and distribution of Coho Salmon (*Oncorhynchus kisutch*), Chinook Salmon (*O. tshawytscha*), Steelhead/Rainbow Trout (*O. mykiss*), Coastal Cutthroat Trout (*O. clarkii clarkii*), and Pacific Lamprey (*Entosphenus tridentatus*). These surveys used the same before–after–control–impact (BACI) eDNA sampling layout and effort implemented during the 2022–2023 baseline study, which was designed to test the hypothesis that removal of Kellogg Dam will increase the occurrence and distribution of anadromous fishes. A power analysis completed during the baseline study estimated that our eDNA survey effort may provide a 95% probability of detecting approximately 3,000 grams of each target species within 1,000 meters of each of the eleven sampling locations[1].

This memo presents an additional year of eDNA data from the "before" period within the impact sampling unit (Kellogg Creek–Mt. Scott Creek watershed). Results are summarized descriptively using two primary metrics: (1) standardized eDNA concentration (normalized by volume of water filtered) and (2) the spatial and seasonal frequency of positive detections. Aside from the power analysis, this memo does not include hypothesis testing, effect-size estimation, or other formal statistical inference. The full BACI statistical analysis will be completed following dam removal.

As documented previously, eDNA monitoring in the Johnson Creek control watershed (2020–2022) detected Steelhead/Rainbow Trout, Coho Salmon, and Pacific Lamprey, with historical visual observations confirming the presence of Chinook Salmon. During the 2022–2023 baseline surveys, eDNA detections indicated the presence of Coho Salmon, Chinook Salmon, and Steelhead/Rainbow Trout upstream of Kellogg Dam, while Pacific Lamprey eDNA was not detected. During the extended 2024–2025 survey, eDNA from Coho Salmon, Chinook Salmon,

[1] In an ideal BACI design, control and impact watersheds are sampled concurrently during both the before and after phases. Johnson Creek, the "control" site was sampled once during the before phase but not concurrently with all Kellogg Creek surveys. Johnson Creek will be sampled once during the after phase following dam removal at Kellogg Creek.

Steelhead/Rainbow Trout, and Coastal Cutthroat Trout was detected upstream of Kellogg Dam, whereas Pacific Lamprey eDNA was again not detected.

Across the extended baseline period, detections of anadromous salmonids were generally infrequent, spatially limited, and characterized by low eDNA concentrations. Observed eDNA quantities for anadromous salmonids, including Chinook Salmon, Coho Salmon, and Steelhead/Rainbow Trout, typically near or below the limit of quantification (LOQ) of the qPCR assays used in this study. The LOQ represents the lowest concentration at which eDNA can be reliably quantified with acceptable precision and accuracy; detections below this threshold indicate presence but yield uncertain concentration estimates. In contrast, Coastal Cutthroat Trout—a ubiquitous resident salmonid—produced consistently higher eDNA quantities that exceeded the LOQ and was detected broadly across sites and seasons. The lower relative eDNA quantities observed for anadromous species therefore likely reflect limited occupancy and lower relative abundance within the watershed during much of the sampling period, consistent with Kellogg Dam functioning as a partial barrier to anadromy.

As in the 2022–2023 baseline survey, Steelhead/Rainbow Trout eDNA was detected during each seasonal sampling event in the 2024–2025 extended baseline survey, though not at every site. However, detections were typically low in magnitude and patchy in distribution, and Steelhead/Rainbow Trout eDNA concentrations were notably lower than those observed during the baseline period. Coastal Cutthroat Trout eDNA, which was not assayed during the baseline study, was detected during all sampling events across the project area, with a somewhat reduced spatial distribution observed in April 2025, when detections were largely confined to upper Mt. Scott Creek. . This observation may be associated with spring spawning movements.

Pacific Lamprey eDNA was not detected during any sampling event in Kellogg Creek during the extended survey, nor was it detected during the baseline surveys. The contrast between repeated detections in the nearby Johnson Creek control watershed and non-detection in Kellogg Creek could reflect passage issues in Kellogg Creek.

During quality-control review, we discovered that the Steelhead/Rainbow Trout qPCR assay developed by Duda et al. (2021) was inadvertently used during the April and July 2025 sampling

events, whereas all other sampling periods were analyzed using the Brandl et al. (2015) assay. To restore methodological consistency across the dataset, all samples from the April and July 2025 events were subsequently reanalyzed using the Brandl et al. (2015) assay. Detections from both assays were retained as valid indicators of Steelhead/Rainbow Trout presence; however, only results from the Brandl et al. (2015) assay were used to report relative eDNA concentrations and generate spatial visualizations. Importantly, correlation of Steelhead/Rainbow Trout detections between the two assays was low. This discordance likely reflects the fact that observed eDNA quantities fell near or below assay quantification limits, consistent with low relative abundance of Steelhead/Rainbow Trout in Kellogg Creek during the sampling period.

In addition, a December 2024 contract modification added a new sampling site in the Mt. Talbert Natural Area upstream of site 9, which was incorporated beginning with the January 2025 sampling event. Together, the baseline and extended eDNA surveys provide a robust foundation for evaluating changes in eDNA detection patterns following removal of Kellogg Dam.

Introduction

Kellogg Dam (Figure 1) is a 16-foot-high and 22-foot-wide concrete structure located at the confluence of Kellogg Creek and the Willamette River in the Portland, Oregon metropolitan area (Figure 2). Constructed in 1858, the dam has remained inactive since 1890 and no longer serves a water-management function. Due to its poorly functioning fish ladder, Kellogg Dam may act as a partial barrier to native migratory fishes for much of the year, limiting access to spawning and rearing habitats within the Kellogg Creek–Mt. Scott Creek watershed. The North Clackamas Watersheds Council, in coordination with the City of Milwaukie, American Rivers, and the Oregon Department of Transportation, is pursuing removal of Kellogg Dam, with construction anticipated around 2028. Dam removal is expected to restore access to upstream habitats for anadromous species, including Pacific salmon (*Oncorhynchus* spp.), Steelhead/Rainbow Trout (*O. mykiss*), and Pacific Lamprey (*Entosphenus tridentatus*).

Environmental DNA (eDNA) analysis is an established tool for assessing the occurrence and distribution of Pacific salmon, Steelhead, and Pacific Lamprey in Pacific Northwest watersheds (Duda et al. 2021). eDNA surveys reduce many of the logistical constraints and costs associated with traditional visual or capture-based methods (Wilcox et al. 2016). Environmental DNA consists of genetic material shed into the environment through processes such as respiration, feeding, and reproduction, and can be collected noninvasively through water sampling (Wilcox et al. 2016). Field sampling is typically conducted using a portable pump and filtration system, with individual sampling events generally completed within 15–30 minutes, depending on site conditions and sampling design.

The data presented in this memo come from a follow-up study to baseline eDNA surveys conducted in 2022 and 2023. The baseline study employed a before–after–control–impact (BACI) experimental design to test the hypothesis that removal of Kellogg Dam will increase the occurrence and distribution of Pacific salmon, Steelhead/Rainbow Trout, and Pacific Lamprey in Kellogg Creek, Oregon. Extended baseline sampling conducted in 2024 and 2025 continues this effort and expands the “before” period of the BACI study. Because Kellogg Dam has not yet been removed, this memo presents additional findings from the pre-removal period (2022–2025) within the impact unit of the watershed (Kellogg Creek–Mt. Scott Creek) (Table 1).

Pacific salmon, Steelhead/Rainbow Trout, and Pacific Lamprey are the focal species of both the 2022-2023 baseline and 2024-2025 extended studies. However, the 2024–2025 extended study includes eDNA detections of Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*) (Table 2). In January 2025, a contractual modification was implemented to add an additional sampling location on an unnamed tributary to Mt. Scott Creek within the Mt. Talbert Natural Area, located just upstream of Site 9.

Methods

Experimental Design

BACI studies are structured to include sampling at a minimum of two sites, one designated as a "control" site and the other as an "impact" site, both assessed before and after an event. In the context of our study, the Kellogg Creek-Mt. Scott Creek watershed was chosen as the "impact" site due to the planned dam removal in the forthcoming years. As our "control" site, we opted for the ongoing eDNA monitoring of fish by the Johnson Creek Watershed Council in the neighboring Johnson Creek watershed (Figure 2). Since 2017, the Johnson Creek Watershed Council has conducted monitoring at eight sites along the mainstem of Johnson Creek, during both spring and fall. Johnson Creek shares similarities in terms of size, urbanized conditions, and proximity to the Kellogg Creek-Mt. Scott Creek watershed but is unimpacted by a dam. Yet, there are important differences between the watersheds to consider, including notable groundwater contributions from Crystal Springs, a tributary to Johnson Creek, which contrasts to the runoff-based hydrological characteristics of Kellogg Creek. Surveys completed in the extended baseline surveys in 2024 and 2025 continued following the sampling layout and effort of this BACI design.

Power Analysis

eDNA survey effort determines the probability of detecting (PoD) organisms in the environment. PoD can be increased by filtering more water, sampling more sites, and implementing more quantitative PCR (qPCR) replicates. The desired PoD must be balanced, however, with available time and resources. To maximize PoD, given the resources available for this study, we used the ARTEMIS R package (Espe et al. 2022) to model eDNA detections and predict our PoD based on various survey efforts. ARTEMIS provides a statistical workflow to simulate and model qPCR data

to generate predictions, implement power and precision analysis, and estimate PoD. Ideally, the input data for the ARTEMIS analysis would include eDNA detections from a "live car" study implemented in the specific system, in this case, Kellogg Creek, to increase the likelihood that the modeled conditions reflect the actual target site of inference. Live car studies entail the placement of cages containing predetermined quantities of target species within a river environment. However, in the absence of such data, the next best option is to use pre-existing live car data (i.e., surrogate data) from previous studies.

Since we did not have live car data available from Kellogg Creek, we used live car data from two separate studies completed by Cramer Fish Sciences (CFS): Pacific Lamprey eDNA in the upper Yakima River, WA, and Ballyhoo (*Hemiramphus brasiliensise*) eDNA in the Lower American River, CA. Additionally, we incorporated qPCR data shared by the Johnson Creek Watershed Council. Live car data from these projects were chosen due to relative similarity of the surrogate river environments, such as water depth and water quality compared to Kellogg Creek. We used the surrogate live car data to model PoD if 3.0L were filtered across two or three filters per site assuming a biomass of 3,000 grams of a target species was present. We modeled sampling distances of 1000, 1500 and 2000 meters away from the eDNA source. As a point of reference, a biomass of 3,000 grams is approximately equal to 84 age-2 mid-Columbia River steelhead smolts (Peven et al. 1994). We evaluated PoD for these three sampling distance scenarios and chose a sampling effort that aligned with the project's scope and budget while achieving the highest PoD. Our analysis indicated that the PoD exceeded 95% when we collected two filters positioned 1000 meters from the DNA source. Consequently, we adopted the following sampling approach: filtering 2-3 liters of water through two filters per sampling site, with each site spaced 1000 meters apart along the length of Kellogg Creek. The specific sampling locations are displayed in Figure 2.

eDNA Field Sampling

Field sampling followed procedures described in (Bergman et al. 2016), modified to use Millipore Sterivex™ PVDF 0.45µm sterile filter unit (Millipore Sigma). Filtration occurred using approximately 3 meters of surgical tubing. At each site, water from Kellogg Creek was filtered at

an approximate depth of 8–10 cm below the surface using sterile Masterflex peroxide cured silicon tubing - L/S 15 (Cole Parmer), and a portable Masterflex L/S Easy-Load II peristaltic pump (Cole-Parmer) powered by a cordless hand drill. Water samples were filtered through a single Millipore Sterivex™-GP 0.45µm sterile filter unit until 1000 ml of water was filtered or until filters became too congested to filter water. The total volume of water sampled was measured using a graduated flask. To ensure that field equipment was free of contamination, DNA field control samples were taken for each sampling day. Each field control consisted of Sterivex™ filtered ultra-pure water and processed in the same fashion as the field samples. Samples were immediately sealed and placed on ice. Samples were transferred to a freezer (-17.78°C) within 24 hours. Samples were mailed overnight on ice to the CFS Molecular Biology (Genidaqs) Laboratory in West Sacramento, CA for laboratory processing.

Timing of eDNA Sampling

To capture seasonal variation in the occurrence and distribution of eDNA, we sampled every three months across 2024 and 2025, including during October 2024, January 2025, April 2025, July 2025. These four seasonal replicates span both the spawning and rearing periods of Pacific Salmon, Steelhead Trout/Rainbow Trout, Coastal Cutthroat Trout, and Pacific Lamprey. Pacific Salmon are fall spawners whereas Steelhead, Coastal Cutthroat, and Pacific Lamprey are spring spawners. Yet, juveniles of all species and adult Steelhead, Coastal Cutthroat, and Pacific Lamprey may be present year-round.

Laboratory Analysis

We used fluorescence-based quantitative real-time Polymerase Chain Reaction (qPCR) and species-specific qPCR assays to detect the five focal species: Pacific Lamprey (Carim et al. 2017), Coho Salmon (Duda et al. 2021), Chinook Salmon (Laramie et al. 2015), and Rainbow Trout/Steelhead (Brandl et al. 2015; Duda et al. 2021[2]), Coastal Cutthroat Trout (Duda et al.

[2] The qPCR assay described in Brandl et al. (2015) was the primary assay used to detect Rainbow Trout/Steelhead throughout the study. A laboratory error resulted in the Duda et al. (2021) assay being applied during the April and July 2025 sampling events. All samples from those events were subsequently reanalyzed using the Brandl assay to maintain consistency across sampling periods. Detections from both assays were considered valid indicators of Rainbow Trout/Steelhead presence; however, only results from the Brandl assay were used for reporting relative eDNA concentrations and spatial visualizations.

2021). As determined by the ARTEMIS analysis, we implemented three technical qPCR replicates (i.e., repeated measures) for each species per filter for a total of six qPCR replicates per species per site. A qPCR replicate comprises a 10 µl reaction volume composed of 2x TaqMan™ Environmental Master Mix 2.0 (Thermo Fisher Applied Biosystems®), with optimal initial primer concentrations (90 uM) and probe-specific initial concentrations (12.5uM). Thermocycling is performed using a QuantStudio 3 (Thermo Fisher Applied Biosystems®) with a standardized profile. Quantification results, presented as "quantification cycle (Cq)," indicate the PCR cycle at which a fluorescent signal threshold is met. High DNA concentrations, resulting in lower Cq values, are indicative of stronger detections with a sample considered positive for the target species if any technical replicate Cq is <40. The Cq values are then used in an assay-specific "standard curve" equation to estimate the concentration (ng/uL) of eDNA in each reaction. To account for variation in the volume of water filtered, we standardized each estimated eDNA concentration by dividing it by the volume of water filtered.

O. mykiss Assay Consistency and Reanalysis

Throughout the Kellogg Creek study, detections of Rainbow Trout/Steelhead were intended to be generated using the species-specific qPCR assay developed by Brandl et al. (2015), which targets a 59-base-pair fragment of the mitochondrial cytochrome oxidase I (COI) gene. During internal data review of the 2025 dataset, it was discovered that samples collected during the April and July 2025 sampling events were initially analyzed using a newer assay developed by Duda et al. (2021), which targets an 86-base-pair fragment of the mitochondrial cytochrome-b (CytB) gene. Because these assays target different mitochondrial regions and fragment lengths, detection results are not directly interchangeable and may yield differences in detection frequency and magnitude. To maintain methodological consistency across all sampling periods and ensure comparability with previous years of the study, all April and July 2025 samples were reanalyzed using the Brandl et al. (2015) assay prior to final data synthesis and reporting. The initial detections generated using the Duda et al. (2021) assay remain valid evidence of Rainbow Trout/Steelhead presence; however, results from the Brandl et al. (2015) assay were used for all concentration estimates, spatial analyses, and figures presented in this report.

Results and Discussion

We discuss results in terms of what species were detected, how many filters tested positive at each site–season–year combination, and the eDNA concentrations normalized by volume of water filtered. Like the 2022–2023 baseline study, eDNA surveys conducted during the extended study between October 2024 and July 2025 detected Coho Salmon, Chinook Salmon, and Steelhead/Rainbow Trout upstream of Kellogg Dam, while Pacific Lamprey eDNA was not detected during any sampling event. We did not assay Coastal Cutthroat Trout eDNA during the baseline study; however, in the extended study, Coastal Cutthroat Trout was the most widely detected fish species, with eDNA detected at all sites except site 6 (Figure 3). Mean eDNA concentrations for Coastal Cutthroat Trout were approximately two orders of magnitude higher (mean = 4.59×10^{-9} ng/µL; 95% CI: 3.41×10^{-9}–5.77×10^{-9}) than the combined mean for all other species detected during the extended study (mean = 5.59×10^{-11} ng/µL; 95% CI: 4.31×10^{-11}–6.86×10^{-11}) (Figure 4), likely reflecting higher relative abundance of this ubiquitous resident species compared to anadromous salmonids.

Detections of anadromous salmonids were generally infrequent, spatially limited, and characterized by low standardized eDNA concentrations (Figure 4). Observed eDNA quantities for Chinook Salmon, Coho Salmon, and Steelhead/Rainbow Trout were near or below the limit of quantification (LOQ) of the qPCR assays used in this study, indicating presence but uncertain concentration estimates. The substantially lower relative eDNA quantities observed for anadromous species therefore likely reflect limited occupancy and lower relative abundance within the watershed during much of the sampling period, consistent with Kellogg Dam functioning as a partial barrier to anadromy.

Heat maps illustrating standardized eDNA concentration (as an index of relative magnitude) for each species are presented in Figures 5 through 27. The extended study provides additional, albeit indirect, evidence for the presence of anadromous Coho Salmon and Chinook Salmon upstream of the dam.

