Addendum ADNe
Octobre 2024

Validation
de la présence de l'anguille d'Amérique au bas d'obstacles infranchissables

Réalisé par
l'Organisme de bassins versants de Kamouraska, L'Islet et Rivière-du-Loup

Obakir
ORGANISME DE BASSINS VERSANTS
de Kamouraska, L'Islet et Rivière-du-Loup

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Méthodologie

Effort d'échantillonnage

Certaines modifications ont été amenées au plan d'échantillonnage en fonction des recommandations provenant du MELCCFP. Afin d'améliorer les chances de détections, le nombre de stations témoins sur la rivière Goudron a été nettement diminué dans le but de concentrer l'effort d'échantillonnage sur la rivière Cacouna. Plus précisément, on cherche à valider la présence de l'espèce en amont et en aval du barrage hautement détérioré à St-Épiphane, ainsi que de la chute Ouellet, un autre obstacle potentiellement infranchissable plus en amont. L'emplacement précis de la station témoin ainsi que des six stations de suivi après modification sont présentés dans le tableau 1. Ils sont également représentés sous forme de cartes aux figures 3 et 6 de la section Résultats. Afin de mettre en contexte le nouveau plan d'échantillonnage, la localisation des trois sites retenus (un témoin, deux de suivi) sont présentées à la figure 1.

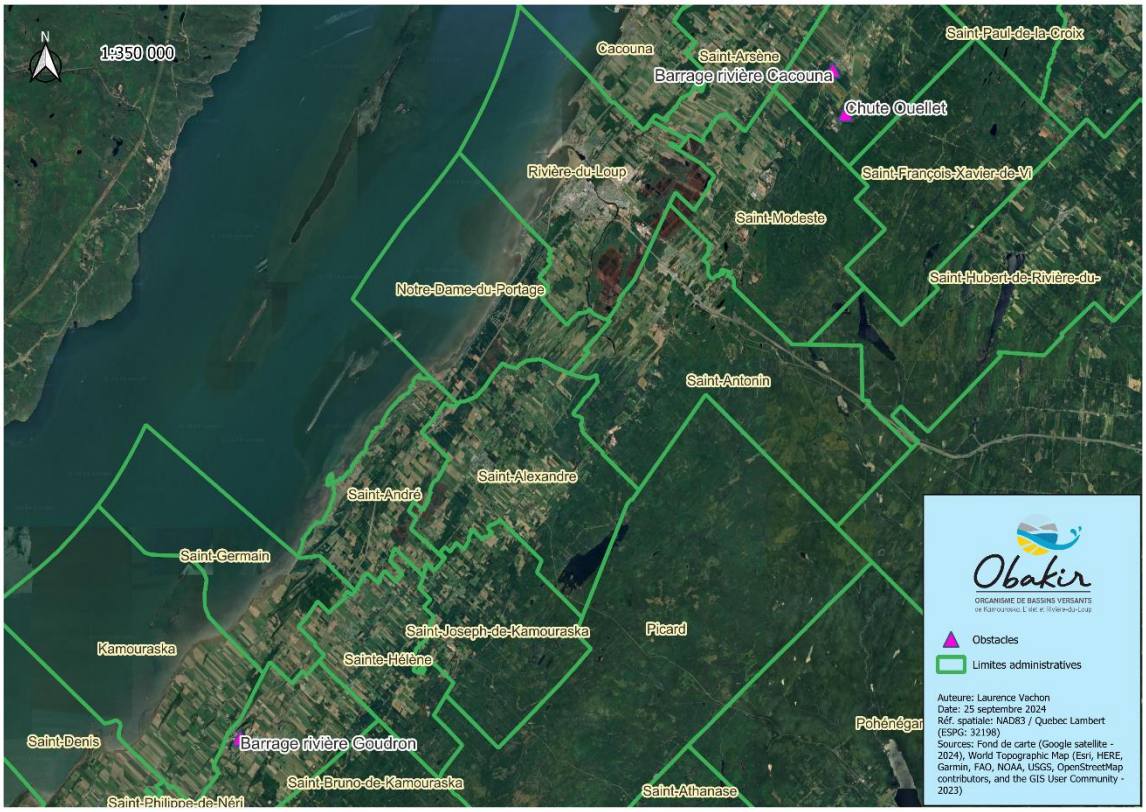


Figure 1. Carte de localisation des sites d'échantillonnages sur les rivières Goudron et Cacouna

Tableau 1. Coordonnées des stations d'échantillonnage d'ADNe et principaux obstacles à la libre circulation de l'anguille d'Amérique

Identifiant	**Descriptif**	**Longitude**	**Latitude**
Barrage rivière Cacouna	Obstacle	616044.8847755624	5314521.3714786535
Barrage rivière Goudron	Obstacle	582067.0411239692	5272268.1601067549
Chute Ouellet	Obstacle	616947.1485380904	5311935.4037539857
Station Cac_01	Aval chute Ouellet	616935.9447530652	5311936.1701292144
Station Cac_02	Amont chute Ouellet	616972.8736375618	5311921.9671875024
Station Cac_03	Aval barrage rive gauche	616033.8981521282	5314531.7683677590
Station Cac_04	Aval barrage rive droite	616039.2135583512	5314535.0465610744
Station Cac_05	Amont barrage rive droite (aval obstacle en bois)	616051.8089555994	5314515.3657096755
Station Cac_06	Amont barrage, rive gauche (~20m en amont du barrage)	616046.8605123369	5314505.4841832547
Station témoin Goud_01	Station témoin	581924.1973129886	5272257.1951268455

Procédure de collecte

Les trois prochaines sections détaillent les différentes procédures utilisées par l'équipe pour l'échantillonnage de l'ADNe, soit les procédures de collecte, filtration et préservation.

1. Identifier tous les flacons d'échantillons à l'aide d'un marqueur permanent. Plus d'une bouteille d'échantillon peut être nécessaire, selon le plan d'échantillonnage sur le terrain.
2. Mettre une nouvelle paire de gants propres sur chaque site de collecte et éviter tout contact entre les gants et autre chose que les flacons de collecte.
3. Rincer tous les flacons d'échantillon et leurs couvercles trois fois chacun en collectant l'eau de surface à proximité du lieu de prélèvement des échantillons, en fermant légèrement les couvercles et en secouant les flacons. Jetez cette eau loin du lieu d'échantillonnage (en aval ou sur le rivage/sol).
4. Remplir les flacons d'échantillons avec 1 L d'eau provenant de la surface du site de prélèvement et serrer les couvercles. Placer immédiatement ces bouteilles dans la glacière sur de la glace.

