Salmonids at the Gate: Pre-Restoration eDNA Baseline Monitoring in Mac's Creek, Bainbridge Island

June, 2026

Prepared by:

Wild Fish Conservancy

Wild Fish Conservancy
NORTHWEST

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Introduction

Wild Fish Conservancy (WFC) and Sustainable Bainbridge used environmental DNA (eDNA) to document the distribution of fish in Mac's Creek, a small tributary to the Puget Sound on the south side of Bainbridge Island. Fish habitat connectivity in the watershed is currently fragmented by a series of anthropogenic fish passage barriers, including road and trail crossings and a derelict concrete dam. The project team conducted eDNA sampling at strategic locations during the fall of 2025 and spring of 2026, ahead of the planned removal of the dam in summer 2027. This study characterized existing fish species composition and distribution in Mac's Creek, providing a baseline against which future eDNA results can be compared to evaluate fish passage project effectiveness over time.

Background

The many small tributaries draining into Puget Sound collectively provide essential habitat for salmon and trout at critical life stages. As a network, these streams offer not only spawning habitat for returning adults, but also vital rearing habitat for juvenile salmon and trout — both fish rearing in their natal stream and those seeking refuge in non-natal tributaries — where cooler water temperatures, reduced predation pressure, and abundant invertebrate food resources support growth and survival (Beamer et al., 2013; Flitcroft et al., 2024). Despite their ecological significance, small tributaries have been disproportionately impacted by anthropogenic barriers such as road crossing culverts and derelict dams (Warren & Pardew, 1998). Because of their size, these streams are frequently overlooked in restoration planning and monitoring efforts, allowing fish passage barriers to persist long after their ecological costs have been recognized (Washington Department of Fish and Wildlife [WDFW], 2024).

Restoring fish passage in Puget Sound tributaries is a key component of salmon recovery efforts, allowing for natural recolonization and increased fish population resilience (Pess et al., 2014). In addition to restoring fish access to historical spawning and rearing habitats, benefits of restoring fish passage can include increased distribution of marine-derived nutrients delivered upstream by anadromous fish (Helfield & Naiman, 2001); habitat connectivity improvements for other stream-related fauna including mammals, amphibians, and crustaceans (Sepulveda & Lowe, 2016); and improved delivery of wood, water, and sediment to downstream reaches (Wohl et al., 2015). Despite the widespread implementation of fish passage restoration projects across the Pacific Northwest, post-restoration effectiveness monitoring and recolonization studies are frequently absent from project designs, leaving critical gaps in our understanding of how salmonid populations respond to restored access (Roni et al., 2002). Pre-restoration baseline data are particularly valuable in this context, as they provide the necessary reference point against which post-restoration changes in species presence, distribution, and community composition can be evaluated. Documenting the effectiveness of fish passage restoration projects is essential for evaluating their success and guiding future conservation strategies.

Environmental DNA (eDNA) is a powerful, non-invasive tool for monitoring fish distributions in aquatic ecosystems. Unlike traditional survey methods, which can be labor-intensive and disruptive, eDNA allows researchers to detect species presence through genetic material shed into the environment via skin, mucus, or feces (Goldberg et al., 2015). This approach offers high sensitivity, enabling the detection of rare or cryptic species that might be missed using conventional techniques like visual surveys, seining, trapping, and electrofishing (Lodge et al., 2012). Additionally, eDNA sampling can cover large areas efficiently, providing a cost-effective and scalable means of assessing biodiversity (Cristescu & Hebert, 2018). Its application in fish recolonization studies is particularly valuable for tracking species presence and community composition before and after fish passage restoration.

Mac's Creek

The Mac's Creek watershed, located on the south side of Bainbridge Island, is largely forested and undeveloped. The stream originates at Mac's Pond on the IslandWood campus, a 250-acre property that encompasses the majority of the basin. From Mac's Pond, the creek flows southward through the IslandWood property and onto a Blakely Harbor County Park before discharging into Blakeley Harbor (Figure 1). In 2014, Wild Fish Conservancy conducted water typing surveys throughout Bainbridge Island, identifying 4,700 feet of potential fish habitat in the Mac's Creek Watershed.