Chinook Salmon

During October 2022, Chinook Salmon eDNA was detected at sites 1, 2, 5, 6, and 10, whereas during October 2024 detections occurred at sites 1, 5, 6, 8, 9, and 11, suggesting a wider spatial distribution during the 2024 spawning period than the 2022 spawning period. Despite this broader spatial extent, standardized eDNA concentrations remained well below assay quantification limits. In January 2023, Chinook Salmon eDNA was detected at two sites (sites 1 and 7), whereas during the January 2025 sampling event, positive detections were observed at sites 3 through 10, as well as at the additional sampling location (ST) on an unnamed tributary to Mt. Scott Creek within the Mt. Talbert Natural Area. No Chinook Salmon eDNA was detected during April 2023; however, in April 2025, detections were observed at sites 9 and 10, the upper portion of the Mt. Scott Creek watershed. During July 2023, Chinook Salmon eDNA was detected at two downstream sites (sites 2 and 3), whereas no Chinook Salmon eDNA was detected during the July 2025 sampling event. Overall, detections outside the fall spawning period were sparse and low in magnitude (Heatmaps: Figures 5-10, Concentrations: Figure 28)

Coho Salmon

Coho Salmon eDNA was detected across a wide spatial distribution during October 2022, when the Willamette River overtopped Kellogg Dam, with detections observed at sites 1, 2, 3, 5, 6, 7, 8, 9, and 11. During an *ad hoc* November 2023 sampling event, when backwatering did not occur, Coho Salmon eDNA was detected at fewer sites (sites 1–3 and 5–7) and at lower relative concentrations, indicating reduced magnitude and spatial extent compared to October 2022. In contrast, during October 2024, Coho Salmon eDNA was detected at only a single site (site 7), representing an apparent reduction in both spatial distribution and relative concentration during the spawning period. During January 2023, Coho Salmon eDNA was detected at two sites (sites 3 and 7), and no detections were observed during April or July 2023. A similar pattern was observed during the 2024–2025 sampling period, with a single detection at site 1 in January 2025 followed by complete absence of detections during April and July 2025. Across sampling events, Coho Salmon detections were infrequent, spatially restricted, and consistently low in magnitude relative to resident species. Across all sampling events, Coho Salmon eDNA was not detected in upper Kellogg Creek. (Heatmaps: Figures 11-15, Concentrations: Figure 29)

Steelhead/RainbowTrout

Steelhead/Rainbow Trout detections are reported using results from both the Brandl et al. (2015) and Duda et al. (2021) qPCR assays; however, spatial heatmaps and relative eDNA concentration summaries are based solely on the Brandl assay to maintain methodological consistency across all sampling periods. Across both the baseline and extended baseline studies, Steelhead/Rainbow Trout eDNA was detected during all seasonal sampling events, albeit not at each site, throughout the Kellogg-Mt. Scott Creek watershed, including upper Kellogg creek, upstream of the confluence with Mt. Scott Creek. During the 2022–2023 baseline study, detections were broadly distributed across seasons, with the widest spatial extent observed during winter sampling. In October 2022, Steelhead/Rainbow Trout eDNA was detected at multiple sites, whereas during October 2024 detections were limited to sites 8 and 10, indicating a reduced spatial extent relative to baseline conditions. In January 2023, the widest distribution of detections was observed, with eDNA detected at all sites except site 10. In contrast, during January 2025 detections were observed at fewer locations (sites 1, 2, 6, and 8). Steelhead/Rainbow Trout eDNA was detected at sites 2, 7, and 8 in April 2023, while in April 2025 detections occurred at sites 3 and 7 through 10, including upstream portions of the watershed. During summer sampling, detections were observed at sites 2, 4, and 7 through 9 in July 2023, and at sites 2, 3, 6, 7, 8, 10, and 11 in July 2025, indicating broad spatial occupancy but consistently low eDNA concentrations during the extended study . (Heatmaps: Figures 16-23, Concentrations: Figure 30)

Concordance between the Brandl et al. (2015) and Duda et al. (2023) assays was low during the extended baseline period. In April 2025, Steelhead/Rainbow Trout eDNA was detected at eight sites across the two assays, yet only a single site (site 8) yielded a positive detection using both assays. Similarly, in July 2025, detections occurred at seven sites across the two assays, with only one site (site 10) shared between assays (Figure 31).

All Steelhead/Rainbow Trout eDNA detections during these sampling events fell well below the LOQ for both assays. Consequently, the low agreement between assays likely reflects stochastic variation in qPCR amplification when target DNA copy numbers are very low. This pattern is consistent with low relative abundance of Steelhead/Rainbow Trout within the watershed during

the extended baseline period, particularly when contrasted with the strong and consistent eDNA signal observed for the ubiquitous resident Coastal Cutthroat Trout.

Pacific Lamprey

We did not detect Pacific Lamprey eDNA during any sampling events during the baseline study. Similarly, no Pacific Lamprey eDNA was detected during the 2024-2025 extended period. Pacific Lamprey have been detected in the control site at Johnson Creek during 2020-2022 (Figure 32). It is possible that the fish ladder on Kellogg Creek is a barrier to Pacific Lamprey, or at least during the time periods that we sampled. Given the high probability of detection based on our sampling effort (see eDNA Sampling Effort section above), it is unlikely that we failed to detect Pacific Lamprey present in the study area.

Coastal Cutthroat Trout

Coastal Cutthroat Trout eDNA was detected during all seasonal sampling events conducted between October 2024 and July 2025, with detections observed across much of the Kellogg Creek–Mt. Scott Creek watershed. In October 2024, Coastal Cutthroat Trout eDNA was detected at multiple sites distributed throughout the watershed, including both lower and upper reaches, indicating widespread occupancy during fall sampling. A similar spatial pattern was observed in January 2025, with detections occurring across several sites and extending into upstream portions of the watershed. During April 2025, detections of Coastal Cutthroat Trout eDNA were more spatially restricted, with detections primarily observed in the upper portion of the Mt. Scott Creek watershed. This contraction in spatial distribution during spring may reflect seasonal movement associated with spawning behavior, as Coastal Cutthroat Trout typically spawn in late winter to spring and may move upstream to access suitable spawning habitats (Trotter 1989). In contrast, during July 2025, Coastal Cutthroat Trout eDNA was again detected across a broader range of sites, including both lower and upper portions of the watershed, with exceptionally high concentration observed at Site 11, highlighting abundant and consistent eDNA quantities associated with this resident species relative to anadromous salmonids . (Heatmaps: Figures 24-27, Concentrations: Figure 33)

Conclusion

In conclusion, eDNA surveys conducted between October 2024 and July 2025 provide an extended “before” dataset that builds directly on the 2022–2023 baseline study and strengthens the foundation for a BACI analysis following removal of Kellogg Dam. Positive eDNA detections of anadromous salmonids indicate that Kellogg Dam does not fully block upstream passage, although detections were generally low in magnitude and limited in frequency outside the fall spawning period. Presence of Pacific Lamprey in Johnson Creek, paired with the continued absence of Pacific Lamprey eDNA in Kellogg Creek, suggests this species may be affected. Coastal Cutthroat Trout eDNA was also detected during all seasonal sampling events, with shifts in spatial distribution that are consistent with seasonal movements for spawning and summertime thermal refugia. Pacific Lamprey eDNA was not detected during any 2024–2025 sampling event in Kellogg Creek, despite repeated detections in the Johnson Creek control watershed. While eDNA from Oregon Floater was detected at one site and Western Pearl Shell at several sites during the baseline study, mussel species were not evaluated as part of the 2024–2025 continuation study. Taken together, the low frequency of detections, consistently low eDNA concentrations, and values falling well below assay LOQ indicate that anadromous salmonids likely occurred sporadically and/or at low relative densities within the Kellogg Creek–Mt. Scott Creek watershed during much of the extended baseline period. This inference is supported by the strong and persistent eDNA signal observed for the ubiquitous resident Coastal Cutthroat Trout, which contrasts sharply with the weak and sporadic signal from anadromous species. Collectively, results from the 2024–2025 surveys complement and extend the baseline findings by increasing temporal coverage of the “before” condition and improving resolution of seasonal and spatial patterns in fish presence. These data underscore the importance of continued post-removal monitoring to evaluate changes in species occurrence and distribution following dam removal and will serve as a critical reference point for assessing ecological responses within the Kellogg Creek watershed. These patterns provide important context for evaluating biological sensitivity during future in-water activities and will serve as a critical reference point for assessing ecological responses following dam removal.

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Tables

Table 1. GPS coordinates of all sampling locations during 2024 and 2025.

Site Number	Latitude	Longitude
1	45.4381263	-122.6381885
2	45.4307807	-122.6261195
3	45.4256619	-122.6177763
4	45.420612	-122.600116
5	45.4279694	-122.6079457
6	45.4317896	-122.5960284
7	45.4277464	-122.5797825
8	45.4284751	-122.5636664
9	45.4301977	-122.5456561
ST	45.4298628	-122.544419
10	45.4356007	-122.5386905
11	45.4426573	-122.531469

Table 2. List of all species tested between 2022 and 2025.

Species	Species Common Name	Species Scientific Name
RBT	Steelhead/Rainbow Trout	*Oncorhynchus mykiss*
CHN	Chinook Salmon	*Oncorhynchus tshawytscha*
CCT	Coastal Cutthroat Trout	*Oncorhynchus clarkii clarkii*
COHO	Coho Salmon	*Oncorhynchus kisutch*
WPS	Western Pearl shell	*Margaritifera falcata*
OF	Oregon Floater	*Anodonta oregonensis*
PL	Pacific Lamprey	*Entosphenus tridentatus*
PT	Painted Turtle	*Chrysemys picta*
WPT	Western Pond Turtle	*Actinemys marmorata*
WF	Winged Floater	*Anodonta nuttalliana*
WRM	Western ridged mussel	*Gonidea angulata*

Figures

Figure 1. Photographs of the Kellogg Dam fish ladder (left) and view of the complete Kellogg Dam structure just beneath McLoughlin Boulevard in Milwaukie, Oregon.

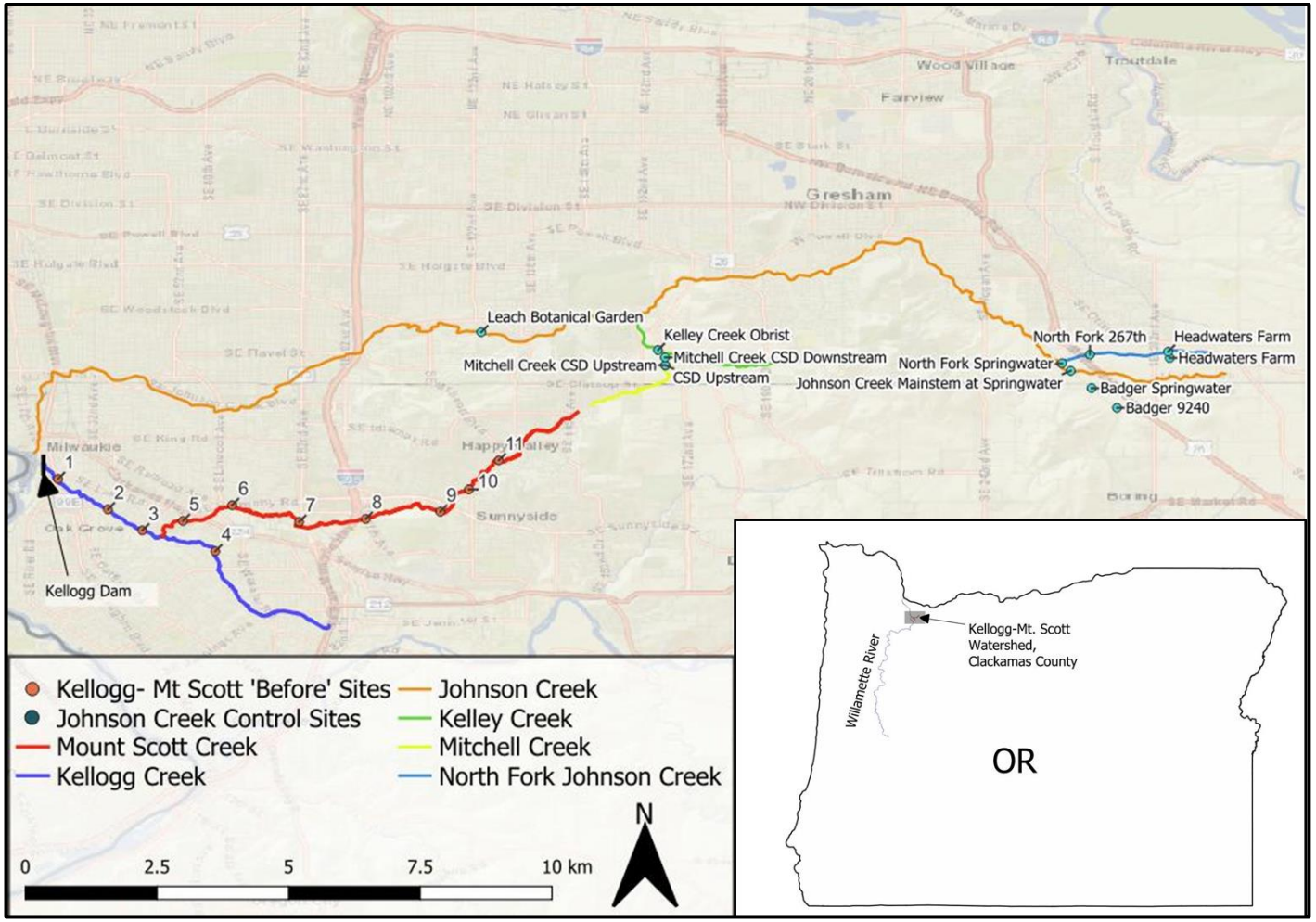

Figure 2. Map of Kellogg Creek-Mt. Scott Creek (i.e., "impact" sampling unit in BACI analysis) and Johnson Creek (i.e., "control" unit) watersheds located in Clackamas County, Oregon. eDNA sampling sites in the Johnson Creek watershed (Johnson Creek, Kelley Creek, Mitchell Creek, and North Fork Johnson Creek) were sampled between 2020 and 2022 by the Johnson Creek Watershed Council as part of their eDNA monitoring program. Kellogg Creek and Mt. Scott Creek were sampled October 2022- July 2023, and October 2024- July 2025. An ad hoc eDNA sampling effort was also implemented in November 2023 targeting Coho Salmon and native mussels.

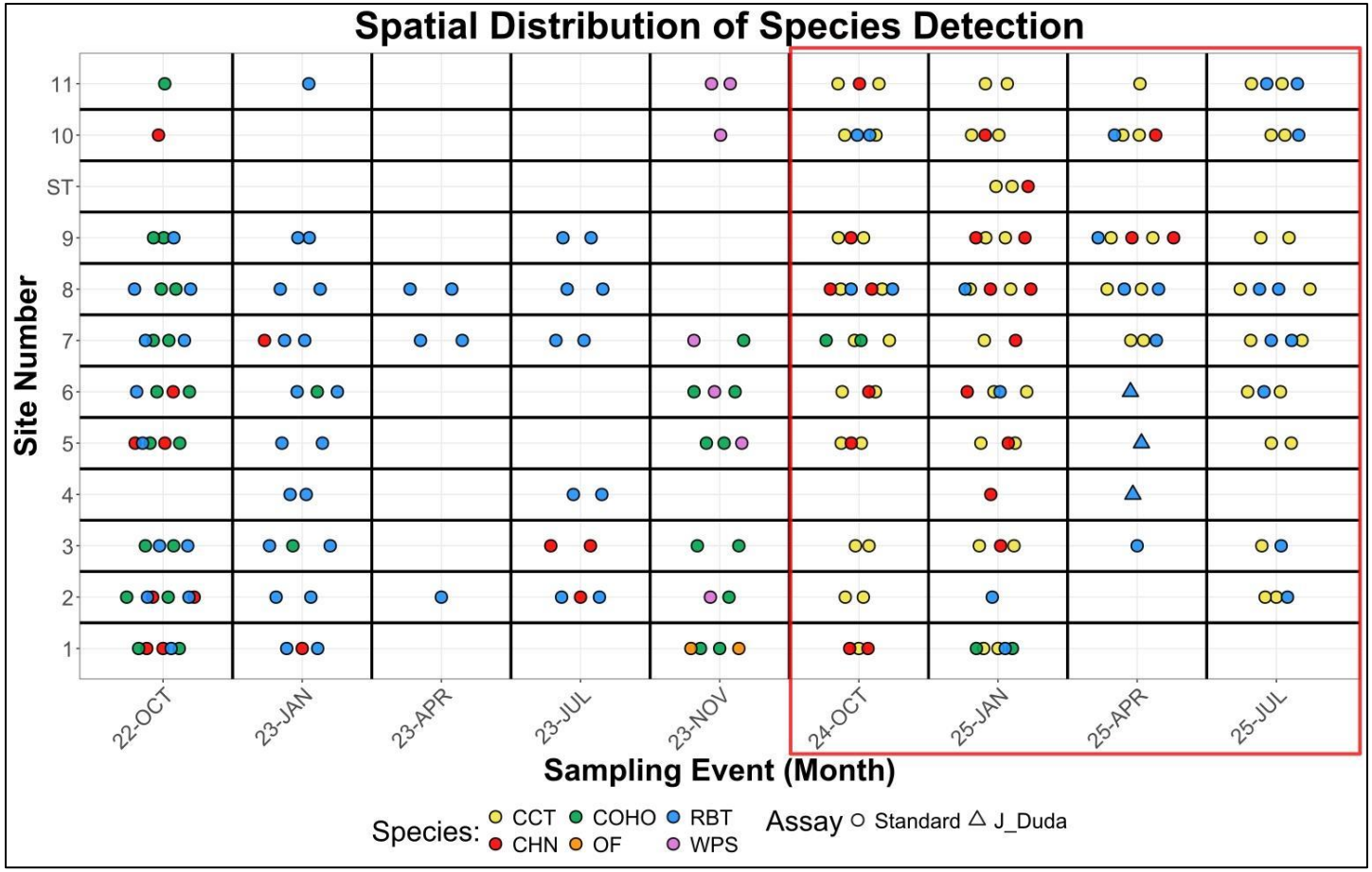

Figure 3. Summary of eDNA detections in the Kellogg Creek-Mt. Scott Creek watershed, identified via qPCR analysis. In the extended study shown within the red box, four out of five of the focal species (Coho Salmon, Chinook Salmon, Coastal Cutthroat Trout, and Steelhead/Rainbow Trout) were detected across eleven sites (12 in January) from October 2024-July 2025. Coho Salmon and Coastal Cutthroat Trout were detected in the additional sampling location (ST) during the January event. Three technical qPCR replicates (i.e., repeated measures) were run for each species per filter. Each point represents amplification of DNA within a Sterivex filter; two filters were collected at each site during each sampling event. For Steelhead/Rainbow Trout, detections made using Duda et al. 2021 remain as valid detection for eDNA presence and appear here as blue triangles.