Procédure de filtration

1. Faire passer le tube de la pompe à la fiole à vide, fixer le bouchon en caoutchouc à la fiole à vide et brancher la pompe à la source d'alimentation.
2. Insérer l'adaptateur d'entonnoir en plastique dans le bouchon en caoutchouc afin que le joint soit hermétique. Retirer le filtre entonnoir de son emballage et fixez-le à l'adaptateur. Faire attention à ne pas toucher l'intérieur de l'entonnoir pour éviter toute contamination.
3. Retourner doucement le flacon de prélèvement d'échantillon plusieurs fois pour mélanger l'échantillon, puis versez lentement l'échantillon dans l'entonnoir du filtre jusqu'à ce que l'entonnoir soit rempli. Noter le volume distribué et l'heure de démarrage du filtre.
4. Allumer la pompe pour commencer la filtration. Ajouter de l'échantillon d'eau par petits incréments à mesure que le vide aspire l'eau dans le flacon pendant

la filtration pour éviter d'avoir à verser de l'eau non filtrée qui pourrait ne pas passer si le filtre est obstrué et que le taux de filtration ralentit ou s'arrête.

5. Une fois la filtration terminée, utiliser la tasse à mesurer pour enregistrer le volume d'eau échantillon qui a traversé la membrane dans le flacon et enregistrer la durée totale de filtration.

Procédure de préservation

1. Identifier une enveloppe de transport appropriée.
2. Avant de retirer chaque filtre d'échantillon de l'entonnoir du filtre, nettoyer deux paires de pinces dans une tasse contenant une solution d'eau de Javel à 50 % pendant au moins une minute, puis les faire tourbillonner dans une autre tasse d'eau déminéralisée/distillée. Ensuite, les placer dans une deuxième tasse d'eau déminéralisée/distillée pendant au moins 30 secondes afin d'empêcher la contamination et les dommages à l'ADNe dus à l'eau de Javel résiduelle.
3. Si l'échantillon est conservé dans de la silice, s'assurer que la membrane est sèche en aspirant de l'air à travers le filtre pendant 3 à 5 minutes avec la pompe à vide une fois que toute l'eau est passée à travers. En cas de conservation dans l'éthanol, la membrane n'a pas besoin d'être complètement sèche.
4. Briser doucement le joint entre l'entonnoir du filtre et l'adaptateur d'entonnoir en tirant l'entonnoir du filtre vers le haut. Ne pas tordre l'entonnoir du filtre, car cela pourrait endommager la membrane de l'échantillon et entraîner une perte d'échantillon.
5. À l'aide de la pince propre, plier soigneusement la membrane filtrante en deux une fois (pour la conservation de la silice) ou deux fois (pour la conservation de l'éthanol) avec le filtrant à l'intérieur du pli.
6. Placer le filtre plié dans l'enveloppe de transport et la mettre dans un sac *Ziplock* avec des billes de silice et fermer le sac. Identifier adéquatement le sac. Pour éviter toute contamination, conserver les enveloppes de transport provenant de différents sites dans différents sacs *Ziplock*.

Expédition

Les échantillons préservés sont ensuite expédiés par transporteur au laboratoire Bureau Veritas, situé en Guelph en Ontario.

Pour plus de détails sur les procédures d'échantillonnage et de laboratoire, se référer aux annexes II à IV.

Résultats

Les figures 2 et 3 ci-dessous présentent les résultats de la station témoin sur la rivière Goudron. La première contient les résultats d'analyse provenant de Bureau Veritas pour la station témoin ainsi que pour le contrôle de qualité (blanc terrain), tandis que la seconde permet de localiser ces résultats par rapport à l'obstacle ciblé.

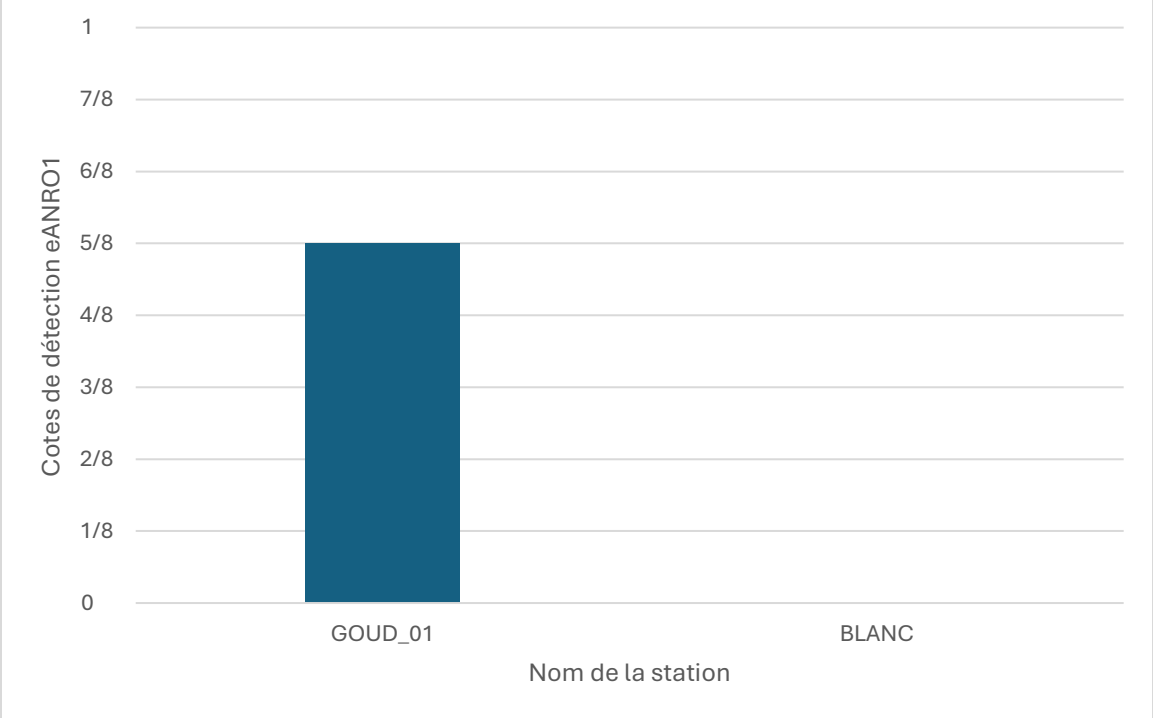


Figure 2. Cotes de détection de la station témoin GOUD_01 et du blanc terrain échantillonnés le 3 juillet 2024 sur la rivière Goudron à Saint-Pascal