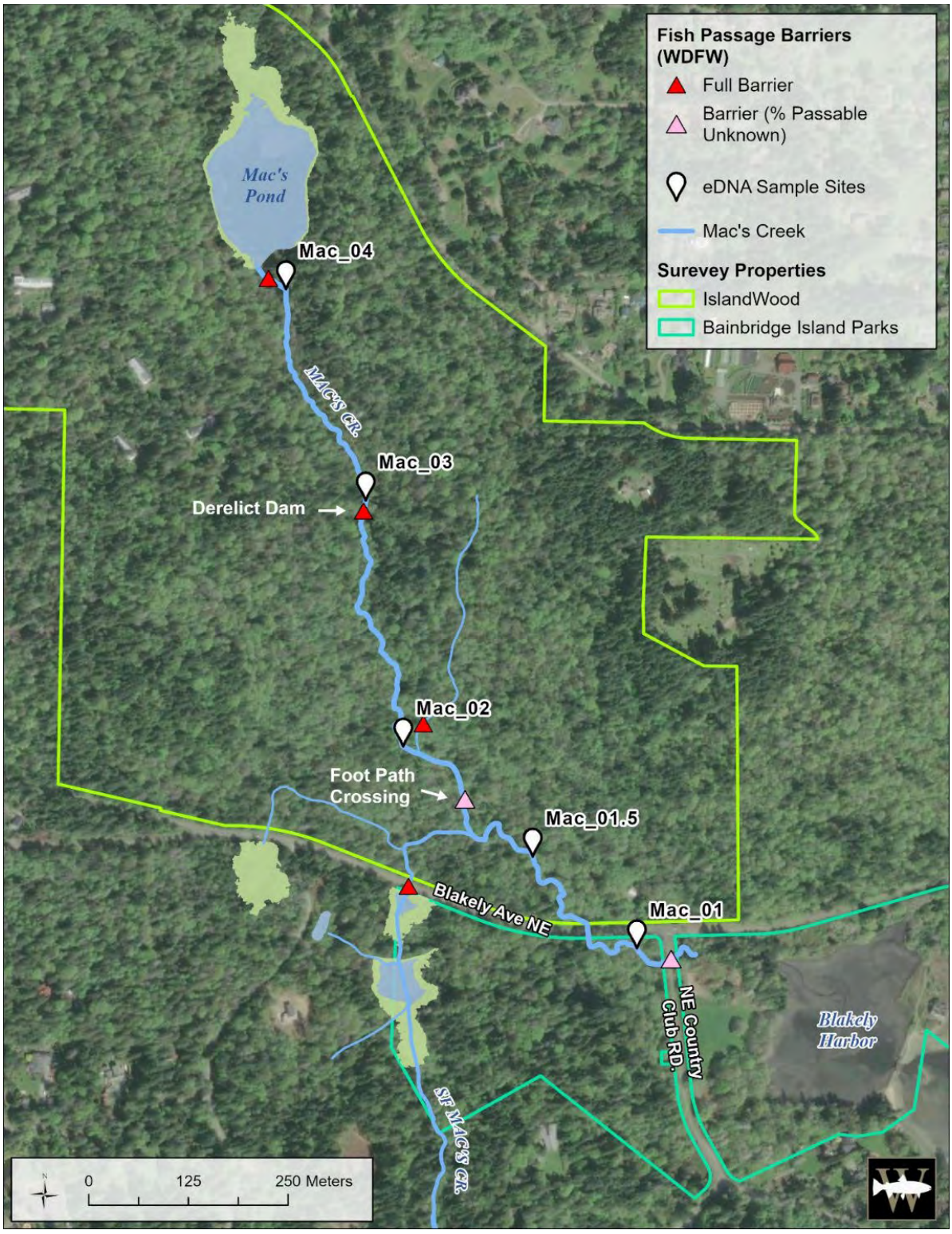

Figure 1. Mac's Creek watershed, with eDNA sample locations and fish passage barriers as classified by the WDFW Fish Passage and Diversion Screening Inventory (FPDSI).