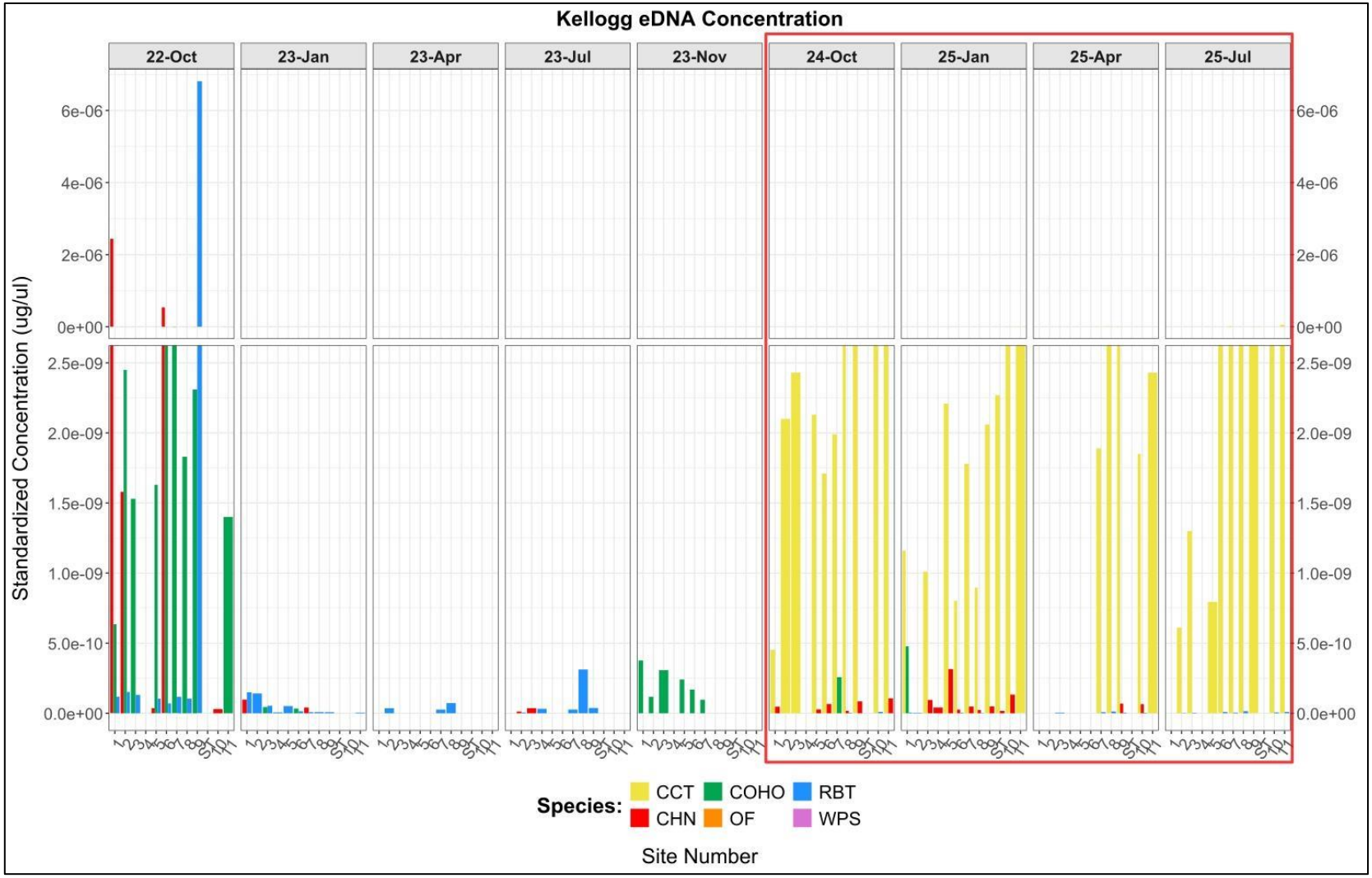

Figure 4. Bar graph of mean eDNA concentration for all samples in the Kellogg Creek-Mt. Scott Creek watershed. Results from the extended study are outlined by the red box. eDNA concentrations were standardized by the volume (µl) of water sampled. Axis breaks at 2.5E-09, 5E-09. Detections of Steelhead/Rainbow Trout using Duda et al. 2021 were omitted from this graph.

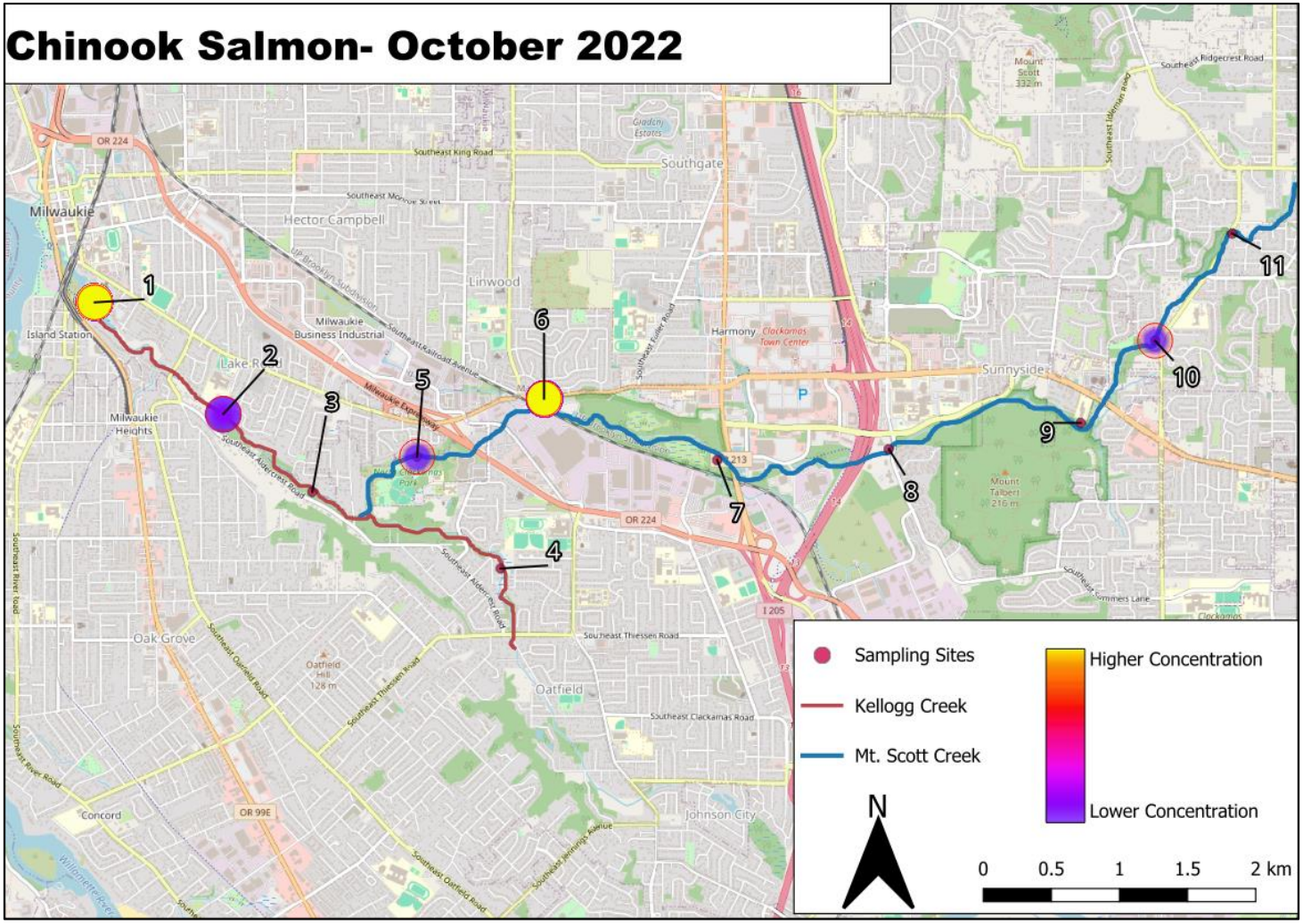

Figure 5. Heatmap depicting the relative site mean concentration of eDNA for Chinook Salmon in October 2022. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

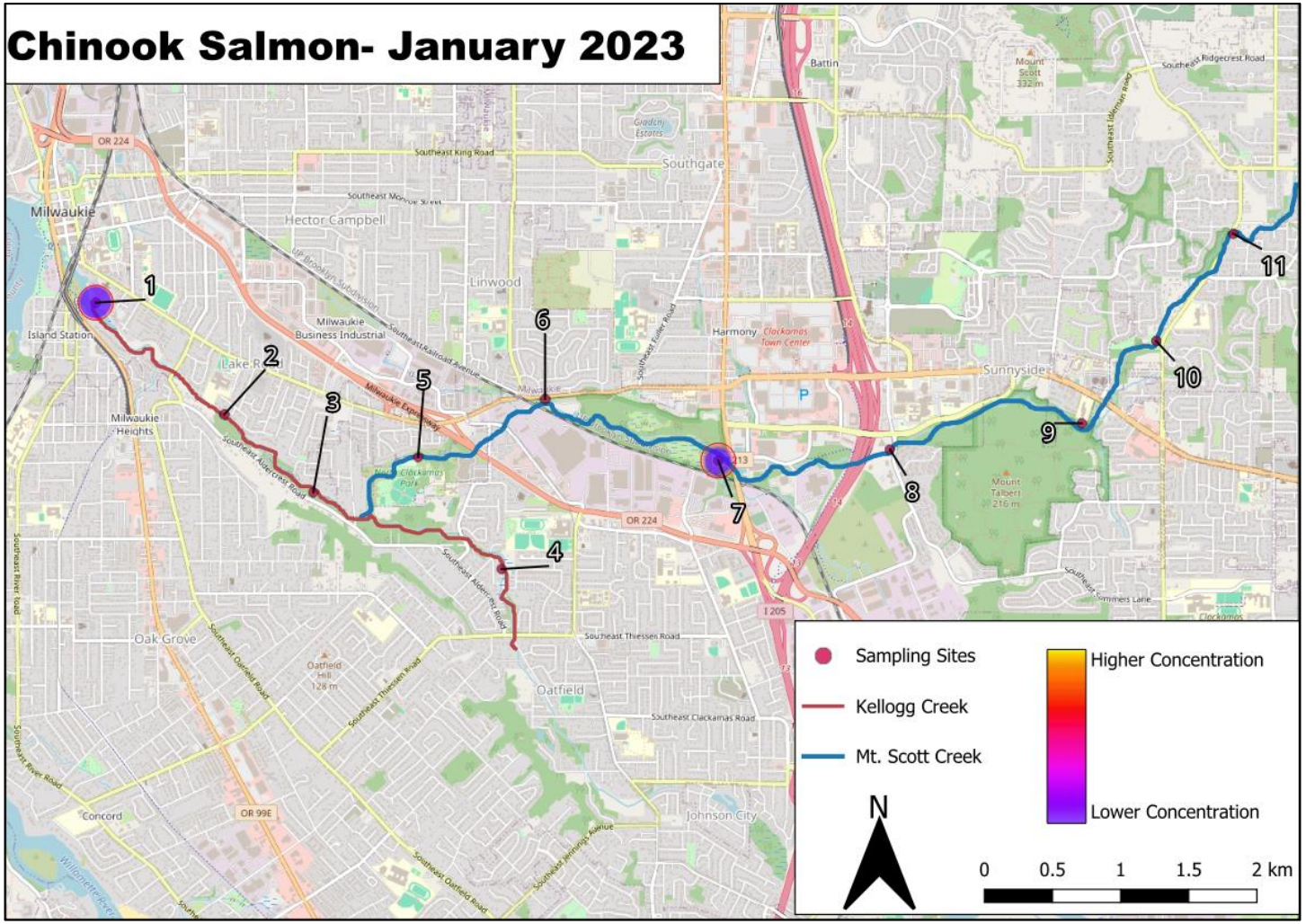

Figure 6. Heatmap depicting the relative site mean concentration of eDNA for Chinook Salmon in January 2023. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

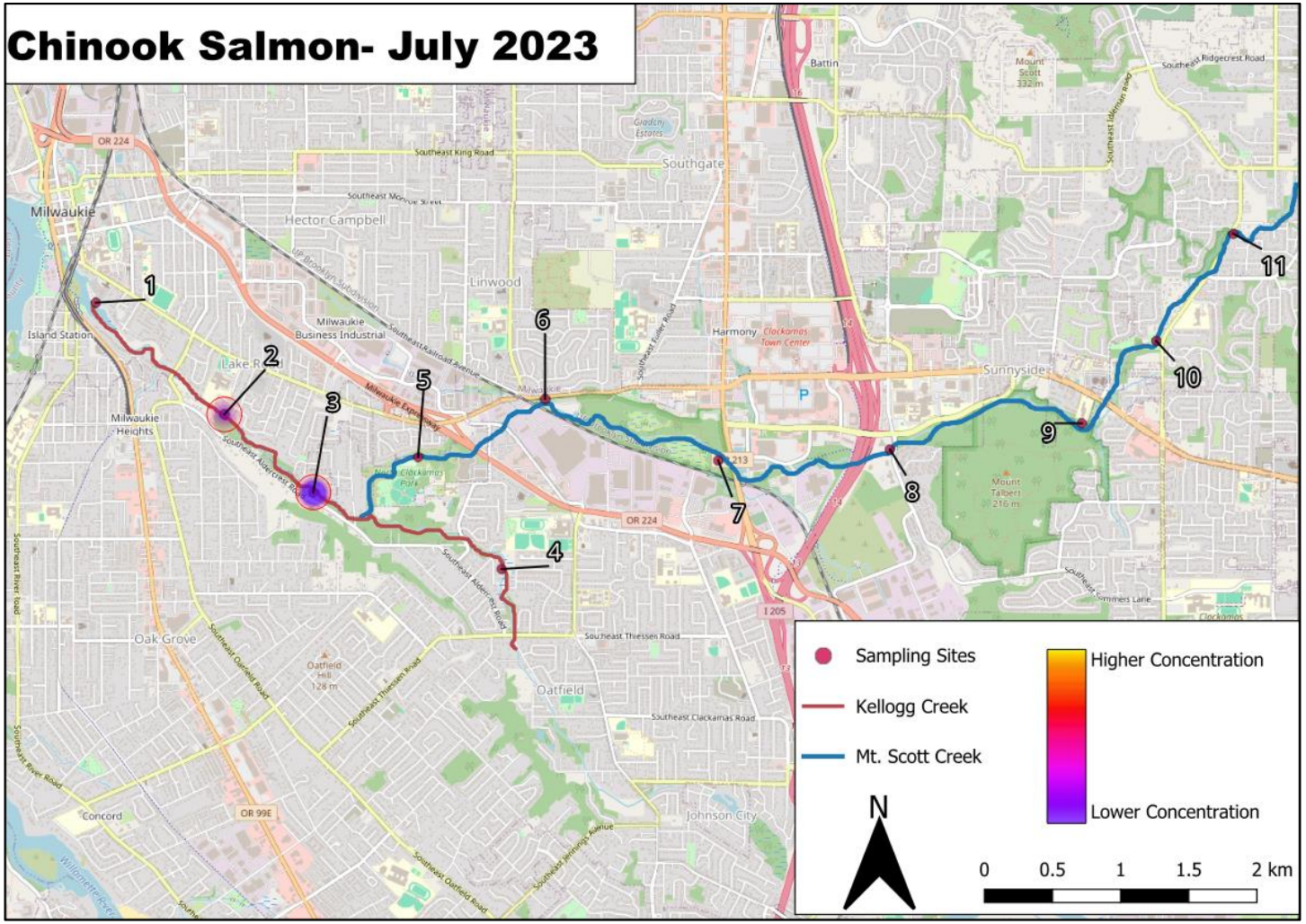

Figure 7. Heatmap depicting the relative site mean concentration of eDNA for Chinook Salmon in July 2023. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

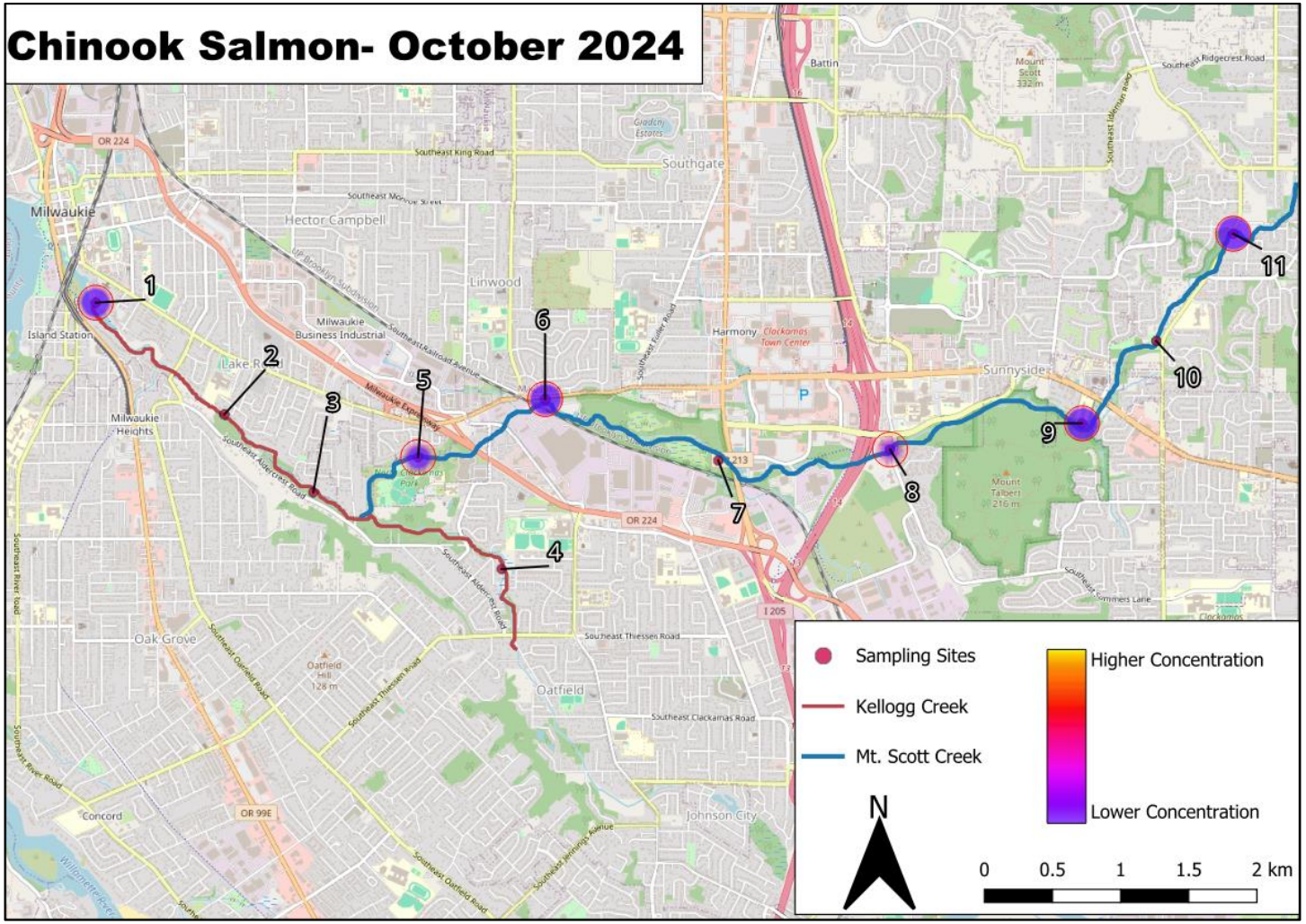

Figure 8**.** Heatmap depicting the relative site mean concentration of eDNA for Chinook Salmon in October 2024. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