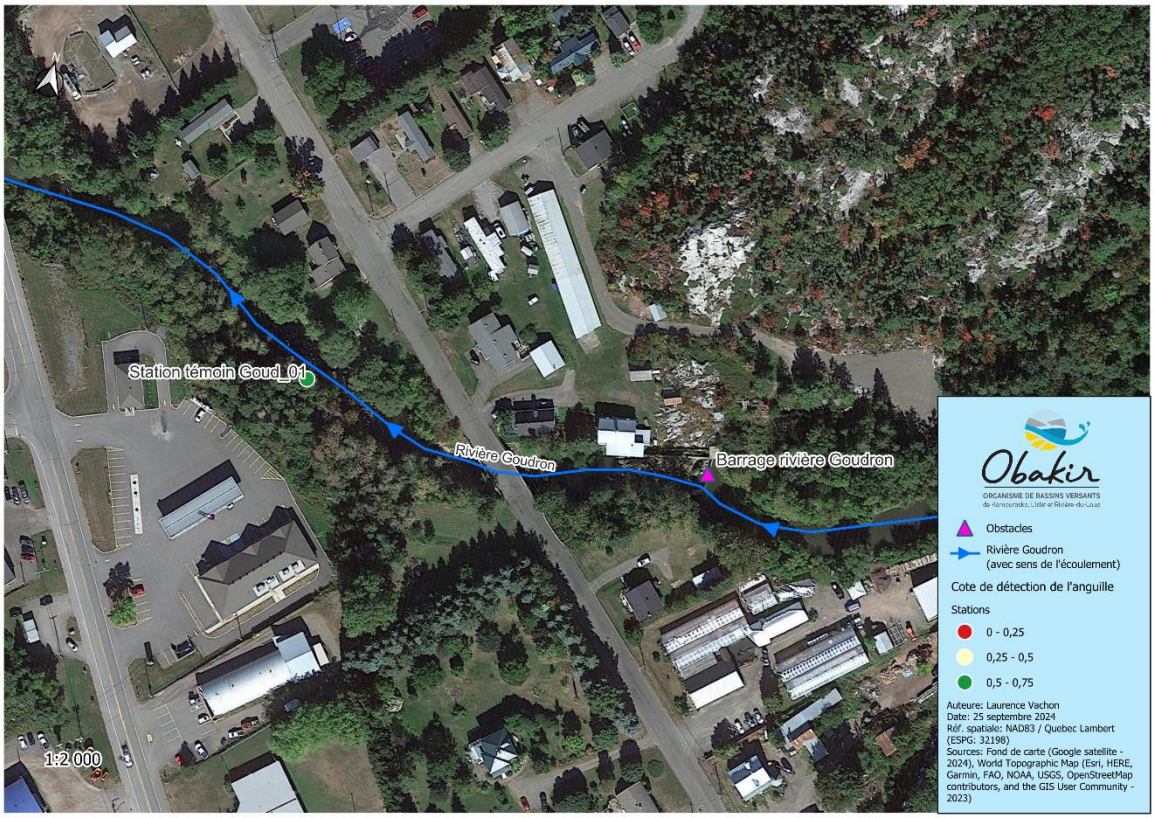


Figure 3. Carte de localisation de la station témoin GOUD_01 et du barrage sur la rivière Goudron

L'analyse de ces résultats nous confirme que la méthode d'échantillonnage est fiable puisque l'ADNe est bel et bien détecté là où les anguillettes ont été observées à l'année 2023 (voir figure 4 et tableau 2), alors que le blanc terrain (ou contrôle de qualité) est exempt de toute détection.