Salmonid species expected to utilize Mac's Creek include Chinook Salmon (*Oncorhynchus tshawytscha*), Coho Salmon (*Oncorhynchus kisutch*), Chum Salmon (*Oncorhynchus keta*), Steelhead/Rainbow Trout (*Oncorhynchus mykiss*), and Coastal Cutthroat Trout (*Oncorhynchus clarkii*) (WDFW FPDSI, 2024). The Washington Department of Fish and Wildlife (WDFW) Fish Passage and Diversion Screening Inventory (FPDSI) database identifies six fish passage barriers within the Mac's Creek watershed, four of which are located on the mainstem. The downstream-most fish passage barrier in the watershed is the culvert running under NE Country Club Road, classified as a barrier of unknown passability due to tidal influence (Figure 2). Moving upstream, the second mainstem barrier is the Spine Trail crossing on IslandWood property, a non-motorized footpath, classified as a barrier of unknown passability. Further upstream, a derelict concrete dam on the mainstem of Mac's Creek, also located on the IslandWood property, is classified as a full barrier, blocking access to approximately 1,000 feet of potential fish habitat in the headwaters (Figure 3). The uppermost mainstem barrier is the outflow structure of Mac's Pond, which also constitutes a full fish passage barrier.

Figure 2.The downstream-most fish passage barrier culvert running under NE Country Club Road.

Figure 3. Derelict dam on IslandWood property.

During WFC's 2014 water typing surveys, no fish were detected above the NE Country Club Road culvert during electrofishing surveys, though Coho Salmon and Cutthroat Trout were documented immediately downstream. WFC flagged this culvert as a high-value restoration opportunity in their Phase III water type assessment summary (Wild Fish Conservancy, 2016), and it has been identified by the City of Bainbridge Island as a priority for future replacement. The derelict dam structure was also flagged as a high-value restoration opportunity by WFC and is now in the planning stages for removal, with removal scheduled for the summer of 2027.

The goal of this study was to establish baseline eDNA data on salmonid presence and distribution in Mac's Creek prior to the removal of these fish passage barriers, providing a reference point for evaluating future recolonization and potential shifts in fish community composition and distribution.

Methods

Study Area

Sampling was conducted at five sites (Mac_01 through Mac_04, and Mac_01.5) spanning the entirety of mainstem Mac's Creek from above the NE Country Club Road culvert to the headwaters below Mac's Pond (Figure 1). Sample sites were strategically positioned above each successive fish passage barrier to allow detection of salmonid presence or absence in relation to specific passage impediments, with sites spaced approximately 250 meters apart. This spacing is consistent with published eDNA detection ranges for Pacific salmonids in small stream systems, where eDNA signal can be reliably detected within hundreds of meters downstream of a source population (Jane et al., 2015). Mac_01 was located 100 ft upstream of the NE Country Club Road culvert, as Coho Salmon and Cutthroat Trout had previously been documented below this barrier during the 2014 WFC water typing surveys, making it the most likely site for initial recolonization detection following barrier removal. Mac_01.5 was added during the second spring 2026 sampling event based on field observations of high-quality salmonid habitat at that location (Figure 4). Mac_02 is above the barrier on the Spine Trail, and Mac_03 is situated above the derelict Islandwood dam, positioned to detect any resident salmonid populations that may persist in the isolated reach between the dam and Mac's Pond. Mac_04 is located at the Mac's Pond outflow structure and was placed to assess whether any fish populations are persisting within the pond itself.

Figure 4. WFC biologist measuring stream width at a Mac's Creek sampling site Mac_01.5 during spring 2026 field surveys.

eDNA Sample Collection

Field sampling was conducted by Wild Fish Conservancy and Sustainable Bainbridge biologists in accordance with field sampling methodologies established by partner laboratories (Figure 5). Samples were collected during four sampling events on November 11 and December 4, 2025 and April 8 and May 7, 2026. The fall 2025 sampling events were timed to overlap with peak Coho Salmon spawning on Bainbridge Island, when adult fish are actively present in neighboring streams and eDNA signal is expected to be highest. The spring 2026 sampling events were designed to capture the emergence and early rearing period of Coho Salmon and trout fry, providing a complementary seasonal window for detecting juvenile salmonid presence.

Figure 5. WFC biologist collecting a qPCR eDNA water sample at Mac's Creek, spring 2026.