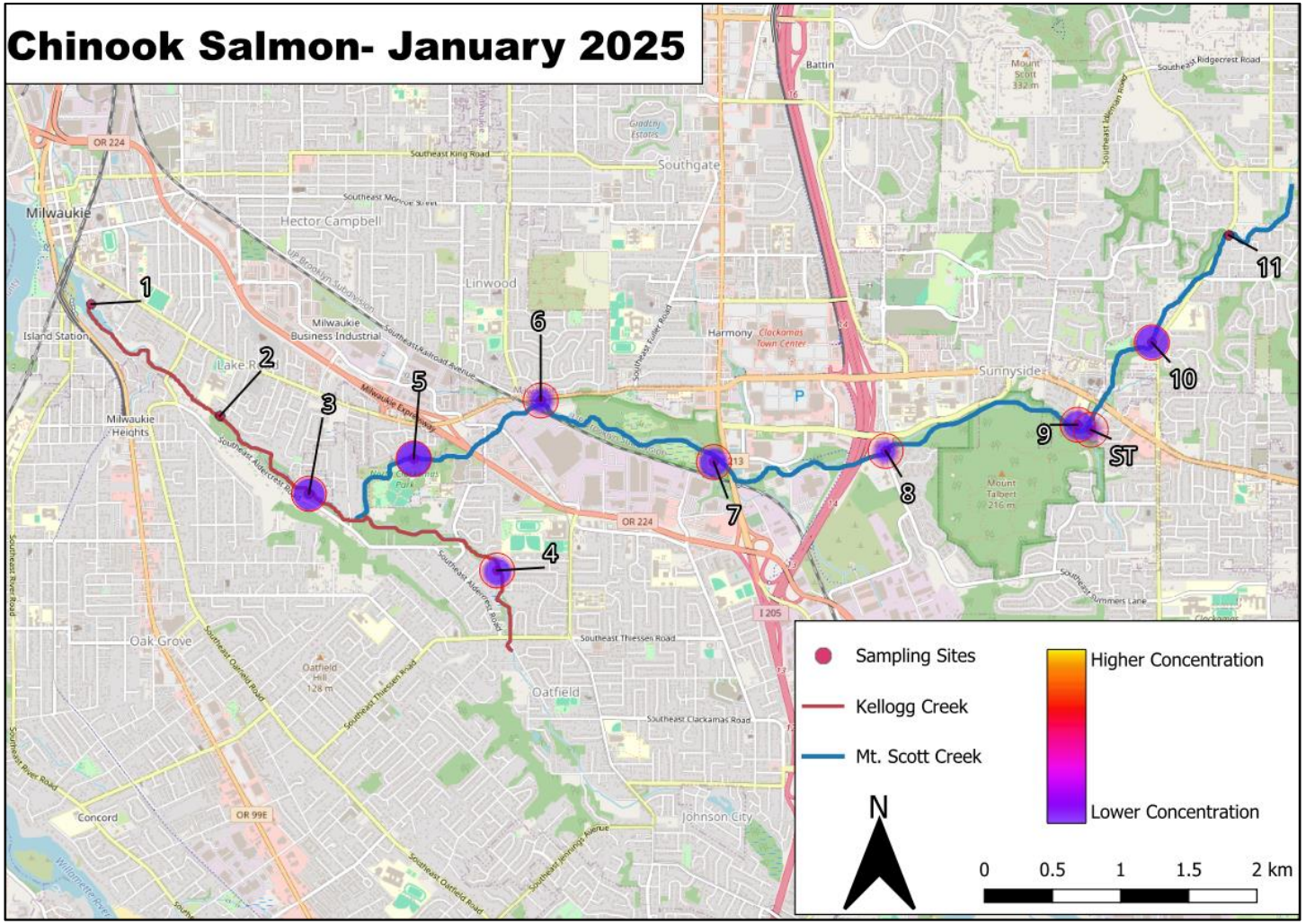

Figure 9. Heatmap depicting the relative site mean concentration of eDNA for Chinook Salmon in January 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

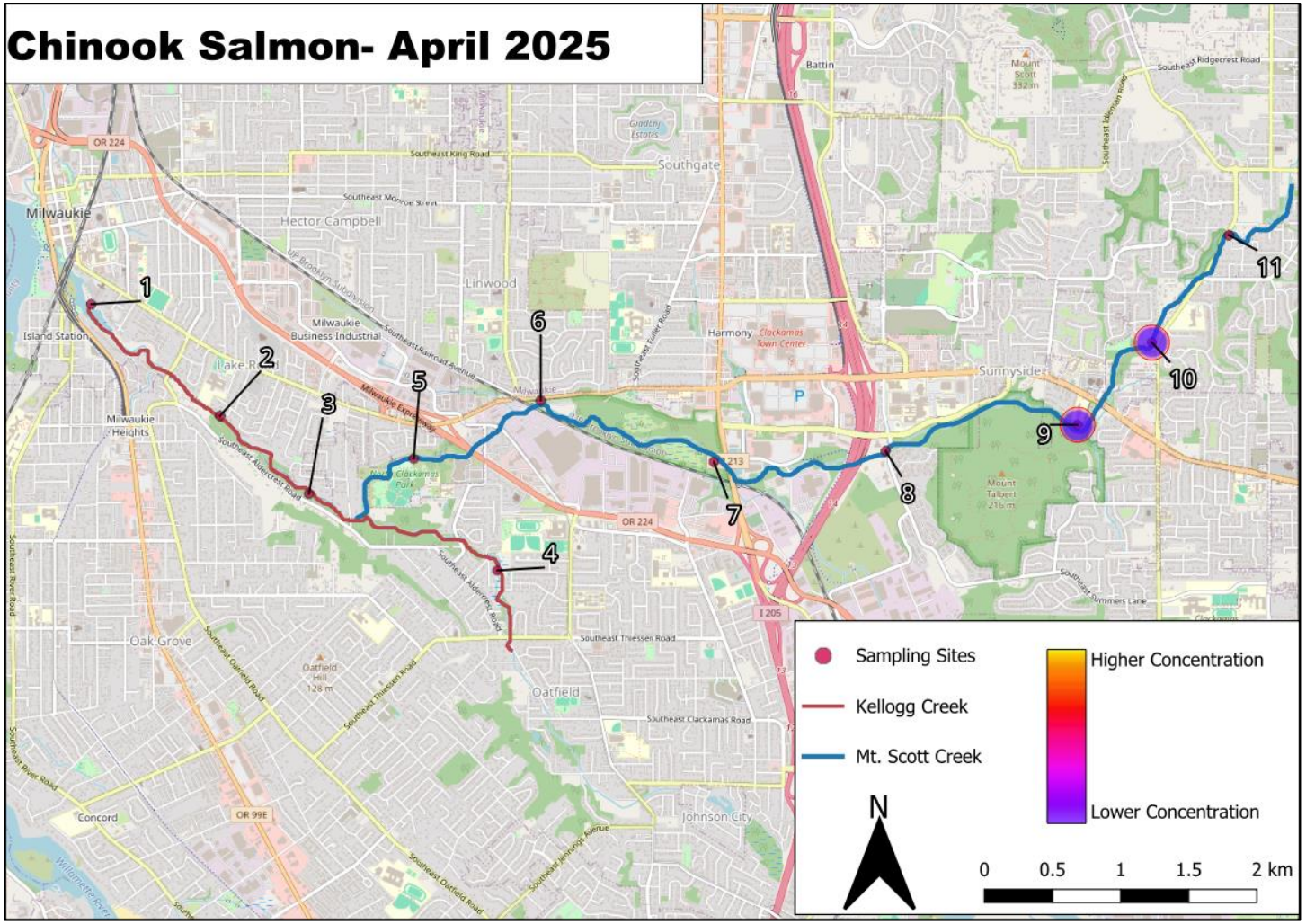

Figure 10. Heatmap depicting the relative site mean concentration of eDNA for Chinook Salmon in April 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

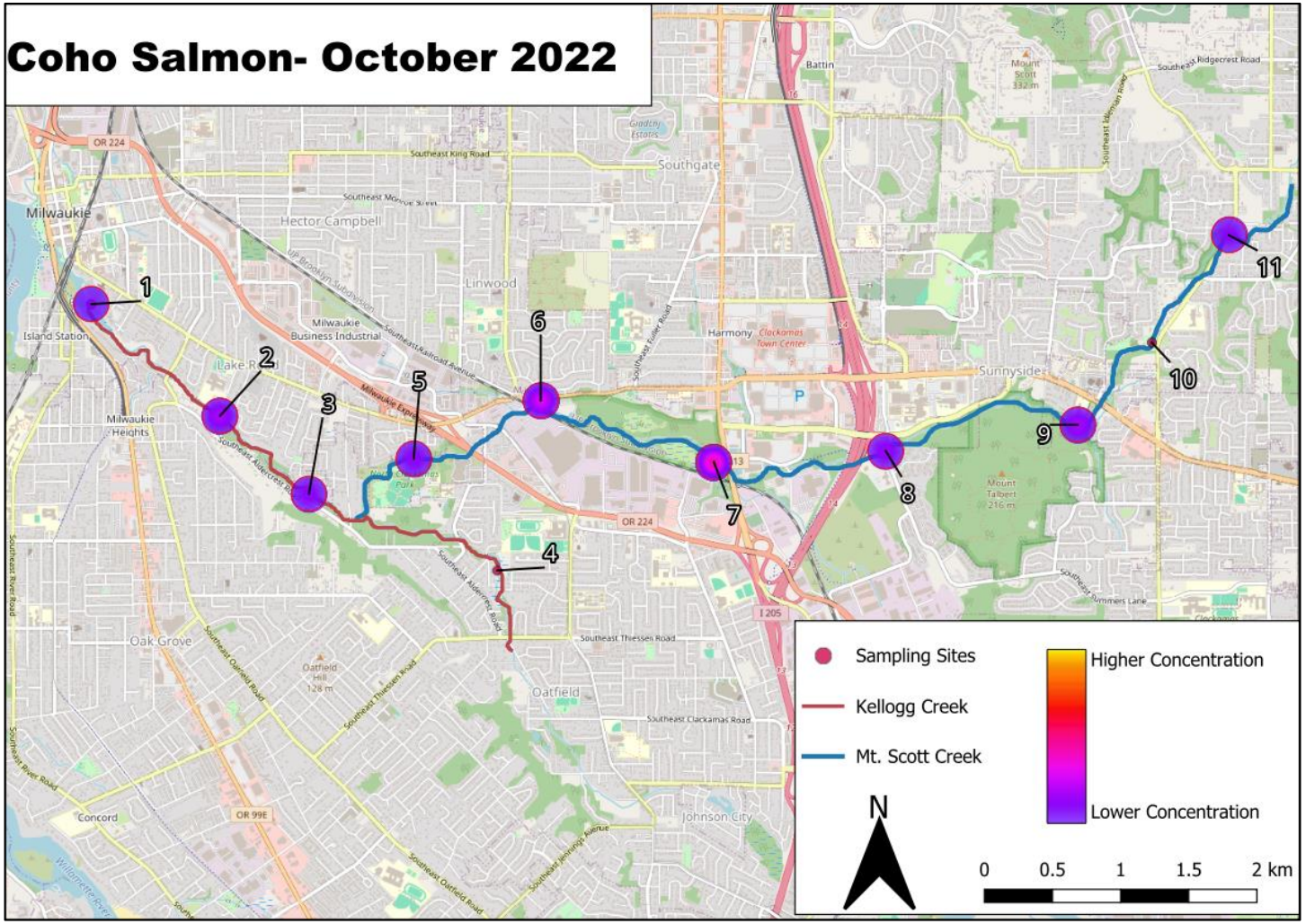

Figure 11. Heatmap depicting the relative site mean concentration of eDNA for Coho Salmon in October 2022. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

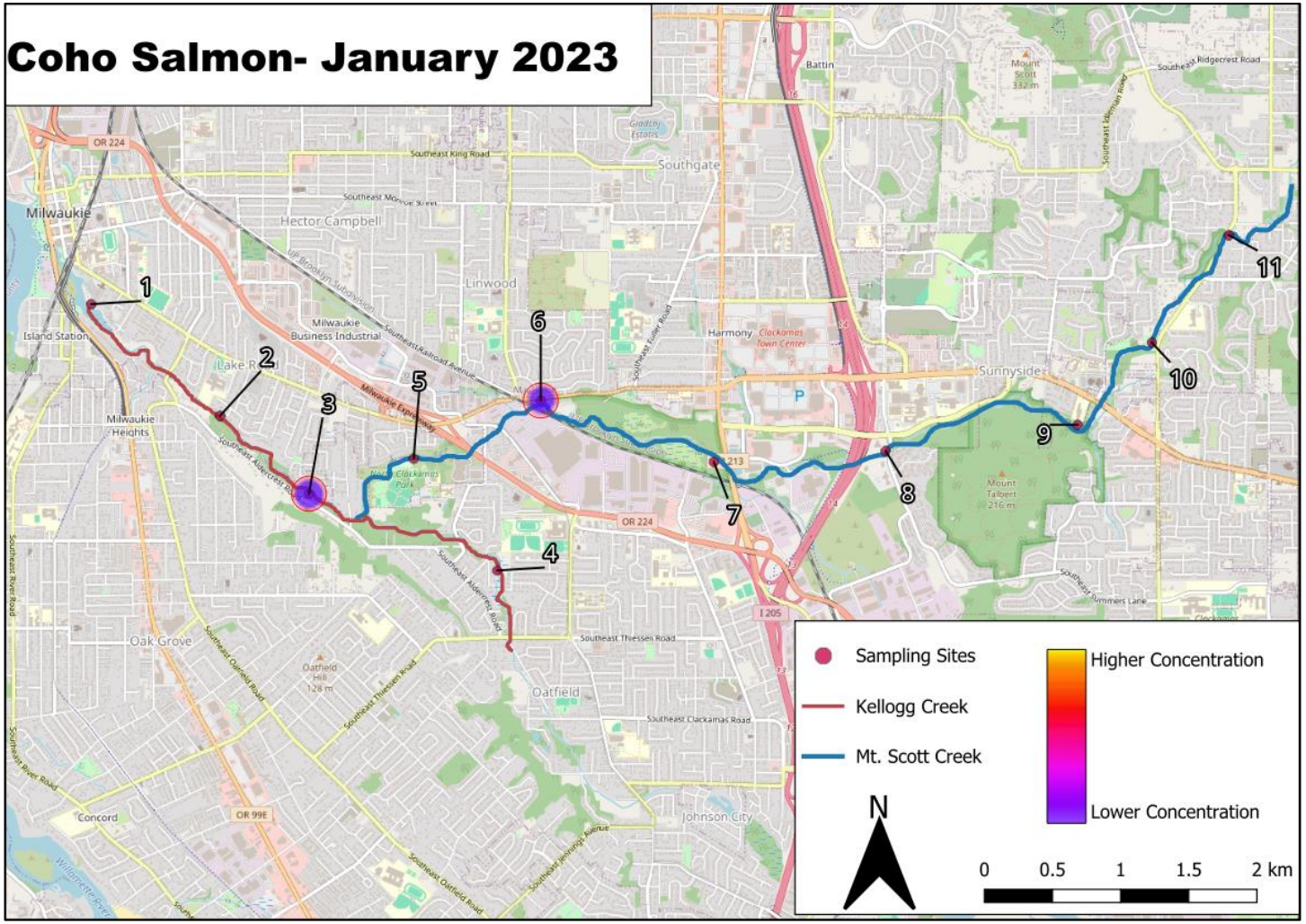

Figure 12. Heatmap depicting the relative site mean concentration of eDNA for Coho Salmon in January 2023. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

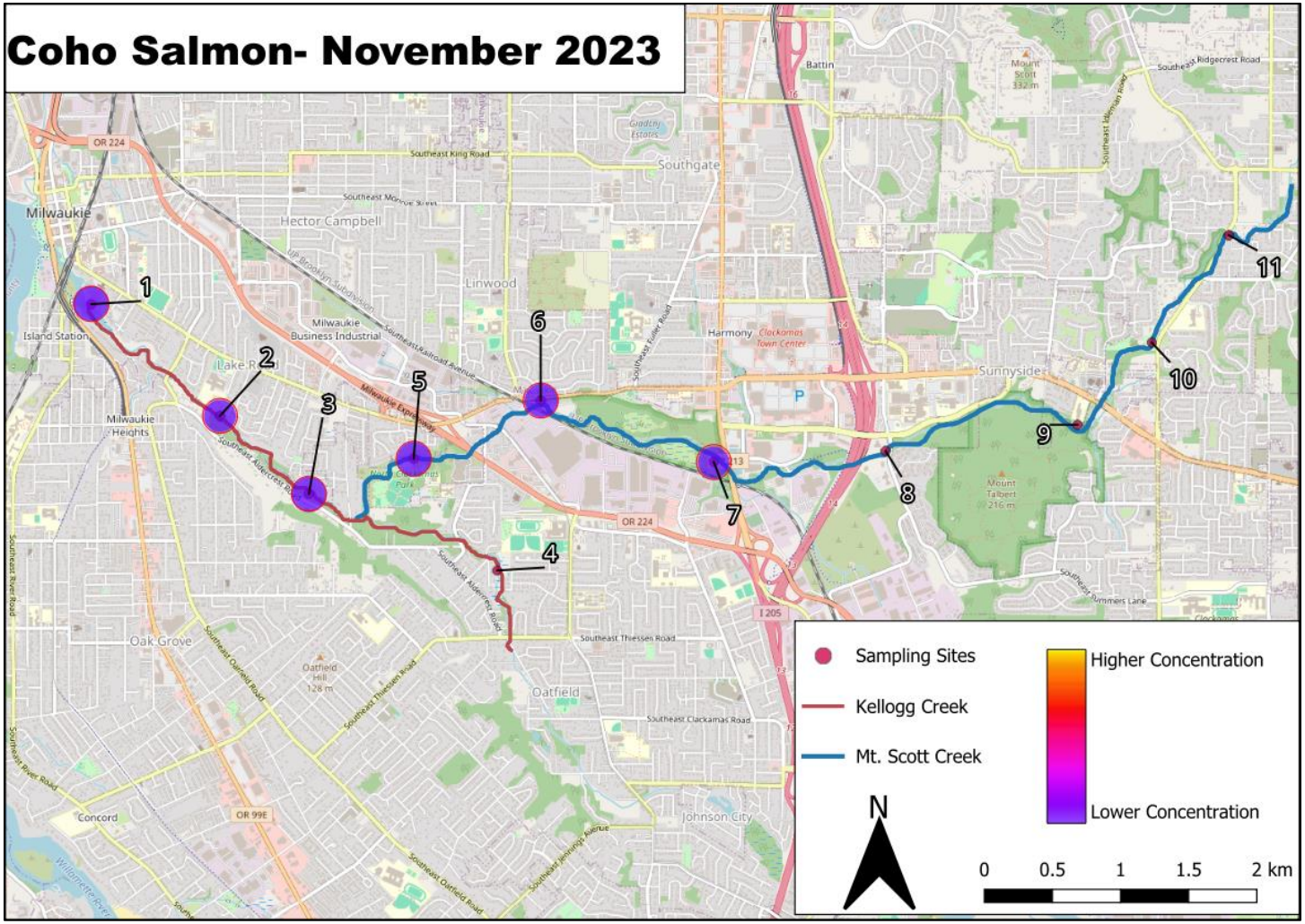

Figure 13. Heatmap depicting the relative site mean concentration of eDNA for Coho Salmon in November 2023. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