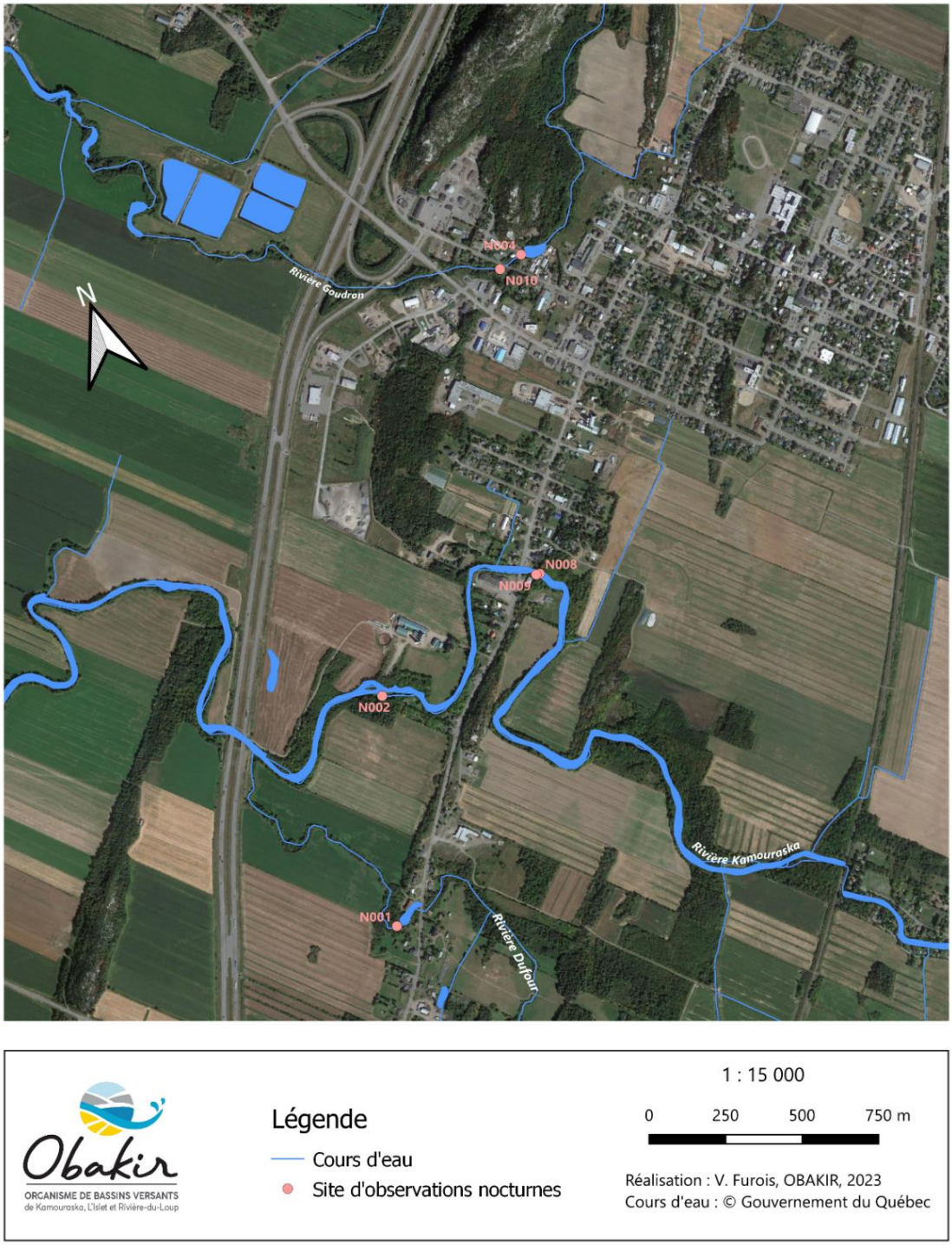


Figure 4. Localisation des sites des observations nocturnes, réalisé en 2022 et 2023, dans le bassin versant de la rivière Kamouraska.

Tableau 2. Résultats des observations nocturnes de la montaison de l'anguille d'Amérique, dans les bassins versants des rivières Kamouraska, Verte et Saint-Jean, en 2022 et 2023.

Bassin versant de la rivière	Rivière	Date	Station no	Capture
Kamouraska	Rivière Kamouraska	2022-07-19	N008	Non
		2022-07-19	N009	Non
		2022-07-29	N002	Non
		2023-07-04	N009	**Oui**
		2023-08-01	N002	Non
	Rivière Goudron	2022-07-19	N004	Non
		2022-07-19	N010	Non
		2022-07-27	N010	Non
		2023-07-03	N004	**Oui**
	Rivière Dufour	2022-07-19	N001	Non
		2022-07-21	N001	Non
		2022-07-27	N001	Non
		2023-07-04	N001	Non
		2023-07-10	N001	Non
Verte	Ruisseau de la Montagne	2022-07-26	N013	Non
	Ruisseau de la Pépinière	2022-07-26	N012	Non
	Rivière Verte	2022-07-27	N014	**Oui**
	Rivière Cacouna	2023-08-02	N015	Non
Saint-Jean	Rivière Saint-Jean	2023-07-05	N007	**Oui**

D’autre part, les figures 5 et 6 présentent les résultats d’analyses des échantillons pris lors des trois visites terrains des six stations sur la rivière Cacouna tout au long de l’été 2024. Grâce à la figure 5, il est également possible d’observer l’évolution temporelle du niveau de confiance quant à la présence d’anguille dans le cours d’eau, ainsi que la moyenne des trois analyses pour chaque station. Les trois moments d’échantillonnages ont été choisis afin de couvrir l’ensemble de la période de migration de l’anguille vers ses habitats de croissance au Bas-Saint-Laurent. Selon le récent recueil des protocoles standardisé du MELCCFP (2023), celle-ci s’étendrait de juin à août pour la rivière du Sud-Ouest (située entre Saint-Mathieu-de-Rioux et le havre du Bic). Quant à elle, la figure 6 sert à localiser ces mêmes résultats dans l’espace, en se fiant exclusivement à la moyenne cette fois.

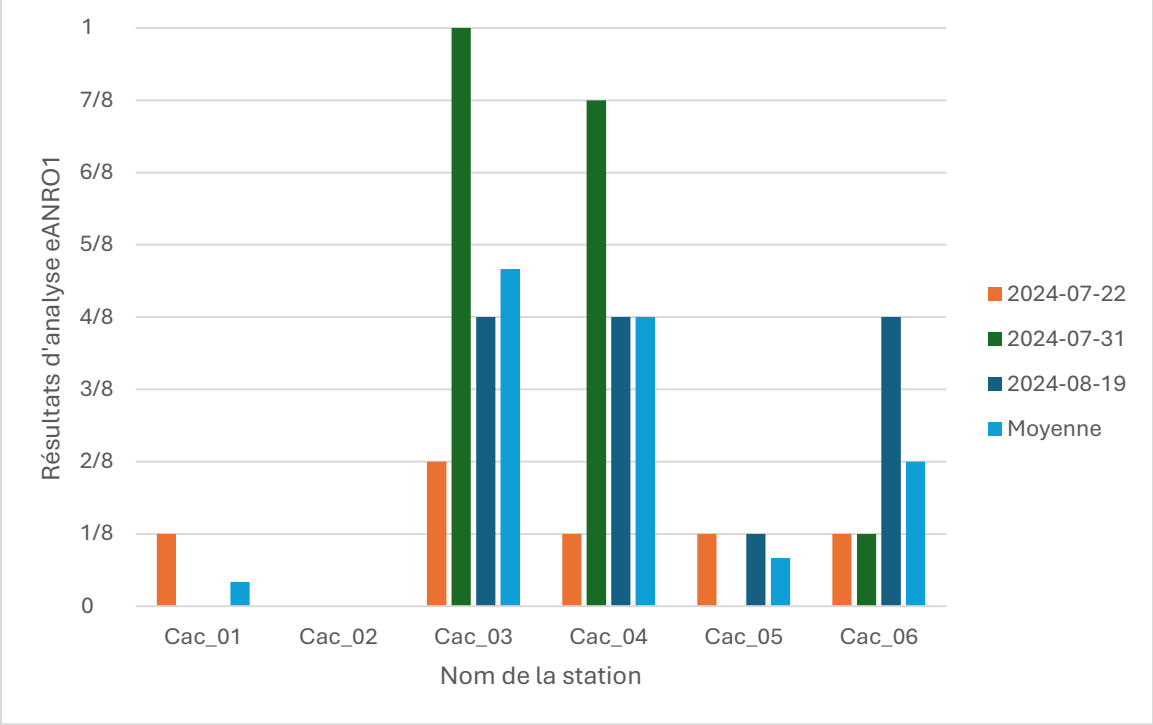


Figure 5. Cotes de détection des stations Cac_01 à Cac_06 échantillonnées du 22 juillet au 19 août 2024 sur la rivière Cacouna