Laboratory Analysis

Two complementary eDNA methods were employed to assess salmonid presence and broader aquatic community composition. Quantitative polymerase chain reaction (qPCR) is a targeted approach that detects and quantifies the DNA of specific species, making it highly sensitive and well-suited for monitoring known species of interest (Wilcox et al., 2013). This method is particularly effective for low-abundance species, as it can amplify trace amounts of DNA for

reliable detection even when present in minimal quantities (Bohmann et al., 2014). qPCR samples were analyzed by the USFS National Genomics Center for the presence of Coho Salmon, Chum Salmon, Cutthroat Trout, and Rainbow Trout.

We used qPCR to test for species based on life history and each site's position relative to the watershed's fish passage barriers. Chum salmon were targeted only during the fall 2025 events, when adults would be spawning in the creek; their fry emerge in late winter and move quickly to the estuary, leaving little subsequent opportunity for detection. Coho and Chum Salmon were targeted only at Mac_01, Mac_01.5 and Mac_02, below the derelict dam. The dam fully blocks anadromous fish, so the reach between the dam and Mac's Pond (Mac_03) was assessed only for resident Cutthroat Trout and Rainbow Trout.

Metabarcoding eDNA uses high-throughput sequencing to identify multiple species simultaneously from a single sample (Taberlet et al., 2012), providing a broader perspective on community composition and biodiversity. Metabarcoding samples were analyzed by Wilderlab to assess broader fish community composition within Mac's Creek. Together, these complementary eDNA methods provided both the precision needed for targeted salmonid detection and a comprehensive view of the broader aquatic community.

Results

eDNA Sample Collection

A total of 24 eDNA samples (thirteen metabarcoding and eleven qPCR) were collected across four sampling events (Table 1). High turbidity conditions were observed during all sampling events, resulting in reduced filtered volumes for metabarcoding samples, with one filter clogging as early as 140 mL. For all qPCR samples, a target volume of 5 liters (5,000 mL) was achieved through the use of multiple filters.

Table 1. Summary of all eDNA samples collected in Mac's Creek, 2025–2026. COHO = Coho Salmon, CHUM = Chum Salmon, CUTT = Cutthroat Trout, RNBT = Rainbow Trout, NGC = National Genomics Center. Vol. refers to the volume of water sampled.

Site	Date	Method	Lab	Vol. (mL)	Species Targeted	Detection
Mac_01	11/11/2025	Metabarcoding	Wilderlab	175	Fish	None
Mac_02	11/11/2025	Metabarcoding	Wilderlab	200	Fish	None
Mac_04	11/11/2025	Metabarcoding	Wilderlab	250	Fish	None
Mac_01	12/04/2025	Metabarcoding	Wilderlab	140	Fish	Staghorn Sculpin
Mac_02	12/04/2025	Metabarcoding	Wilderlab	350	Fish	None

Site	Date	Method	Lab	Vol. (mL)	Species Targeted	Detection
Mac_03	12/04/2025	Metabarcoding	Wilderlab	600	Fish	None
Mac_01	04/08/2026	Metabarcoding	Wilderlab	200	Fish	None
Mac_02	04/08/2026	Metabarcoding	Wilderlab	250	Fish	None
Mac_04	04/08/2026	Metabarcoding	Wilderlab	300	Fish	None
Mac_01	05/07/2026	Metabarcoding	Wilderlab	250	Fish	None
Mac_01.5	05/07/2026	Metabarcoding	Wilderlab	200	Fish	None
Mac_02	05/07/2026	Metabarcoding	Wilderlab	300	Fish	None
Mac_04	05/07/2026	Metabarcoding	Wilderlab	350	Fish	None
Mac_01	11/11/2025	qPCR	USFS NGC	5000	COHO, CHUM, CUTT, RNBT	None
Mac_02	11/11/2025	qPCR	USFS NGC	5000	COHO, CHUM, CUTT, RNBT	None
Mac_03	11/11/2025	qPCR	USFS NGC	5000	CUTT, RNBT	None
Mac_01	12/04/2025	qPCR	USFS NGC	5000	COHO, CHUM, CUTT, RNBT	None
Mac_02	12/04/2025	qPCR	USFS NGC	5000	COHO, CHUM, CUTT, RNBT	None
Mac_03	12/04/2025	qPCR	USFS NGC	5000	CUTT, RNBT	None
Mac_01	04/08/2026	qPCR	USFS NGC	5000	COHO, CUTT, RNBT	None
Mac_01.5	04/08/2026	qPCR	USFS NGC	5000	COHO, CUTT, RNBT	None
Mac_02	04/08/2026	qPCR	USFS NGC	5000	COHO, CUTT, RNBT	None