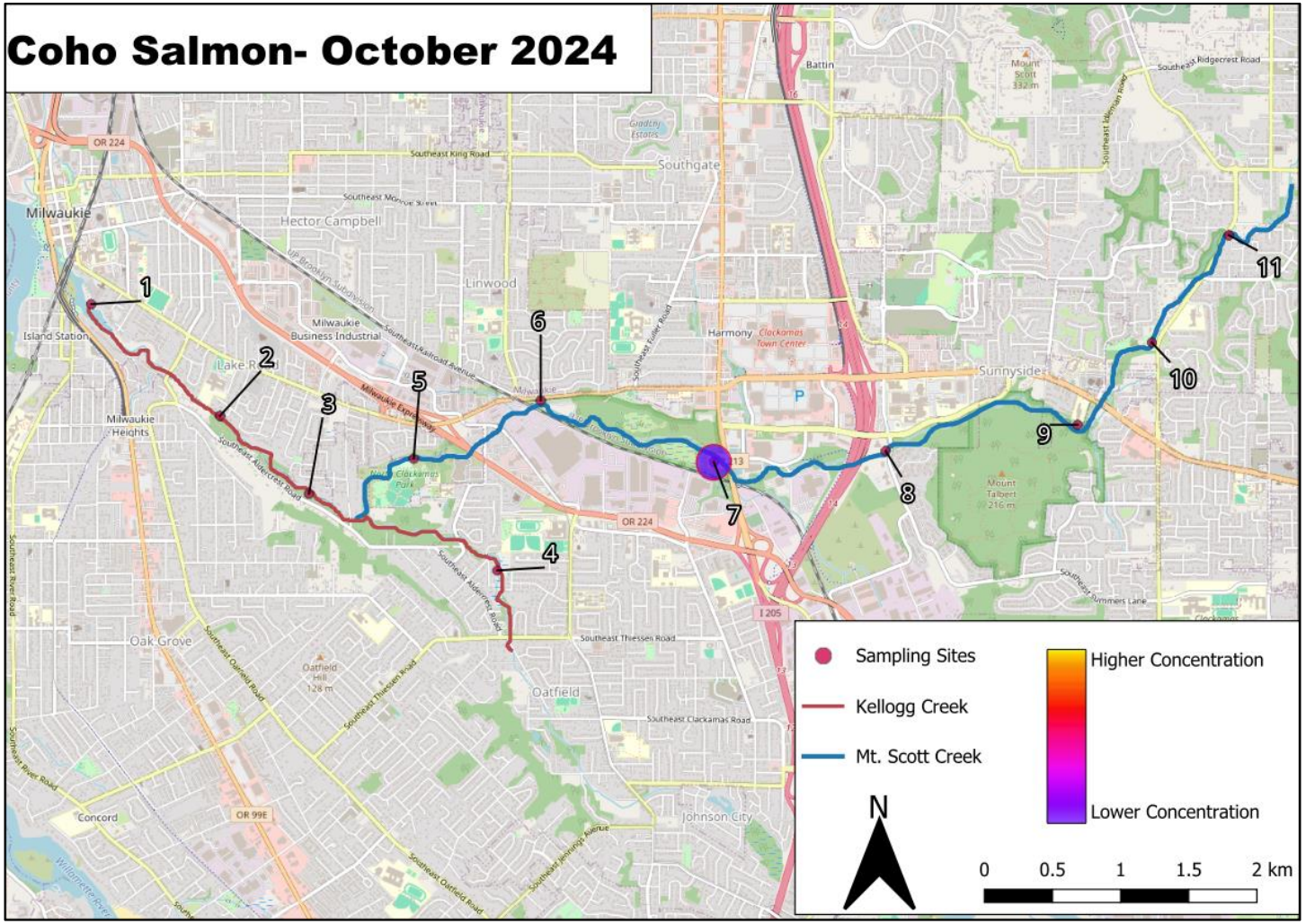

Figure 14. Heatmap depicting the relative site mean concentration of eDNA for Coho Salmon in October 2024. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

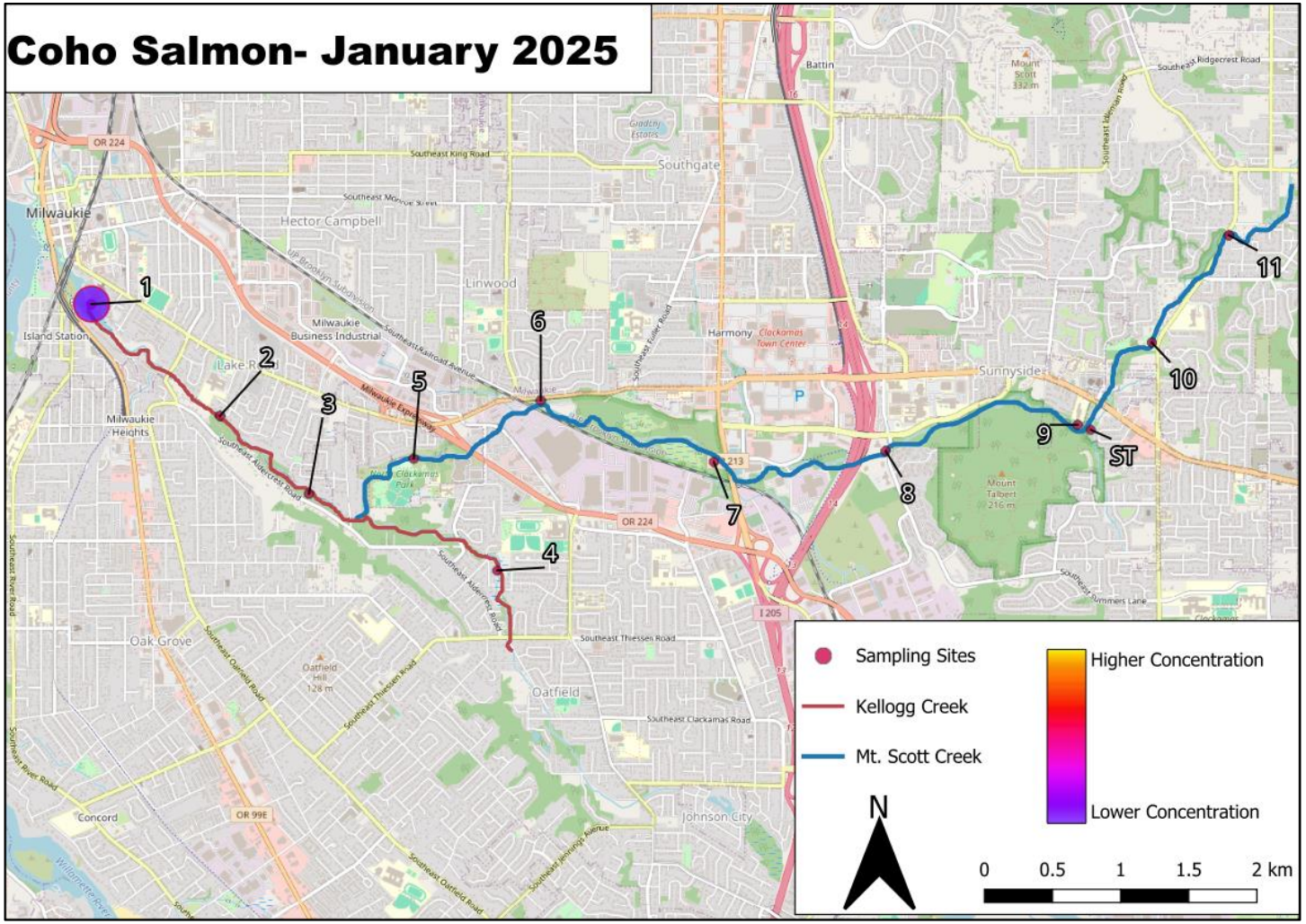

Figure 15. Heatmap depicting the relative site mean concentration of eDNA for Coho Salmon in January 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

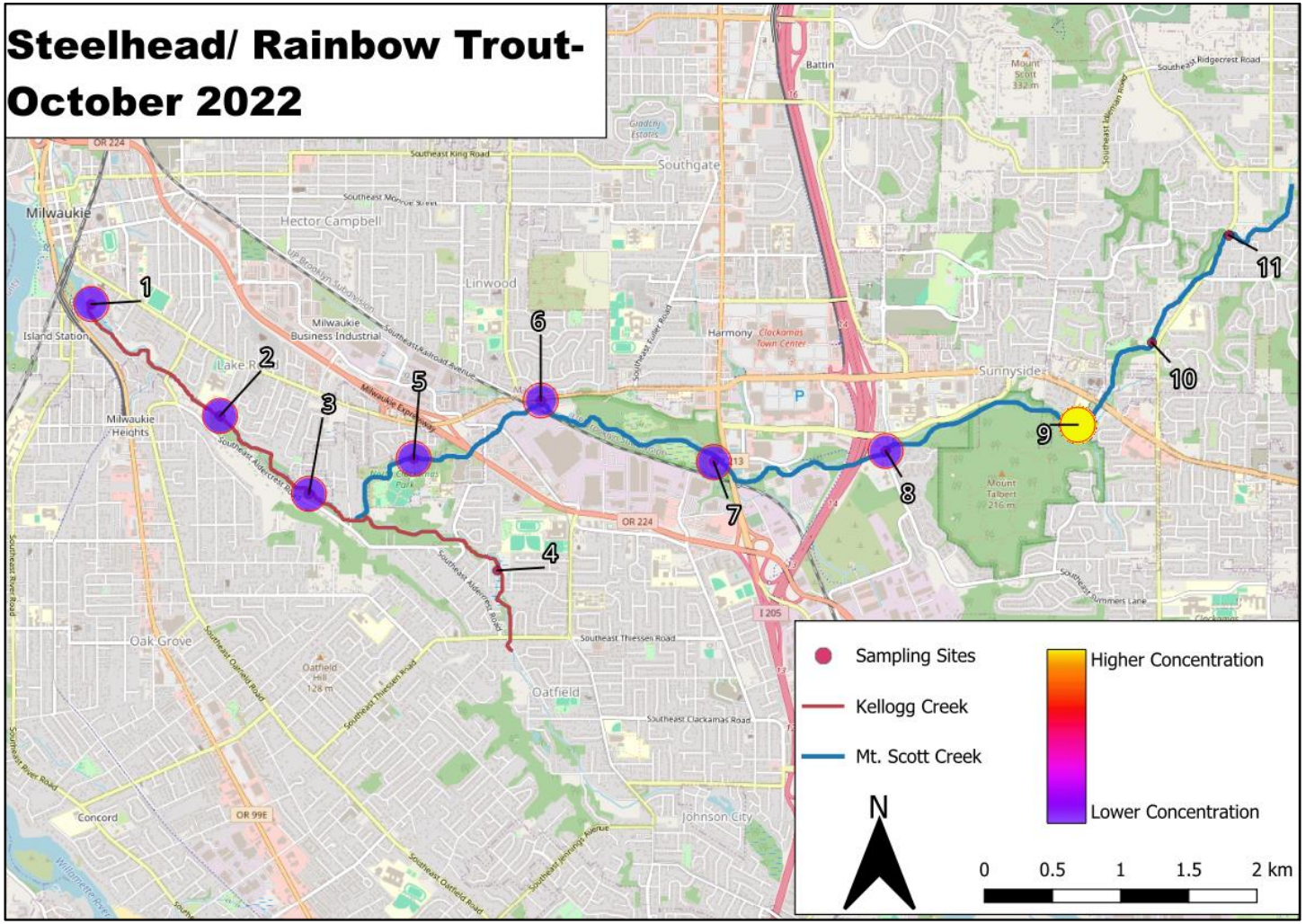

Figure 16. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in October 2022. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

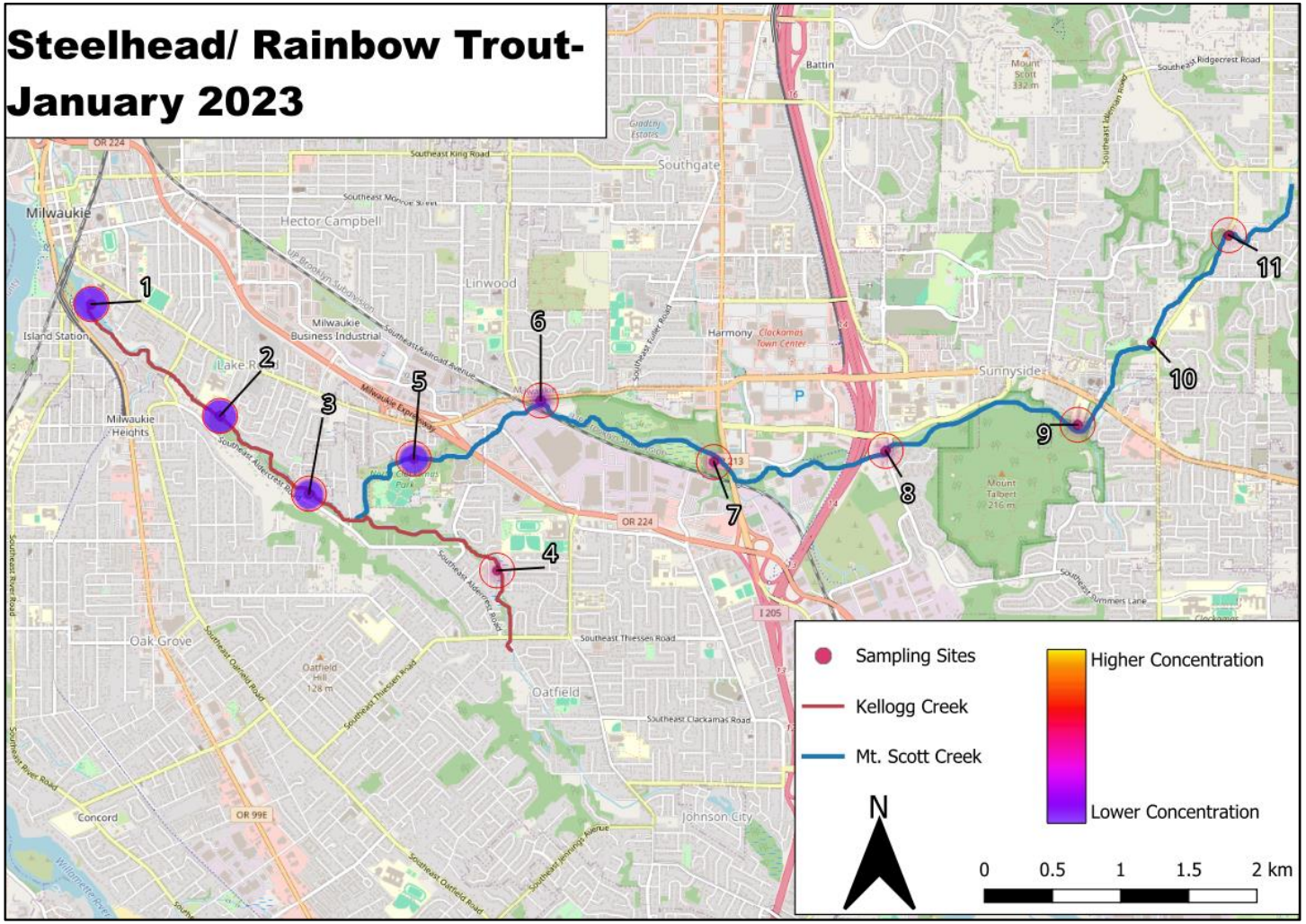

Figure 17. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in January 2023. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

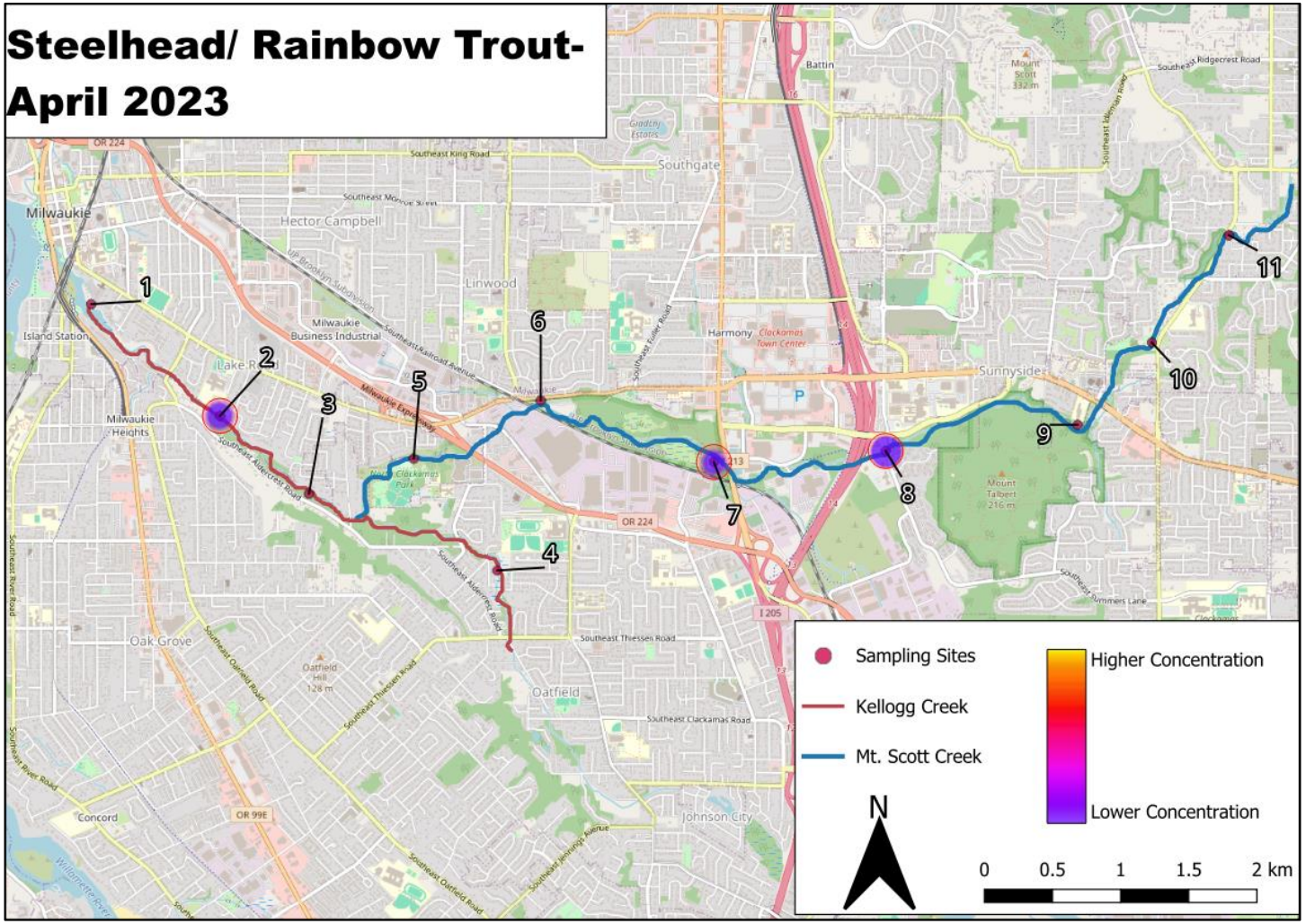

Figure 18. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in April 2023. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