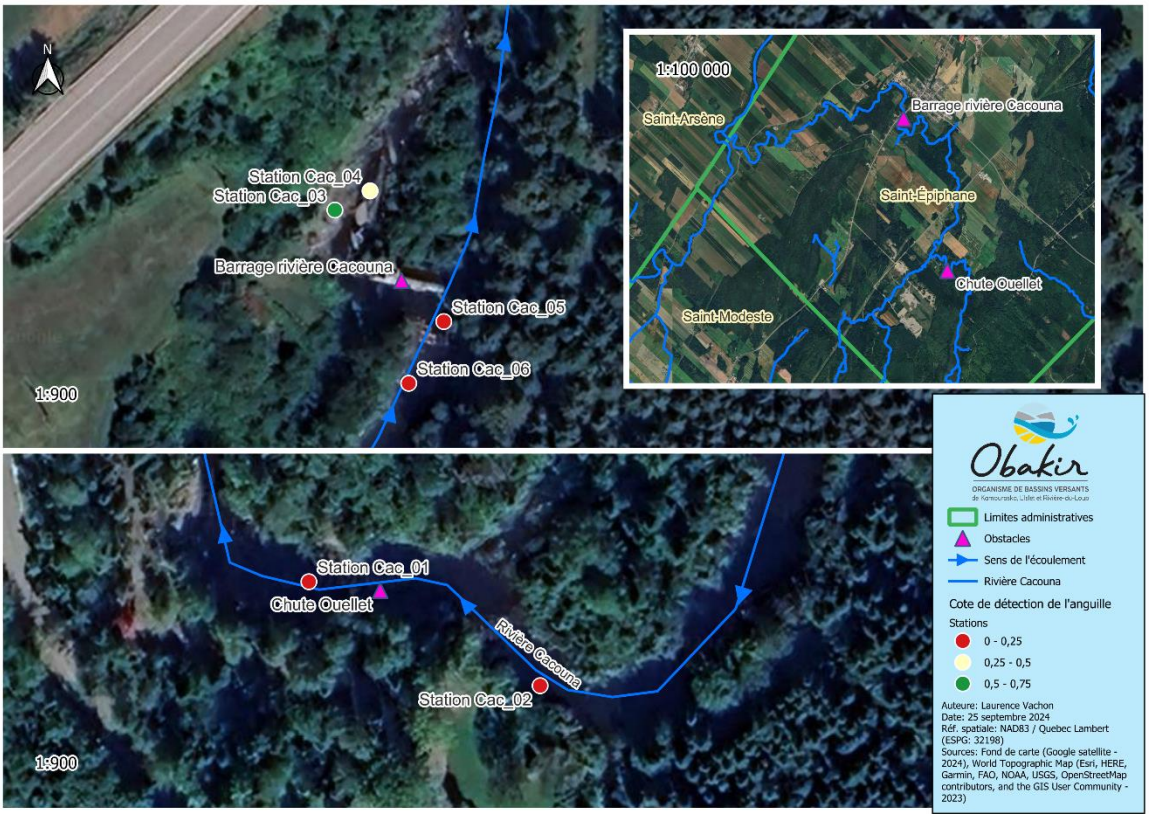


Figure 6. Carte de localisation des stations de suivi et des obstacles sur la rivière Cacouna

Ces dernières figures permettent de confirmer avec un niveau de confiance relativement élevé la présence d'anguille d'Amérique en aval du barrage sur la rivière Cacouna (stations Cac_03 et Cac_04). En effet, ce sont les deux stations présentant les cotes de détection les plus élevées avec une détection maximale de 8/8 pour Cac_03 et de 7/8 pour Cac_04. Autrement dit, les « réplicas techniques » des échantillons prélevés le 31 juillet ont pratiquement tous permis la détection de traces d'ADN environnemental, ce qui permet de supposer qu'il s'agit du pic de montaison des anguillettes.

Conclusion

Les résultats de l'échantillonnage d'ADNe présentés ci-dessus portent à croire que le barrage de la rivière Cacouna (no X0000650) représenterait un obstacle très sélectif à la montaison de l'anguille ou « franchissable avec réserve ». En effet, seul un (1) réplica technique sur huit (/8) a permis de détecter la présence d'ADN d'anguille. De plus, une anguille aurait été observée en 1991 en amont du barrage construit en 1915 (MELCCFP, communication personnelle, 2024). Cette conclusion est plus ou moins cohérente avec les observations faites par l'équipe d'AECOM en

2024. La note technique remise par la firme à l'OBV mentionnait notamment que « *la cote de franchissabilité du barrage X0000650 théorique en amont donné par Tremblay et coll. (2011 et 2016) est de 4, ce qui signifie infranchissable* ». Les photographies du site prises par l'équipe d'OBAKIR à l'été 2023 (voir figure 7, 8 et 9) permettent de supposer que « les voies de contournement, sur les rives, ne semble pas propice à l'anguille ». En effet, *« l'absence de voie humide dans une pente acceptable, la vélocité de l'écoulement de l'eau dans la brèche principale, la présence d'un écart de chute, sont des éléments qui laissent présumer que*

Figure 7. Vue du barrage X0000650 sur la rivière Cacouna (OBAKIR, 2023)

l'infrastructure est infranchissable avec réserve pour cette espèce (AECOM, 2024). » Ainsi, au-delà de l'interprétation photographique, des mesures de la hauteur et de la longueur actuelles de l'infrastructure devraient être prises, afin de valider la cote de franchissabilité attribuée.