Site	Date	Method	Lab	Vol. (mL)	Species Targeted	Detection
Mac_01	05/07/2026	qPCR	USFS NGC	5000	COHO, CUTT, RNBT	None
Mac_01.5	05/07/2026	qPCR	USFS NGC	5000	COHO, CUTT, RNBT	None

qPCR Results

No salmonid eDNA was detected in any of the eleven qPCR samples analyzed by the USFS National Genomics Center. Coho Salmon, Chum Salmon, Cutthroat Trout, and Rainbow Trout were all absent or below the detection threshold at all sites and during all four sampling events. Full qPCR sample collection metadata, including field coordinates and sample ID tags, are provided in Appendix B.

Metabarcoding Results

No salmonid eDNA was detected in any of the thirteen metabarcoding samples analyzed by Wilderlab. One fish detection was captured during the study period — Pacific Staghorn Sculpin (*Leptocottus armatus*) was detected at Mac_01 during the December 4, 2025 sampling event. Mac_01 is the lowest sampling site in the watershed, located just above the tidal zone, and the detection of Pacific Staghorn Sculpin at this site is consistent with the known distribution of this estuarine species in the lower reaches of Puget Sound tributaries.

The metabarcoding analyses detected DNA from a diverse assemblage of amphibians, mammals, and birds across all sites and sampling events. Amphibian detections included Pacific treefrog (*Pseudacris regilla*), red-legged frog (*Rana aurora*), rough-skinned newt (*Taricha granulosa*), Pacific newts (*Taricha* spp.), big salamanders (*Ambystoma* spp.), ensatina (*Ensatina* spp.), and chorus frogs (*Pseudacris* spp.). Mammal detections included American beaver (*Castor canadensis*), northern river otter (*Lontra canadensis*), northern raccoon (*Procyon lotor*), black-tailed deer (*Odocoileus hemionus*), North American deer mouse (*Peromyscus maniculatus*), cinereus shrew (*Sorex cinereus*), and vole (*Clethrionomys* spp.). Bird detections included song sparrow (*Melospiza melodia*), Hutton's vireo (*Vireo huttoni*), band-tailed pigeon (*Patagioenas fasciata*), dabbling ducks (*Anas* spp.), and woodpecker (*Dryocopus* spp.). A summary of vertebrate detections by site and sampling event is provided in Appendix A.

Discussion

No salmonid eDNA was detected at any site in Mac's Creek during either the fall 2025 or spring 2026 sampling events using either qPCR or metabarcoding methods. These results are consistent with the findings of Wild Fish Conservancy's 2014 water typing surveys, which documented fish

habitat but no fish above the barrier culvert at NE Country Club Road, and support the conclusion that salmonids remain absent from the watershed due to the presence of that barrier. The absence of salmonid eDNA across all sites, methods, and sampling events provides a robust pre-restoration baseline against which future recolonization can be assessed.

Pacific Staghorn Sculpin (*Leptocottus armatus*) was detected at Mac_01 during the December 4, 2025 sampling event. While this detection is broadly consistent with the known estuarine distribution of this species in lower Puget Sound tributaries, it should be interpreted with caution. The detection was relatively weak and occurred in only one of the four sampling events at Mac_01. It is possible that the eDNA signal was not the result of a live fish present in the stream, but rather an instance of trophic deposition — for example, a bird or mammal consuming a staghorn sculpin and depositing biological material at or near the sampling site. Secondary deposition of eDNA from predator activity is recognized in stream eDNA studies and underscores the importance of interpreting single, low-confidence detections carefully (Merkes et al., 2014).

These baseline data will serve as a critical reference point for evaluating the effectiveness of upcoming fish passage restoration in Mac's Creek. The IslandWood dam is scheduled for removal in summer 2027, which will restore access to approximately 1,000 feet of potential salmonid habitat in the headwaters of Mac's Creek. However, the full ecological benefit of this restoration will not be realized unless fish passage is also addressed at the two additional mainstem barriers downstream of the dam, as identified in the WDFW FPDSI database. The barrier culvert at NE Country Club Road remains the primary obstacle to salmonid access in the lower watershed, blocking an estimated 4,700 feet of habitat. The Spine Trail crossing on Islandwood property, also classified as a barrier, represents an additional impediment that could prevent full recolonization of the watershed even if the culvert is replaced. Given that the Spine Trail is a non-motorized footpath on Islandwood property, addressing fish passage at this crossing represents a relatively low-cost opportunity to meaningfully improve fish passage and maximize the ecological benefit of the planned dam removal.