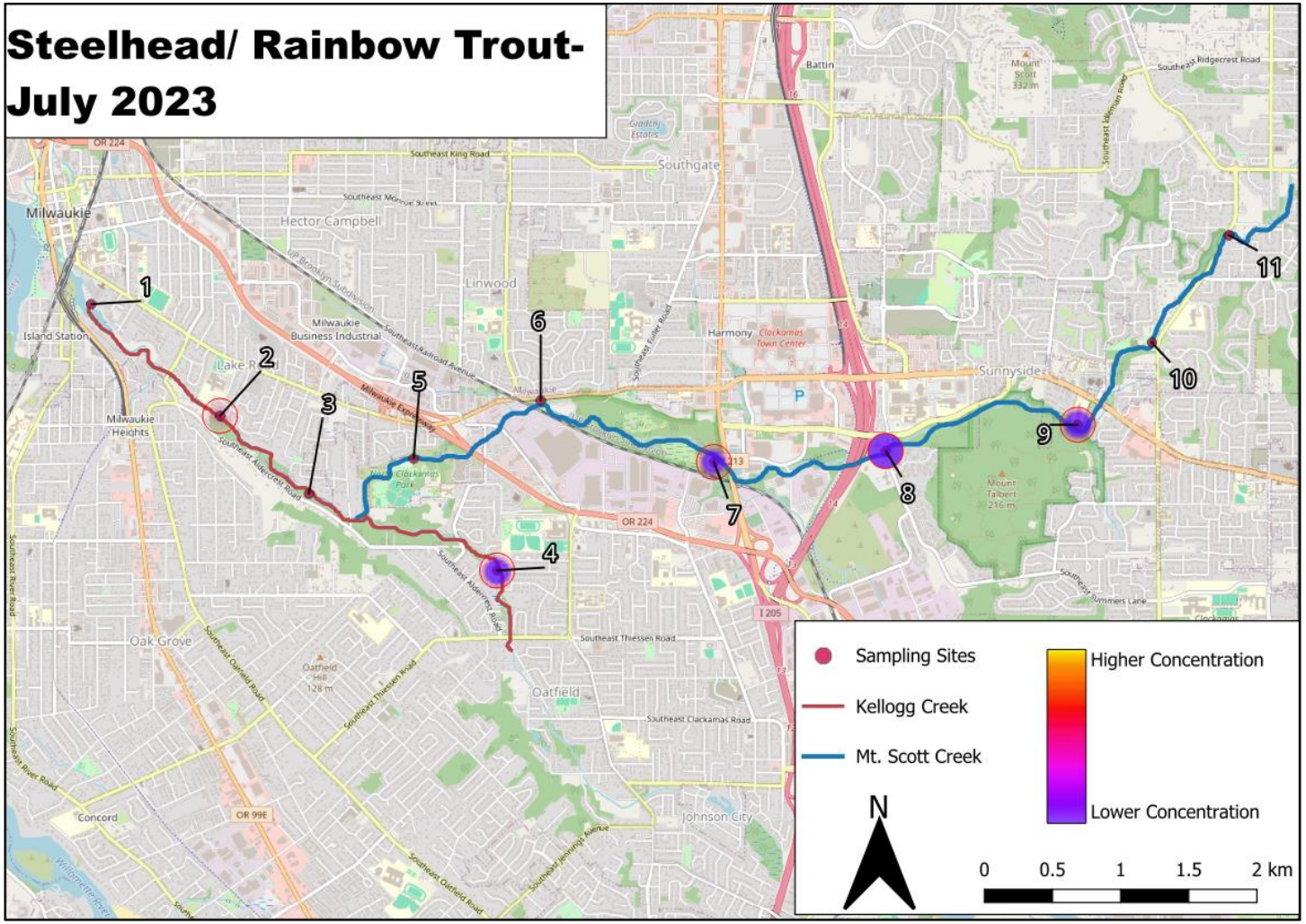

Figure 19. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in July 2023. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

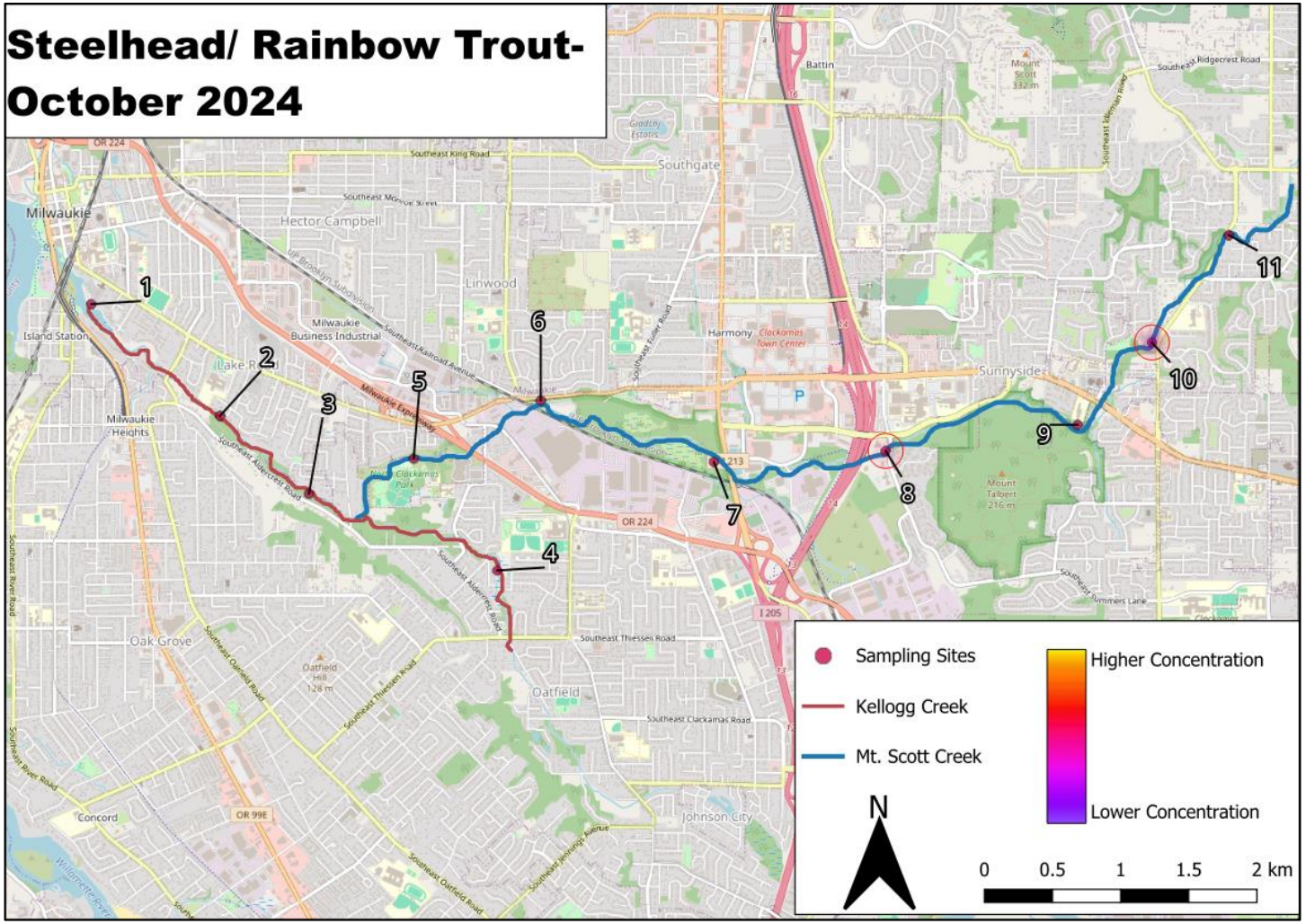

Figure 20. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in October 2024. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

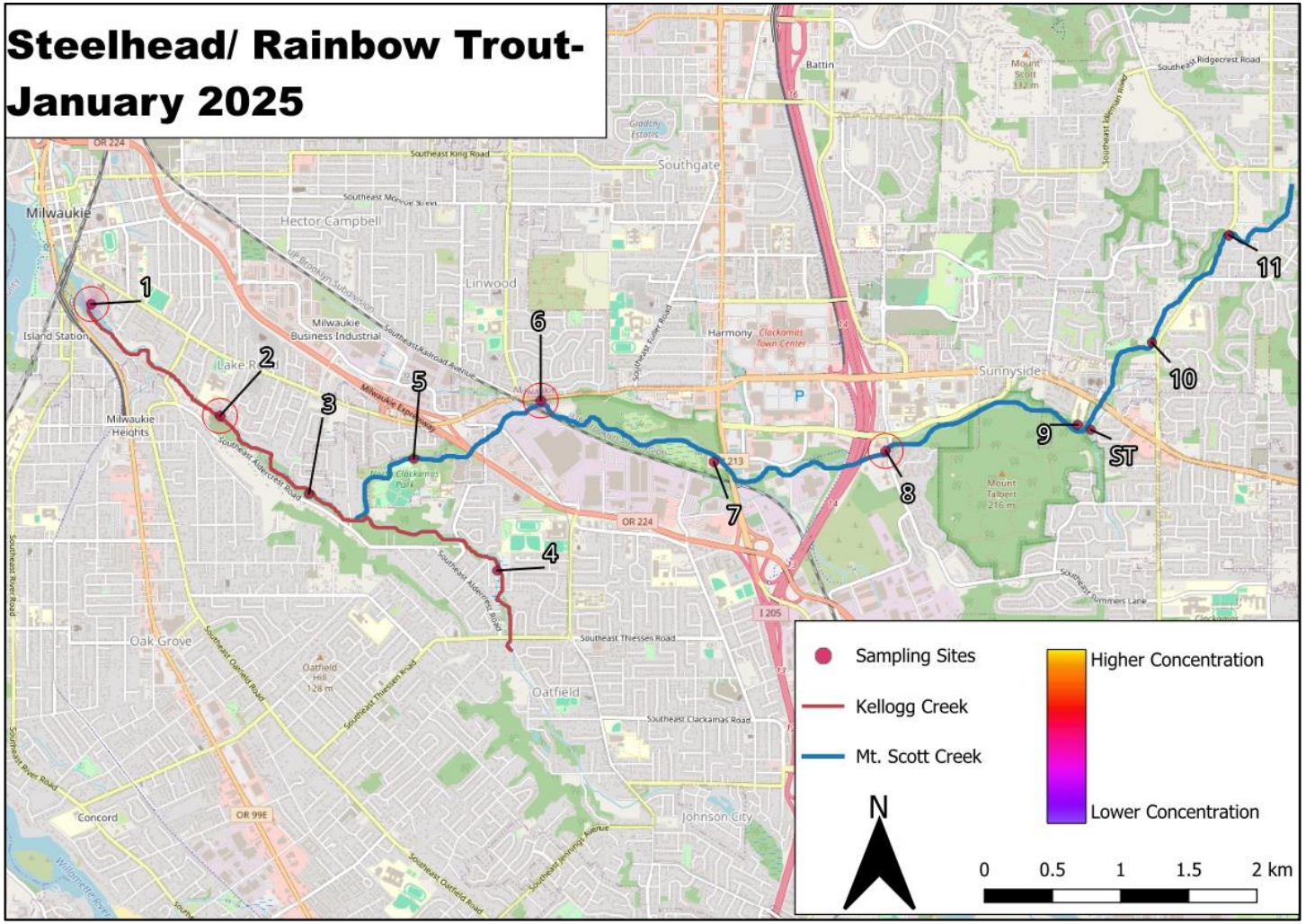

Figure 21. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in January 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

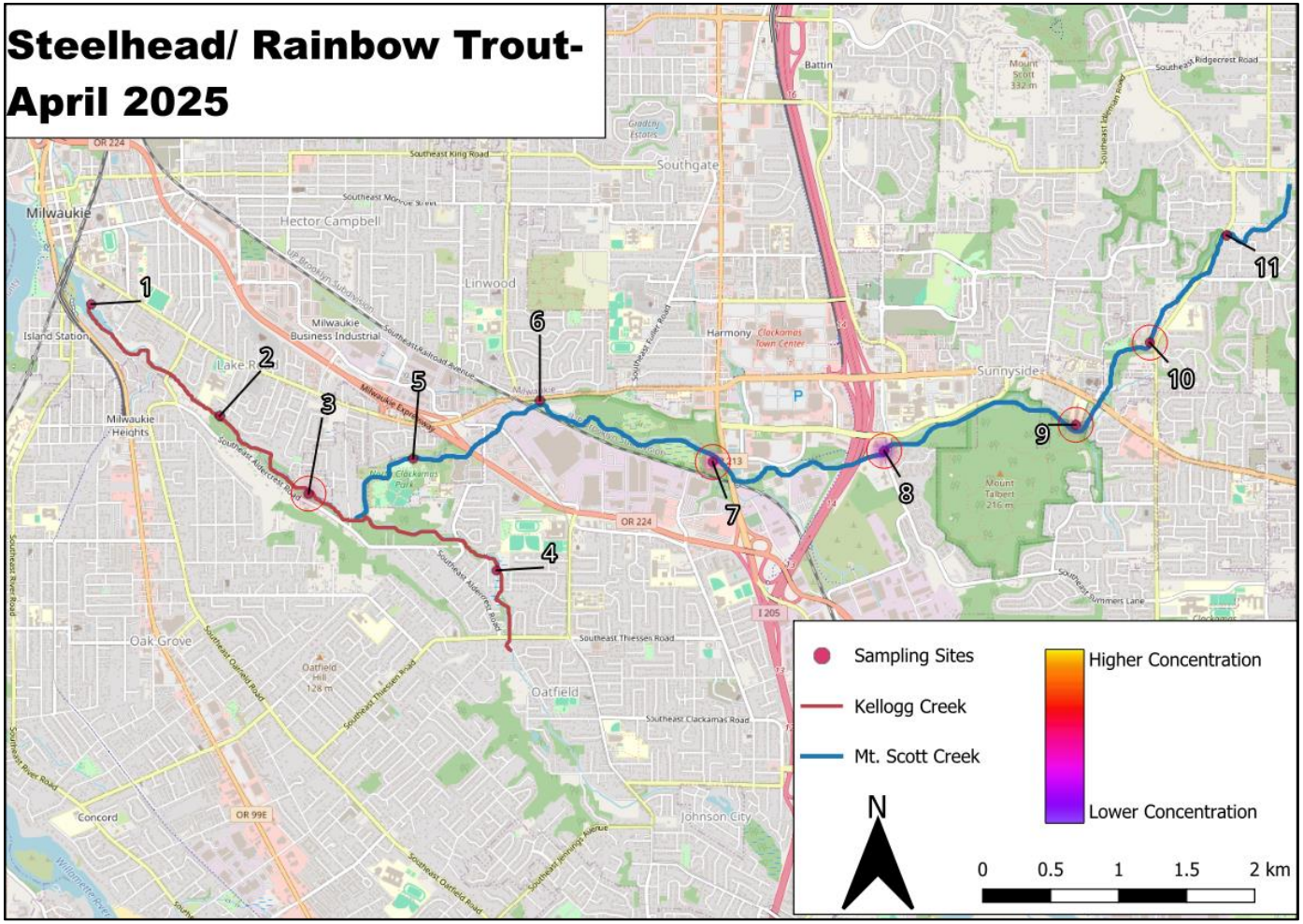

Figure 22. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in April 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected. Detections using Duda et al. 2021 are omitted from this heatmap.

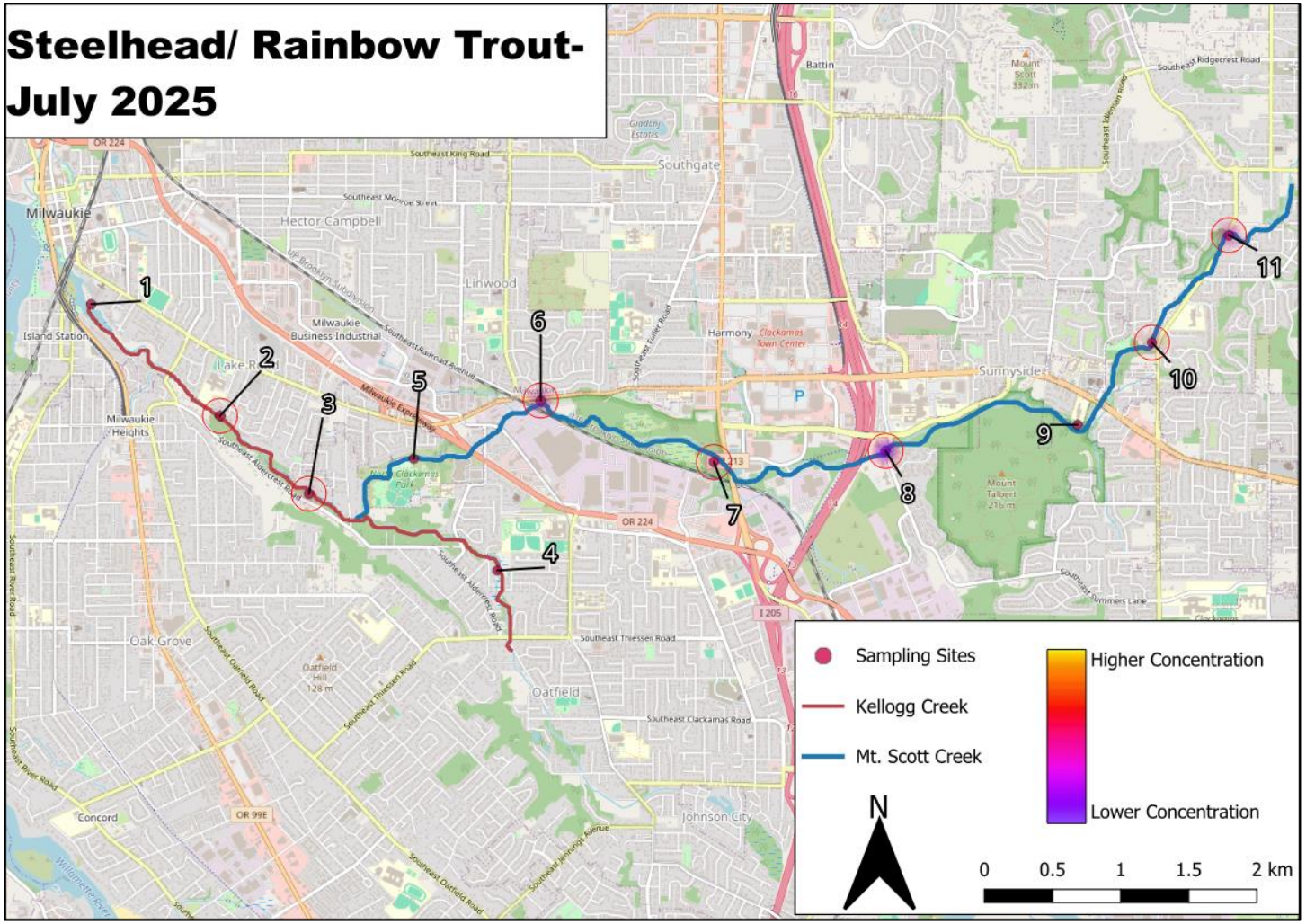

Figure 23. Heatmap depicting the relative site mean concentration of eDNA for Steelhead/ Rainbow Trout in July 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected. Detections using Duda et al. 2021 are omitted from this heatmap.