Par ailleurs, les professionnels d'AECOM (2024) soulevaient également que « l'état très dégradé [du barrage] mène directement à favoriser un scénario à préconiser et à mettre en avant-plan, soit son démantèlement ou une voie de contournement. » Selon leur estimation, ces interventions permettraient de recouvrir des gains potentiels en habitat [pour l'anguille d'Amérique] d'un minimum de 22,60 ha et

Figure 8. Vue rapprochée du barrage X0000650 de la rivière Cacouna. Il est possible d'observer qu'il existe des fissures dans le barrage (OBAKIR, 2023)

jusqu'à 78,60 ha, tout dépendant de la franchissabilité des ponceaux en amont du barrage. De plus, l'absence de barrage hydroélectrique dans ce bassin versant minimise les risques de mortalité lors de la dévalaison de l'anguille argentée (AECOM, 2024).

Recommandations

La présence d'anguille d'Amérique validée en aval du barrage de la rivière Cacouna qui semble très sélectif à la montaison de l'espèce justifient d'entamer les démarches nécessaires afin de démanteler l'infrastructure. Cette intervention permettrait de rétablir la connectivité faunique pour

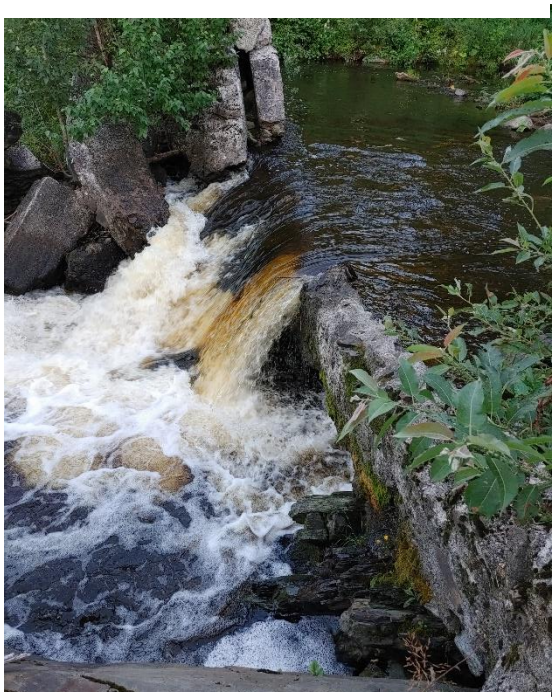


Figure 9. Rive droite du barrage X0000650 de la rivière Cacouna. Il est possible d'observer que l'eau s'écoule sous le muret de la rive droite (OBAKIR, 2023)

l’anguille et potentiellement d’autres espèces tel que l’omble de fontaine et la perchaude.

L’équipe d’OBAKIR recommande donc :

1. De valider la présence/absence et la franchissabilité d’obstacles naturels et anthropiques encore non-visités en amont du barrage. Notamment, il serait intéressant d’évaluer la cote de franchissabilité de la chute identifiée en amont du ponceau V16 (voir figure 10) et les risques de mortalité qu’elle pourrait représenter lors de la dévalaison de l’anguille argentée. Ces nouvelles données permettraient de s’assurer que l’intervention restitue bel et bien le maximum de gains d’habitats potentiels évalués par la firme AECOM.
2. Mener une étude d’avant-projet, afin d’évaluer les impacts du démantèlement du barrage sur le milieu hydrique récepteur en aval de l’infrastructure à court, moyen et long terme. Notamment, en considérant que « des débris dans le cours d’eau proviennent probablement du barrage lui-même qui s’est dégradé au fil des années. Les structures actuelles sont également à risques d’être détruites par les eaux et de se trouver dans le cours d’eau dans un futur prochain (AECOM, 2024). » Ladite étude d’impact est d’ailleurs un des prérequis pour l’obtention des autorisations requises pour la suppression du barrage, soit notamment une autorisation obtenue en vertu de l’article 22 de la LQE.
3. De former une équipe de projet avec une série de partenaires dont, minimalement, la Première Nation Wolastoqiyik Wahsipekuk et/ou les professionnels de l’Association de gestion halieutique autochtone Mi’gmaq et Wolastoqey**,** chargée de la recherche de financement et la mise en œuvre du projet déposé conjointement.
4. De réaliser les travaux nécessaires à la mise en œuvre de l’approche retenue pour rétablir la libre circulation de l’anguille vers les habitats de croissance potentiels en amont du barrage de la rivière Cacouna.