We recommend that the NE Country Club Road culvert be elevated to a high priority for replacement, particularly given the significant investment being made in upstream habitat restoration. We also recommend that the Spine Trail crossing be assessed and considered for remediation in conjunction with the dam removal project. Future eDNA monitoring following barrier removal and remediation will allow researchers to document the timing and extent of salmonid recolonization, track shifts in fish and wildlife community composition, and assess the long-term success of these restoration efforts. The methods and baseline data established through this study provide a replicable framework for effectiveness monitoring of fish passage restoration projects throughout the Puget Sound region.

References

Beamer, E. M., Henderson, W. T., Brown, C. M., & Henderson, K. A. (2013). Juvenile Chinook salmon rearing in small non-natal streams flanking the Whidbey Basin. Skagit River System Cooperative Research Report.

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., & Yu, D. W. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. Trends in Ecology & Evolution, 29(6), 358–367.

Cristescu, M. E., & Hebert, P. D. N. (2018). Uses and misuses of environmental DNA in biodiversity science and conservation. Annual Review of Ecology, Evolution, and Systematics, 49, 209–230.

Flitcroft, R., Arismendi, I., Santelmann, M., & Johnson, S. (2024). Juvenile salmonids traverse coastal meta-nurseries. Canadian Journal of Fisheries and Aquatic Sciences. [volume, issue, and page numbers or DOI needed]

Goldberg, C. S., Strickler, K. M., & Pilliod, D. S. (2015). Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. Biological Conservation, 183, 1–3.

Helfield, J. M., & Naiman, R. J. (2001). Effects of salmon-derived nitrogen on riparian forest growth and implications for stream-forest interactions. Ecology, 82(9), 2403–2409.

Jane, S. F., Wilcox, T. M., McKelvey, K. S., Young, M. K., Schwartz, M. K., Lowe, W. H., Letcher, B. H., & Whiteley, A. R. (2015). Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. Molecular Ecology Resources, 15(1), 216–227.

Lodge, D. M., Turner, C. R., Jerde, C. L., Barnes, M. A., Chadderton, W. L., Egan, S. P., Feder, J. L., Mahon, A. R., & Pfrender, M. E. (2012). Conservation in a cup of water: Estimating biodiversity and population abundance from environmental DNA. Molecular Ecology, 21(11), 2555–2558.

Merkes, C. M., McCalla, S. G., Jensen, N. R., Gaikowski, M. P., & Amberg, J. J. (2014). Persistence of DNA in carcasses, slime and avian feces may affect interpretation of environmental DNA data. PLoS ONE, 9(11), e113346.

Pess, G. R., Quinn, T. P., Gephard, S. R., & Saunders, R. (2014). Recolonization of Atlantic and Pacific rivers by anadromous fishes: Linkages between life history and the benefits of barrier removal. Reviews in Fish Biology and Fisheries, 24(3), 881–900.

Roni, P., Beechie, T. J., Bilby, R. E., Leonetti, F. E., Pollock, M. M., & Pess, G. R. (2002). A review of stream restoration techniques and a hierarchical strategy for prioritizing restoration in Pacific Northwest watersheds. North American Journal of Fisheries Management, 22(1), 1–20.

Sepulveda, A. J., & Lowe, W. H. (2016). Movement and survival of an amphibian in relation to sediment and culvert design. Journal of Herpetology, 43(1), 108–112.

Stoeckle, B. C., Beggel, S., Auerswald, K., Furthner, A., Winter, A., Behrmann-Godel, J., & Geist, J. (2016). A systematic approach to evaluate the influence of environmental conditions on eDNA detection success in aquatic ecosystems. PLOS ONE, 11(12), e0168718.

Taberlet, P., Coissac, E., Hajibabaei, M., & Rieseberg, L. H. (2012). Environmental DNA. Molecular Ecology, 21(8), 1789–1793.