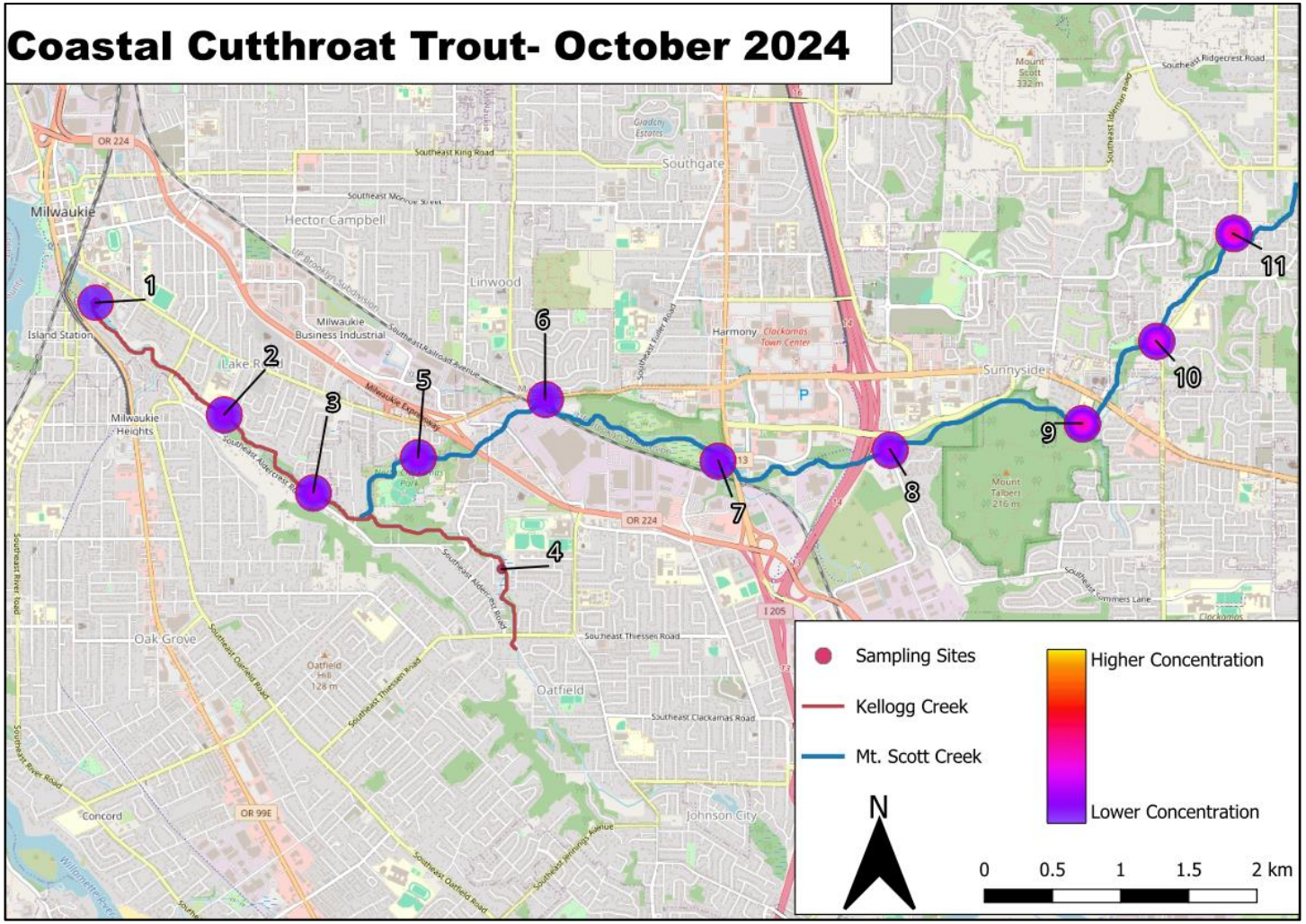

Figure 24. Heatmap depicting the relative site mean concentration of eDNA for Coastal Cutthroat Trout in October 2024. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

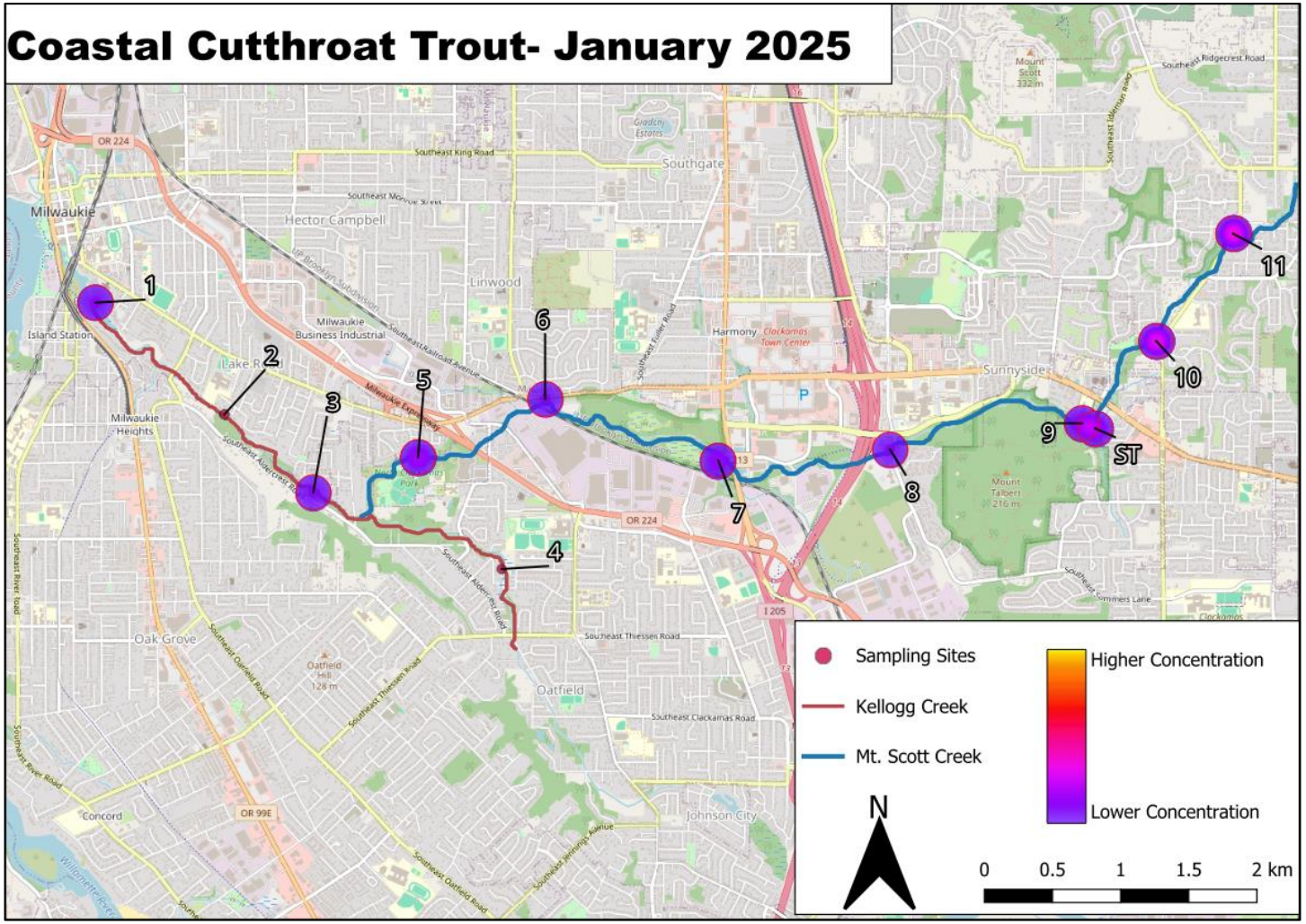

Figure 25. Heatmap depicting the relative site mean concentration of eDNA for Coastal Cutthroat Trout in January 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

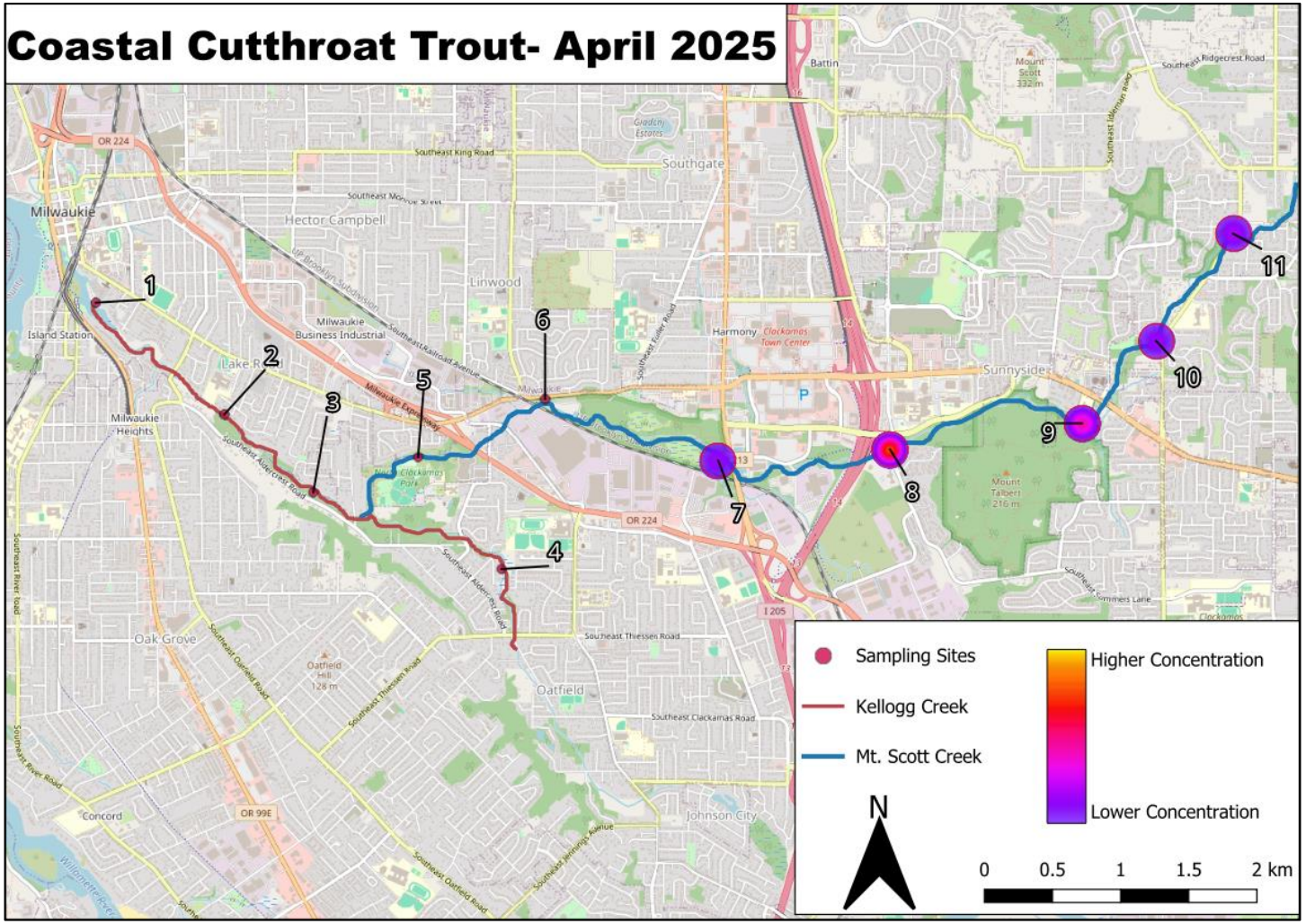

Figure 26. Heatmap depicting the relative site mean concentration of eDNA for Coastal Cutthroat Trout in April 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

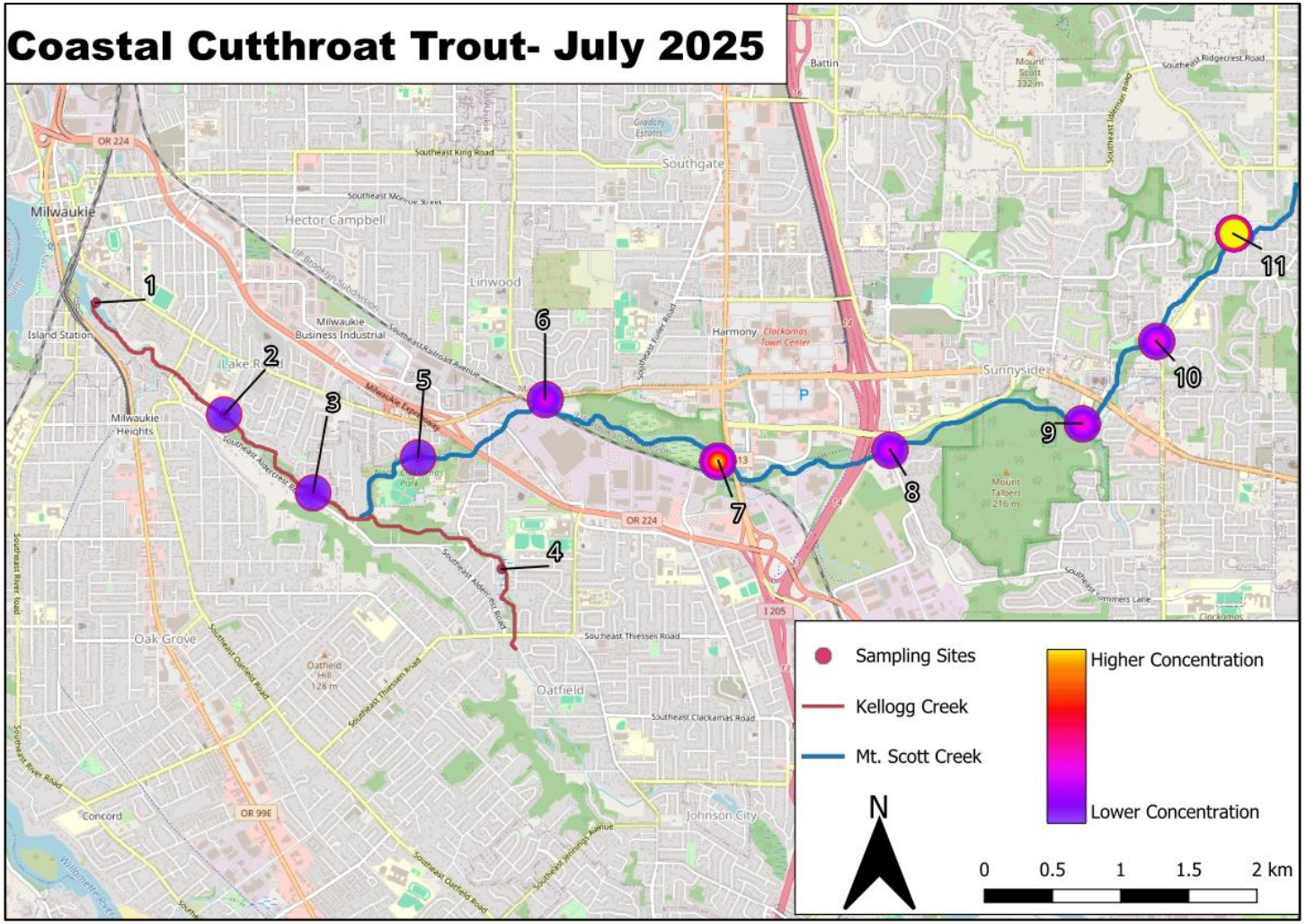

Figure 27. Heatmap depicting the relative site mean concentration of eDNA for Coastal Cutthroat Trout in July 2025. Higher concentrations are depicted as yellow to red, while lower concentrations are depicted from red to purple. Very low concentrations appear nearly transparent purple. For visual assistance, maximum value set for site mean concentration was 1.28E-08; values higher than this appears as all yellow. Red rings indicate where eDNA was detected; absences of a red ring indicate no eDNA detected.

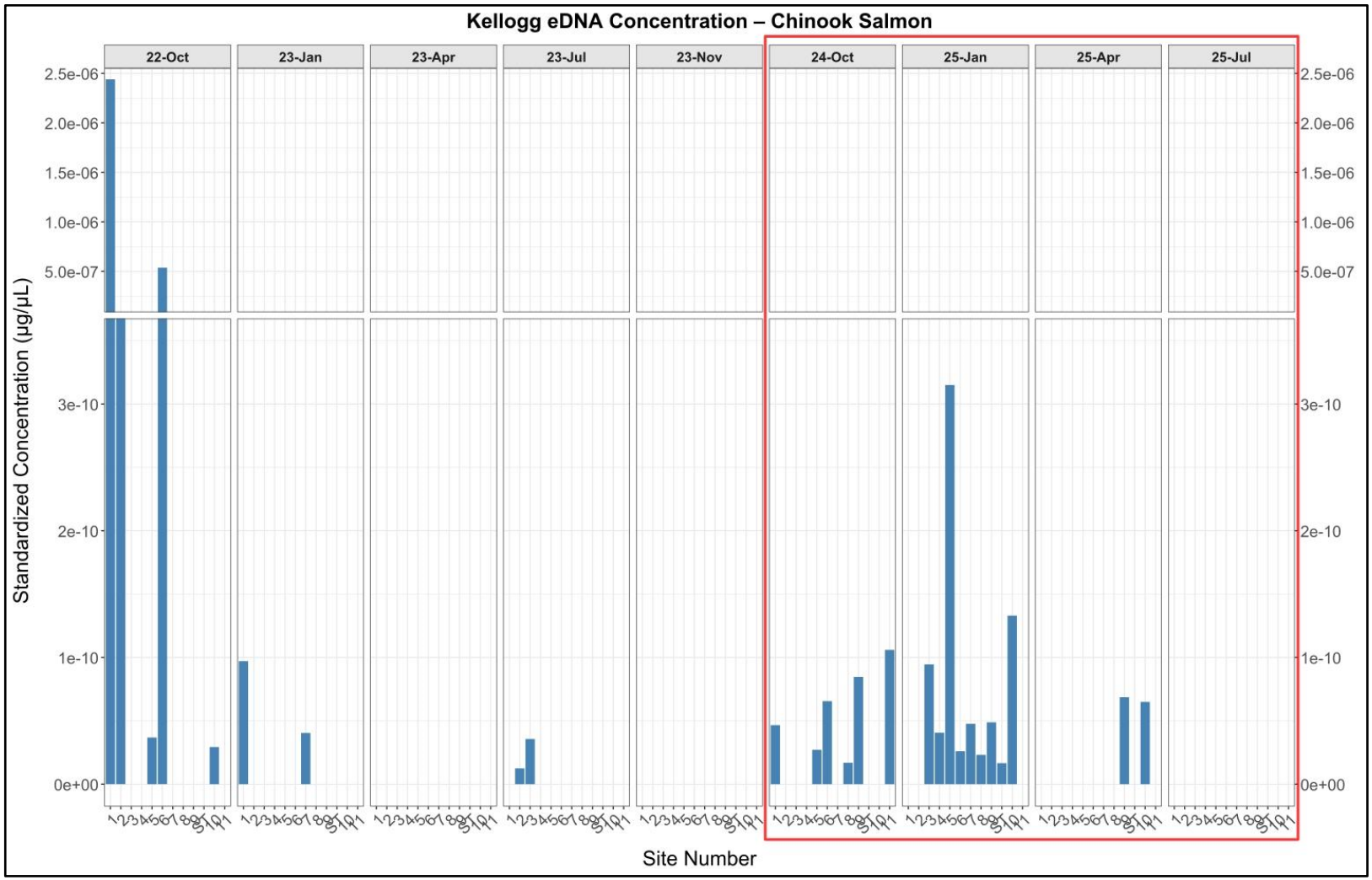

Figure 28. Bar graph of mean eDNA concentration for Chinook Salmon in the Kellogg-Mt. Scott watershed. Results from the extended study are outlined by the red box. eDNA concentrations were standardized by the volume (µl) of water sampled. Axis breaks at 3.5E-10, 2E-07.