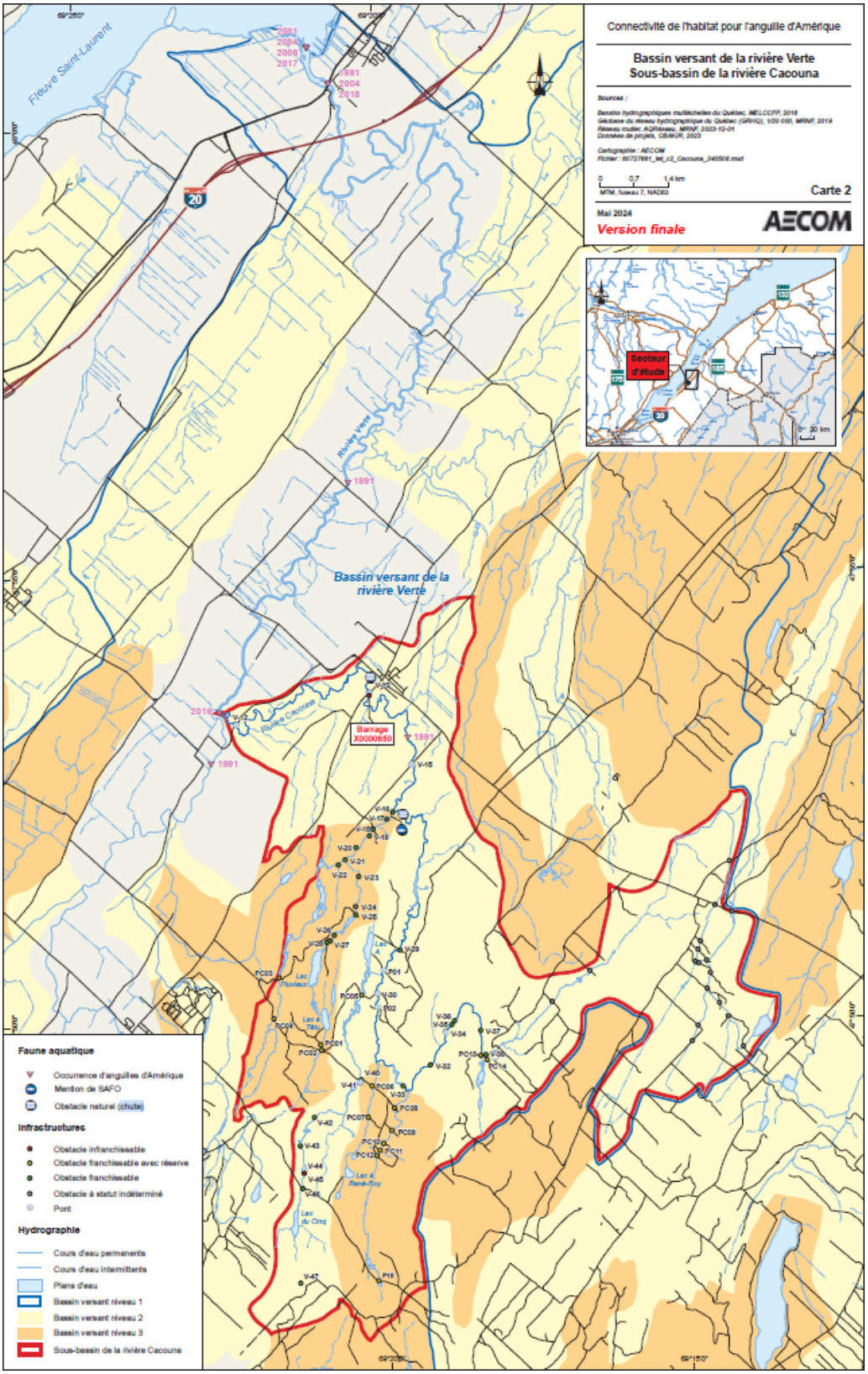


Figure 10. Carte de la connectivité de l'habitat pour l'anguille d'Amérique extraite de la note technique d'AECOM (2024)

Références

AECOM (2024). Note technique - Rivières Cacouna - Anguille d'Amérique, calcul de gains d'habitats et identification de scénarios d'aménagements des barrages, 60727661 – version Finale

LANDRY-MASSICOTTE, L., et J. DUSSUREAULT (2023). Estimation de l'abondance et caractérisation des anguilles d'Amérique (Anguilla rostrata) provenant des transferts dans la pêcherie commerciale de l'estuaire du Saint-Laurent en 2022. Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs, Direction de la gestion de la faune du Bas-Saint-Laurent, 18 pages

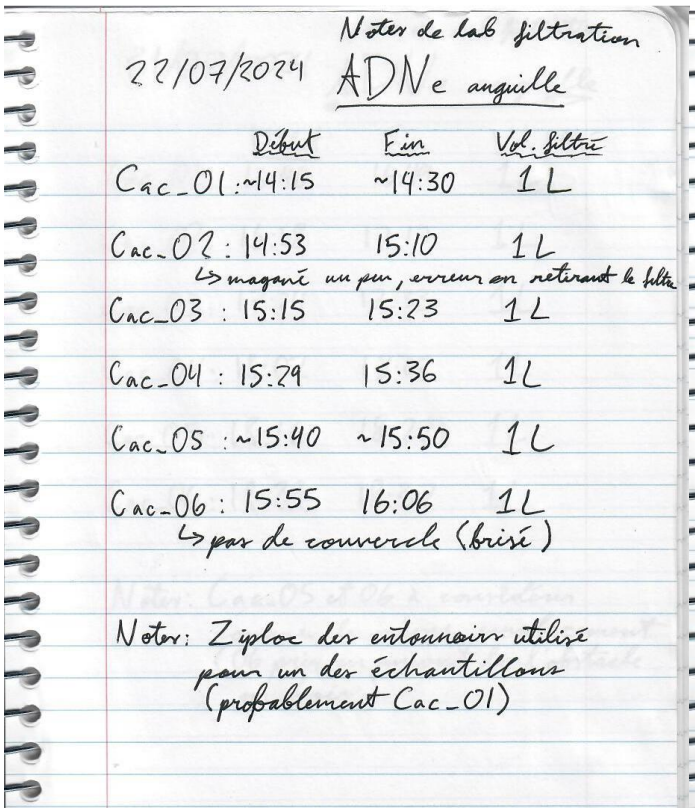
Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs. 2023. Inventaire de l'anguille d'Amérique aux obstacles infranchissables au Québec, recueil des protocoles standardisés. Gouvernement du Québec, Québec. 41 p. et annexes

OBAKIR. 2023. *Validation de la présence de l'anguille d'Amérique au bas d'obstacles infranchissables*. Organisme de bassins versants de Kamouraska, L'Islet et Rivière-du-Loup. Rapport final. 52 p. dont 4 annexes.

Tremblay, V., Cossette, C., Dutil, J.-D., Verreault, G., Dumont, P., 2016, Assessment of upstream and downstream passability for eel at dams, ICES Journal of Marine Science 73 (1):22-32.

Tremblay, V., Cossette, C., Dutil, J.-D., Verreault, G., Dumont, P., 2011, Évaluation de la franchissabilité amont et aval pour l'anguille aux barrages, Rapport technique canadien des sciences halieutiques et aquatiques 2912, 70 p. + 1 annexe.