Warren, M. L., Jr., & Pardew, M. G. (1998). Road crossings as barriers to small-stream fish movement. Transactions of the American Fisheries Society, 127(4), 637–644.

Washington Department of Fish and Wildlife. (2024). Fish Passage and Diversion Screening Inventory (FPDSI). https://geodataservices.wdfw.wa.gov/hp/fishpassage/index.html

Washington Department of Fish and Wildlife. (2024). Fish Passage Performance and Restoration Progress Report.

Wilcox, T. M., McKelvey, K. S., Young, M. K., Jane, S. F., Lowe, W. H., Whiteley, A. R., & Schwartz, M. K. (2013). Robust detection of rare species using environmental DNA. PLOS ONE, 8(3), e59520.

Wild Fish Conservancy. (2016). Wild Fish Conservancy Watertype Assessment Project Summary: West Sound Watersheds Phase III. https://wildfishconservancy.org/wp-content/uploads/2022/05/Westsound-Phase-III-Report_rev.pdf

Wohl, E., Bledsoe, B. P., Fausch, K. D., Hooke, J., Kondolf, G. M., Wohl, L. N., & Yarnell, S. M. (2015). Management of coarse wood in UK and US rivers. Geomorphology, 246, 470–482.

Appendix A.

The following is a complete list of metabarcoding eDNA vertebrate detections by sample site and date. The final column *** indicates Sequence count, representing the number of times a unique taxon was detected in each sample. These counts can be loosely used to predict the relative abundance/biomass of organisms in the local environment, as higher counts represent more DNA detected. Due to the high sensitivity of eDNA testing, low sequence counts should be considered a tentative detection. A full list of all eDNA detections, including taxonomic groups outside of vertebrates, can be made available upon request by contacting Wild Fish Conservancy.

Mac_01 - *Upstream of NE County Club Road*					
		Genus	**Species**	**Common Name**	***
Nov 11 2025	ID: 562308	Canis	-	Dog	765
		Peromyscus	maniculatus	North American deer mouse	67
Dec 4 2025	ID: 562368	Leptocottus	armatus	Pacific staghorn sculpin	980
		Peromyscus	maniculatus	North American deer mouse	536
		Ambystoma	-	Big salamanders	20
		Anas	-	Dabbling ducks	5
April 9 2026	ID: 558578	Ambystoma	-	Big salamanders	4712
		Pseudacris	regilla	Pacific treefrog	4120
		Taricha	-	Pacific newts	565
		Lontra	canadensis	Northern American river otter	429
		Rana	aurora	Red-legged frog	375
		Pseudacris	-	Chorus frogs	332
		Castor	canadensis	American beaver	110
		Melospiza	melodia	Song sparrow	99
		Vireo	huttoni-	Hutton's vireo	7
May 8 2026	ID: 569070	Rana	aurora	Red-legged frog	4851
		Ambystoma	-	Big salamanders	1757
		Taricha	-	Pacific newts	833
		Pseudacris	regilla	Pacific treefrog	574
		Procyon	lotor	Northern raccoon	466
		Peromyscus	-	Deer mice	222
		Patagioenas	fasciata	Band-tailed pigeon	87
		Canis	-	Dog	14

Mac_01.5 - *Lower Mac's Creek*					
		Genus	**Species**	**Common Name**	***
May 8 2026	ID: 569069	Ambystoma	-	Big salamanders	7392
		Pseudacris	regilla	Pacific treefrog	1643
		Rana	aurora	Red-legged frog	1040
		Sorex	cinereus	Cinereus shrew	925
		Procyon	lotor	Northern raccoon	662

Mac_02 - *Above Spine Trail*					
		Genus	**Species**	**Common Name**	***
Nov 11 2025	ID: 562378	Ensatina	-	Ensatina	217
		Canis	-	Dog	18
Dec 4 2025	ID: 562321	Peromyscus	-	Deer mice	3181
		Peromyscus	maniculatus	North American deer mouse	867
		Procyon	lotor	Northern raccoon	577
		Melospiza	melodia	Song sparrow	200
		Tamiasciurus	-	Squirrel	37
April 9 2026	ID: 558577	Ambystoma	-	Big salamanders	12919
		Lontra	canadensis	Northern American river otter	227
May 8 2026	ID: 569070	Procyon	lotor	Northern raccoon	585
		Castor	canadensis	American beaver	460
		Ambystoma	-	Big salamanders	317
		Neurotrichus	-	Shrew	123
		Procyon	-	Raccoons	104
		Odocoileus	hemionus	Black-tailed deer	54