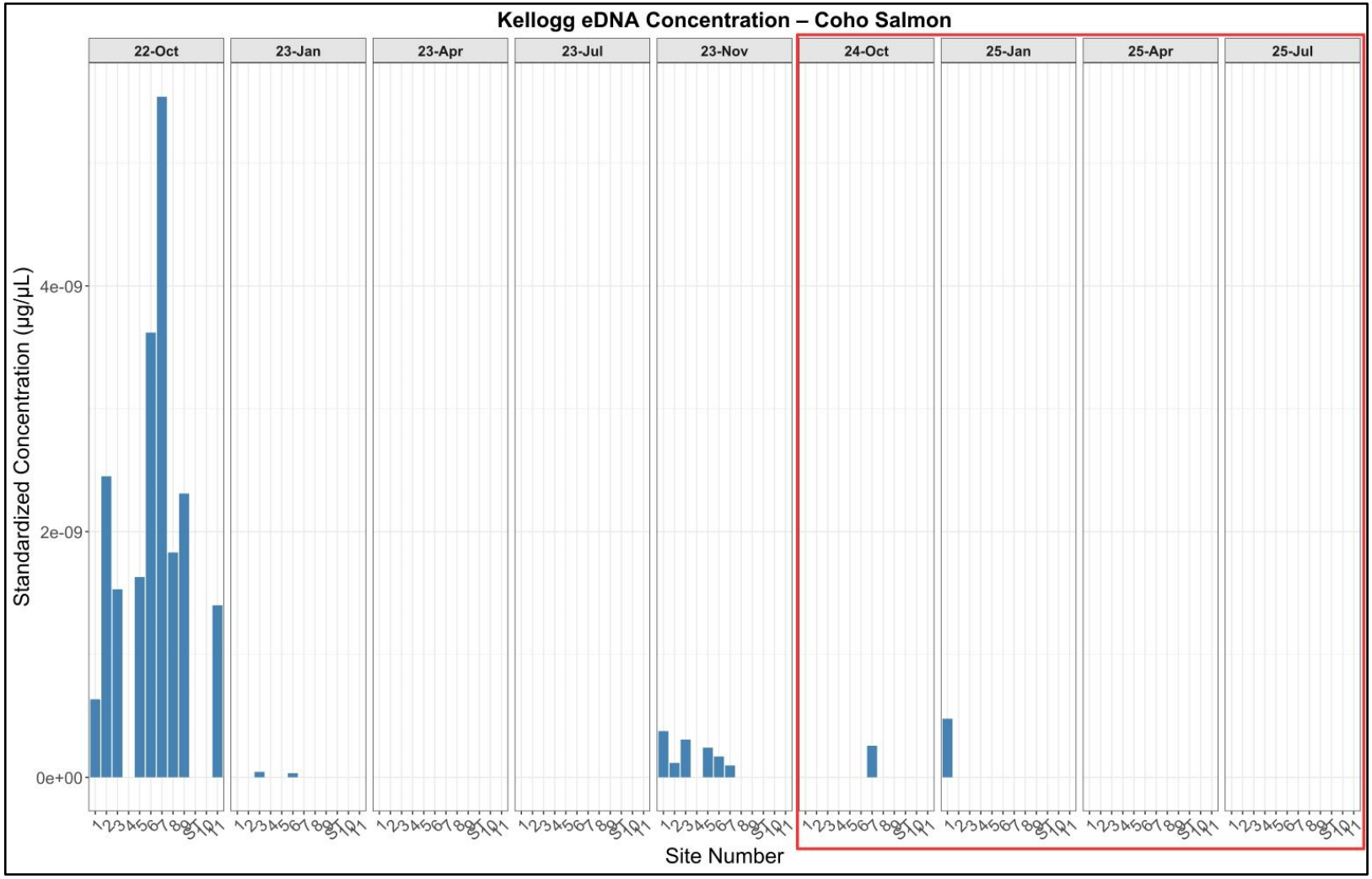

Figure 29. Bar graph of mean eDNA concentration for Coho Salmon in the Kellogg-Mt. Scott watershed. Results from the extended study are outlined by the red box. eDNA concentrations were standardized by the volume (µl) of water sampled.

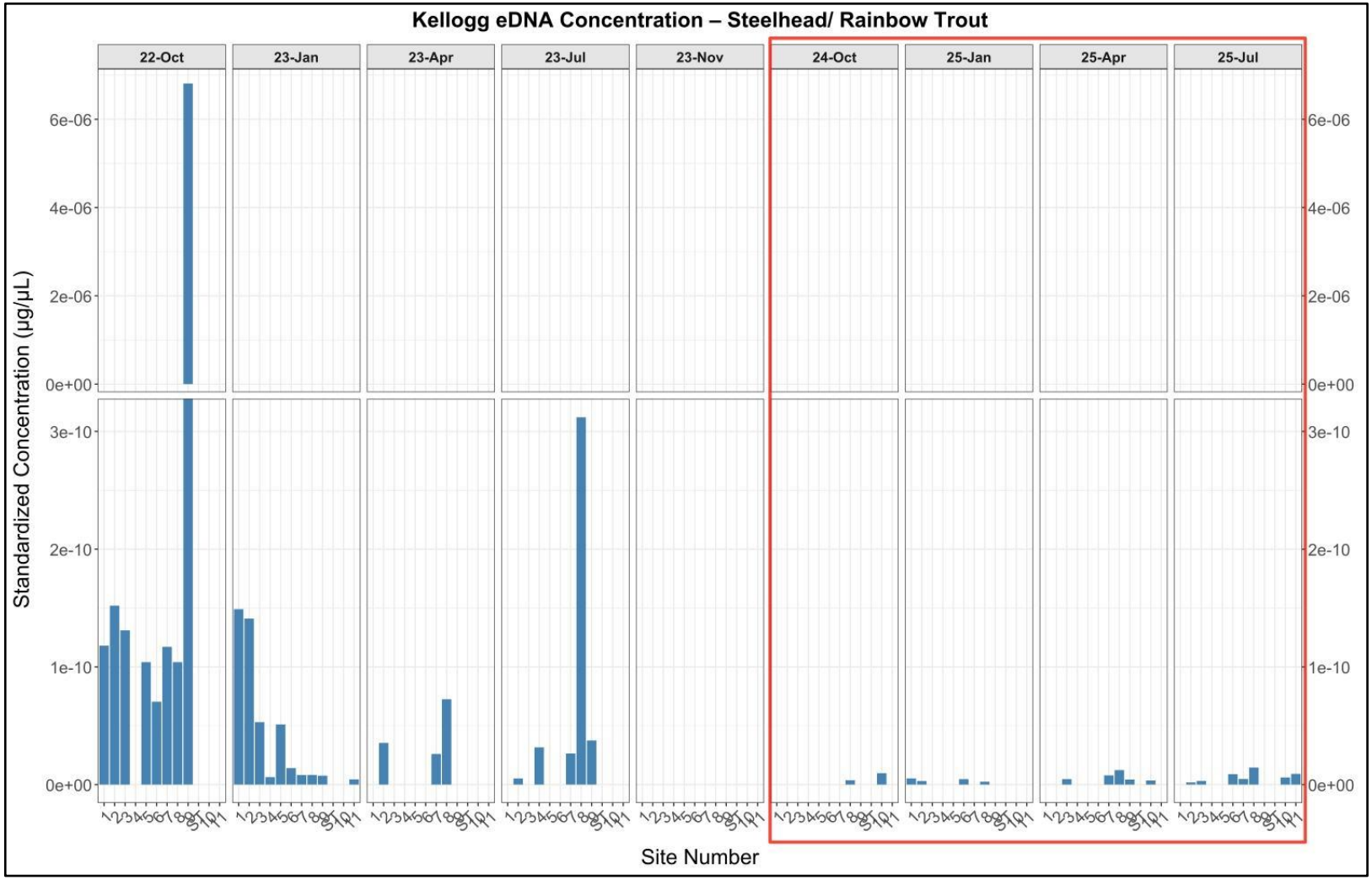

Figure 30. Bar graph of mean eDNA concentration for Steelhead/Rainbow Trout in the Kellogg-Mt. Scott watershed. Results from the extended study are outlined by the red box. eDNA concentrations were standardized by the volume (µl) of water sampled. Axis Breaks at 3.1212E-10, 1.5E-07. Detections using Duda et al. 2021 are omitted from this graph.

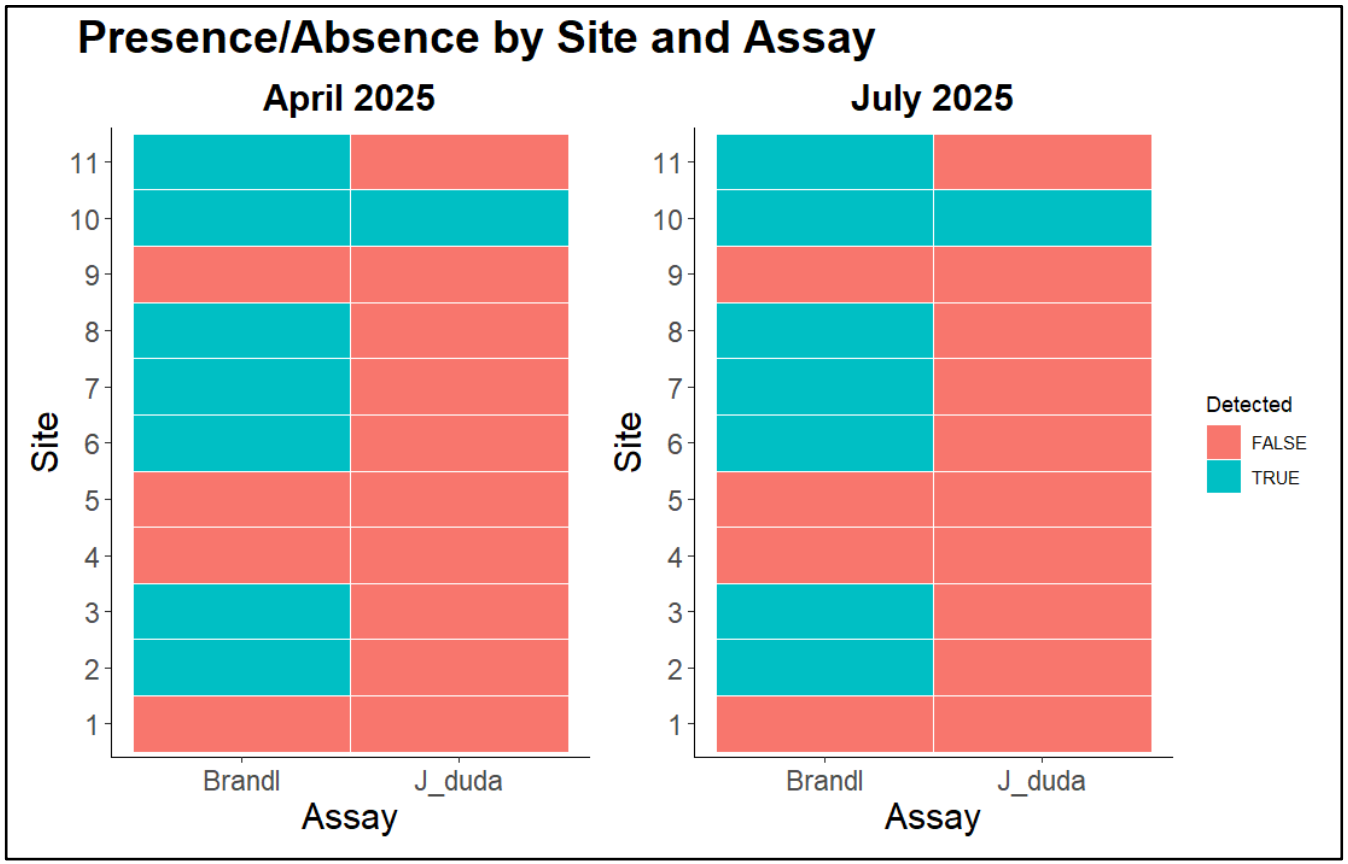

Figure 31. Presence/absence graph comparing results from the two Steelhead/Rainbow Trout assays used during April and July of 2025, Brandl et al 2015 and J_duda et al 2021. Detections made using the J_Duda assay were treated as true detections of presence.

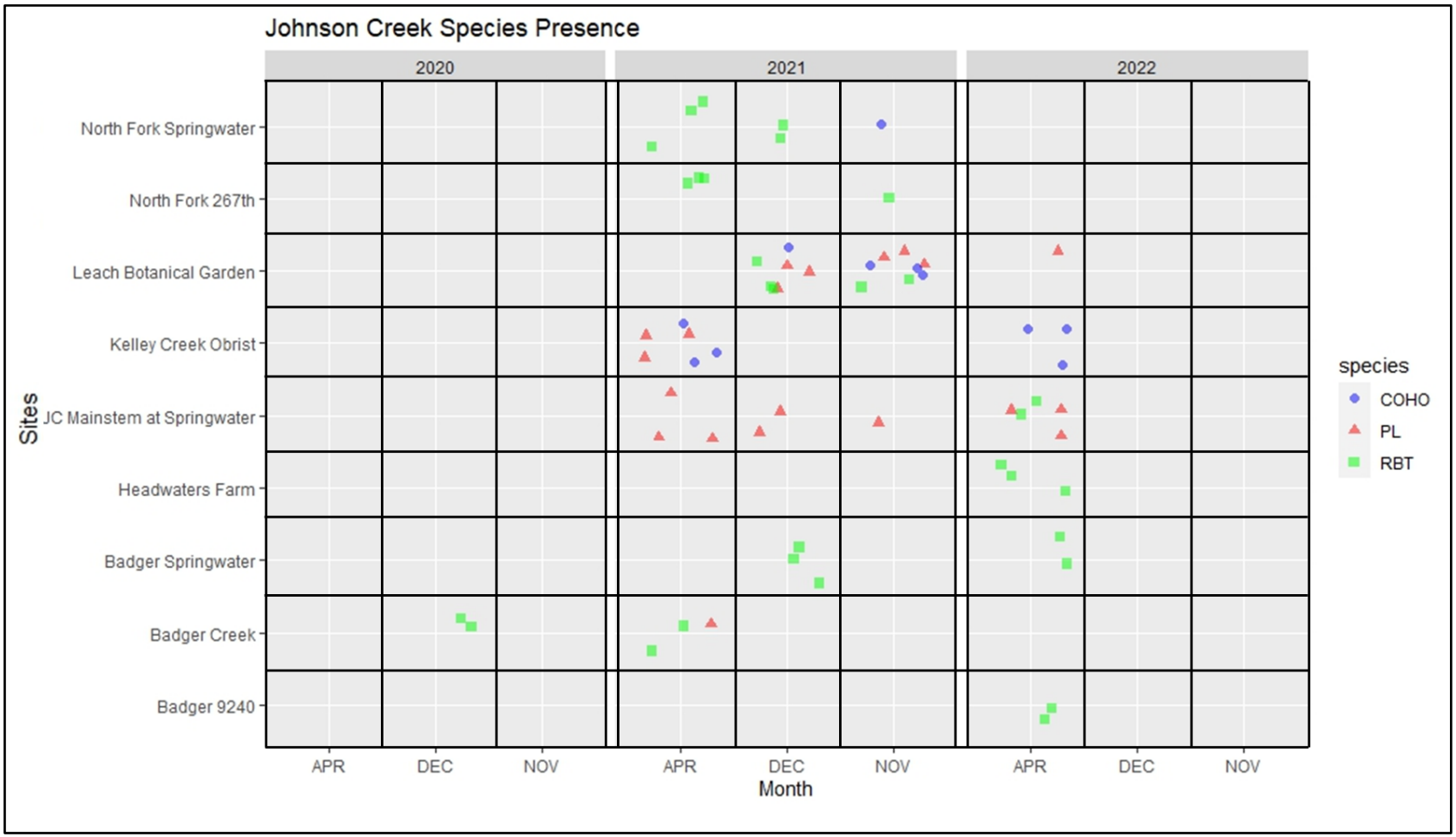

Figure 32. Summary of eDNA detections from the ongoing eDNA monitoring of fish conducted by the Johnson Creek Watershed Council. Data from the Nearby Johnson Creek watershed serves as our "control site" in the BACI analysis.

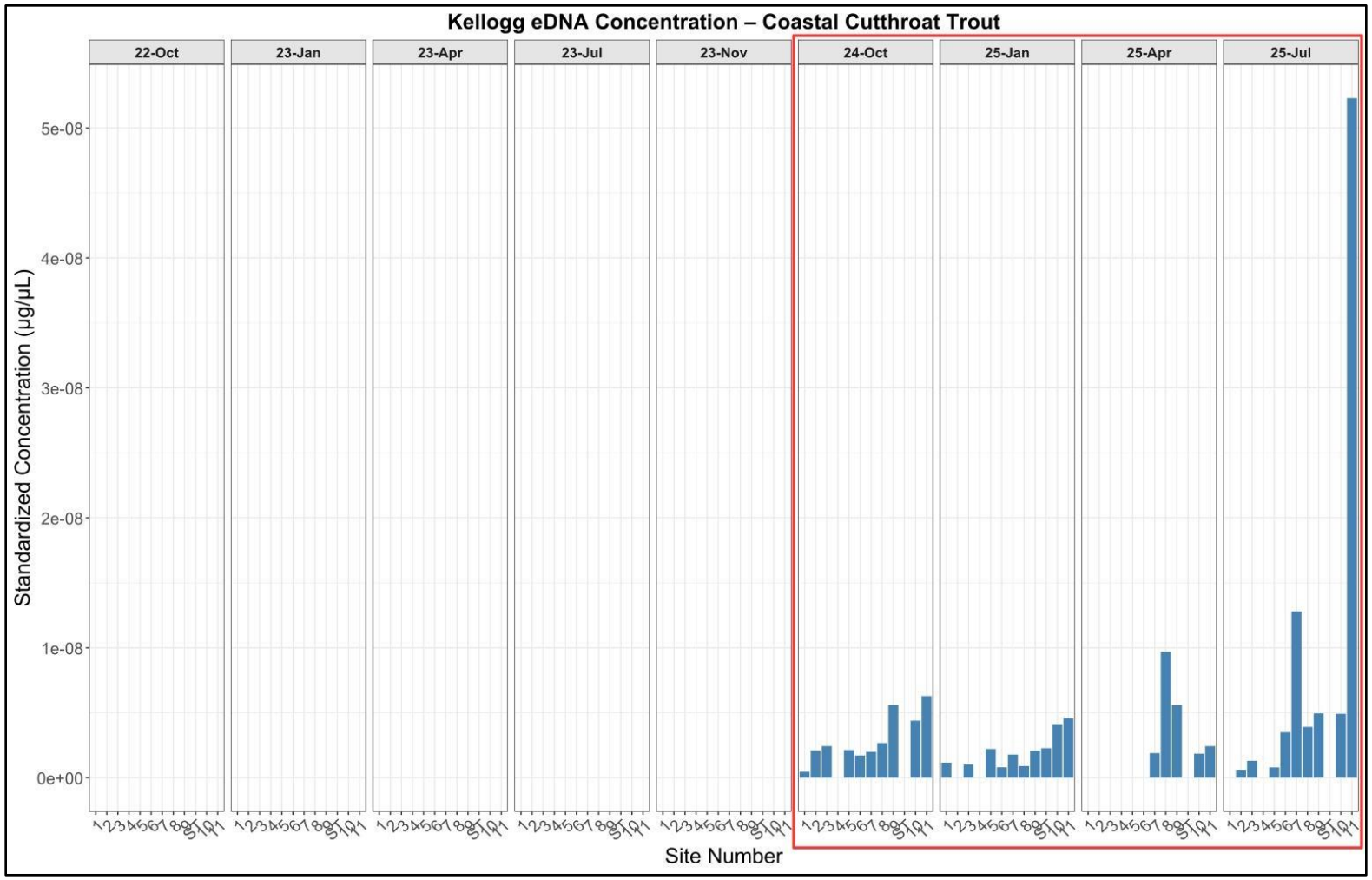

Figure 33. Bar graph of mean eDNA concentration for Coastal Cutthroat Trout in the Kellogg-Mt. Scott watershed. Results from the extended study are outlined by the red box. Coastal Cutthroat was not analyzed as part of the baseline study. eDNA concentrations were standardized by the volume (µl) of water sampled. Coastal Cutthroat Trout was observed at nearly every site, except for April, when detections were isolated to the upper extent of Mt. Scott Creek. The highest concentrations were observed in July 2025, specifically at site 11.