Mac_03 - *above derelict dam*					
		Genus	**Species**	**Common Name**	***
Dec 4 2025	ID: 562343	Peromyscus	maniculatus	North American deer mouse	2572
		Peromyscus	aurora	Deer mice	2336
		Procyon	lotor	Northern raccoon	360
		Melospiza	melodia	Song sparrow	101
		Clethrionomys	-	Vole	32

Mac_04 - *Mac's Pond Outlet*					
		Genus	**Species**	**Common Name**	***
Nov 11 2025	ID: 562309	Castor	canadensis	American beaver	2709
		Ambystoma	-	Big salamanders	34
April 9 2026	ID: 562301	Rana	aurora	Red-legged frog	9580
		Taricha	-	Pacific newts	520
		Castor	canadensis	American beaver	367
		Ambystoma	-	Big salamanders	327
		Anas	-	Dabbling ducks	250
		Odocoileus	hemionus	Black-tailed deer	78
		Pseudacris	-	Chorus frogs	19
May 8 2026	ID: 569057	Taricha	-	Pacific newts	13979
		Taricha	granulosa	Rough-skinned newt	2302
		Pseudacris	regilla	Pacific treefrog	2242
		Castor	canadensis	American beaver	1409
		Rana	aurora	Red-legged frog	506
		Ambystoma	-	Big salamanders	130
		Dryocopus	-	Woodpecker	17

Appendix B.

The following is a complete list of qPCR eDNA sample collection data and detection results, provided by the USDA Forest Service National Genomics Center. The first table summarizes sample collection details by site and date, including coordinates, number of filters, total filtered volume, and sample ID tag. The second table presents species-specific detection results for four target taxa: Coho Salmon, Chum Salmon, Coastal Cutthroat Trout, and Rainbow Trout. A "Y" indicates a positive detection, "N" indicates no detection, and 'n/a' indicates that DNA for that species was not assayed for that sample.

Site Number	Date Collected	Field Latitude	Field Longitude	Num of Filters	Total Volume (L)	ID Tag
Mac_01	11/11/2025	47.59725	-122.52088	1	5	WA_111125_MACS_00
Mac_02	11/11/2025	47.59947	-122.52485	1	5	WA_111125_MACS_01
Mac_03	11/11/2025	47.60223	-122.52557	1	5	WA_111125_MACS_02
Mac_01	12/4/2025	47.59725	-122.52088	1	5	WA_120425_MACS_03
Mac_02	12/4/2025	47.59947	-122.52485	1	5	WA_120425_MACS_04
Mac_03	12/4/2025	47.60223	-122.52557	1	5	WA_120425_MACS_05
Mac_01	4/8/2026	47.59725	-122.52088	2	5	WA_040826_MACS_00
Mac_02	4/8/2026	47.59947	-122.52485	2	5	WA_040826_MACS_01
Mac_03	4/8/2026	47.60223	-122.52557	3	5	WA_040826_MACS_02
Mac_01	5/7/2026	47.59725	-122.52088	2	5	WA_050726_MACS_03
Mac_01.5	5/7/2026	47.59831	-122.52271	2	5	WA_050726_MACS_04

Site Number	Date Collected	Coho Salmon DNA Detected?	Chum Salmon DNA Detected?	Coastal Cutthroat DNA Detected?	Rainbow Trout DNA Detected?
Mac_01	11/11/2025	N	N	N	N
Mac_02	11/11/2025	N	N	N	N
Mac_03	11/11/2025	n/a	n/a	N	N
Mac_01	12/4/2025	N	N	N	N
Mac_02	12/4/2025	N	N	N	N
Mac_03	12/4/2025	n/a	n/a	N	N
Mac_01	4/8/2026	N	n/a	N	N
Mac_02	4/8/2026	N	n/a	N	N
Mac_03	4/8/2026	n/a	n/a	N	N
Mac_01	5/7/2026	N	n/a	N	N
Mac_01.5	5/7/2026	N	n/a	N	N