Annexe I : Notes de laboratoire de filtration (OBAKIR, 2024)



Notes de lab filtration

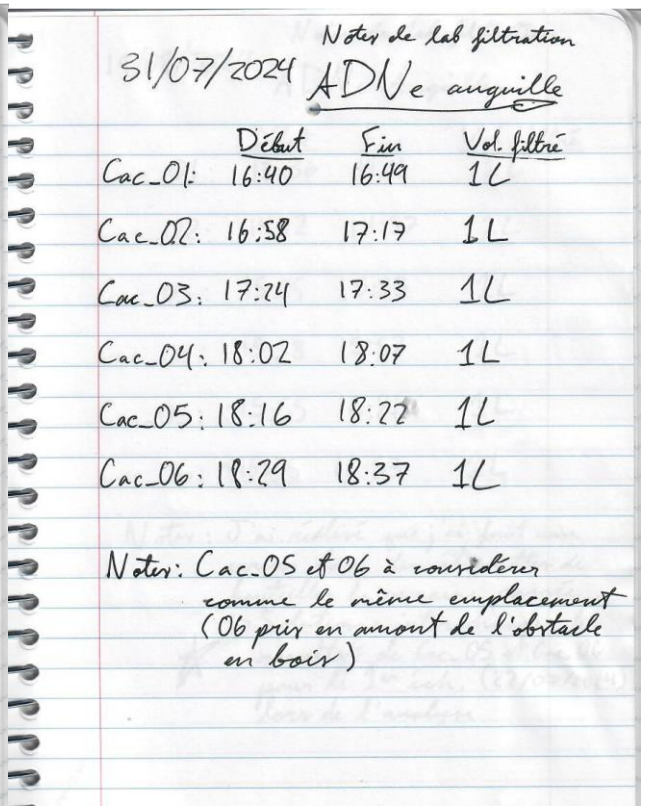
22/07/2024 ADNe anguille

	Début	Fin	Vol. filtré
Cac_01	~14:15	~14:30	1 L
Cac_02	14:53	15:10	1 L
Cac_03	15:15	15:23	1 L
Cac_04	15:29	15:36	1 L
Cac_05	~15:40	~15:50	1 L
Cac_06	15:55	16:06	1 L

↳ magané un peu, erreur en retirant le filtre

↳ pas de couvercle (brisé)

Noter: Ziploc des entonnoirs utilisé pour un des échantillons (probablement Cac_01)

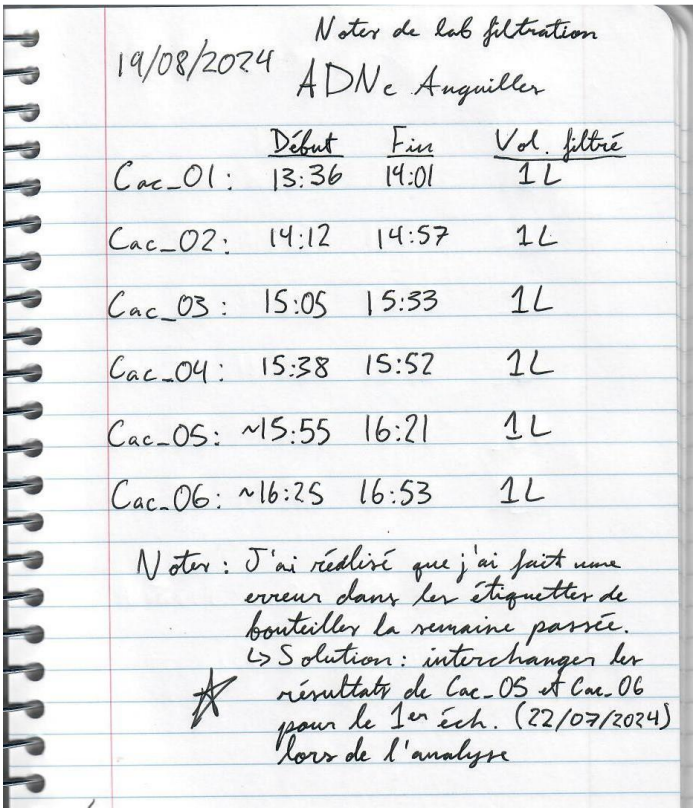


Notes de lab filtration

31/07/2024 ADNe anguille

	Début	Fin	Vol. filtré
Cac_01	16:40	16:49	1 L
Cac_02	16:58	17:17	1 L
Cac_03	17:24	17:33	1 L
Cac_04	18:02	18:07	1 L
Cac_05	18:16	18:22	1 L
Cac_06	18:29	18:37	1 L

Noter: Cac_05 et 06 à considérer comme le même emplacement (06 pris en amont de l'obstacle en bois)



Notes de lab filtration

19/08/2024 ADNe Anguilles

	Début	Fin	Vol. filtré
Cac_01	13:36	14:01	1 L
Cac_02	14:12	14:57	1 L
Cac_03	15:05	15:33	1 L
Cac_04	15:38	15:52	1 L
Cac_05	~15:55	16:21	1 L
Cac_06	~16:25	16:53	1 L

Noter: J'ai réalisé que j'ai fait une erreur dans les étiquettes de bouteilles la semaine passée.
↳ Solution: interchanger les résultats de Cac_05 et Cac_06 pour le 1er éch. (22/07/2024) lors de l'analyse

Annexe II : Certificats d'analyse et méthode qPCR

Attention: **Véronique Furois**
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: anguille
Site Location: N/A
C.O.C. #: 20240708-1
Quote #: N/A
PO#: N/A

Report Date: 2024/07/11
Report #: OB20240711
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20240708-1
Received: 2024/07/08, 10:14 A.M.

Sample Type: Cellulose Nitrate (CN) filter, preserved in silica
Samples Received: 2

Analyses (eDNA Isolation - Species)	Test Requested	Test Performed	Date eDNA Extracted	Date Analyzed IntegritE-DNA™	Date Analyzed Target Species	Laboratory Method	Analytical Method (qPCR Primer/Probe set)
eDNA Isolation and IntegritE-DNA™	2	2	2024-07-08	2024-07-09	N/A	GUE SOP-00056	ePlant5
American Eel (*Anguilla rostrata*)	2	2	N/A	N/A	2024-07-10	GUE SOP-00056	eANRO1

Remarks:

Bureau Veritas Laboratories (Animal DNA Department, DNA Services) is accredited to ISO17025:2017 for eDNA testing.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by industry professionals using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Bureau Veritas Laboratories in writing). All data has met quality control and method performance criteria unless otherwise noted.

Bureau Veritas Laboratories' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Bureau Veritas Laboratories has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Bureau Veritas Laboratories unless otherwise agreed in writing. Bureau Veritas Laboratories is not responsible for the accuracy or any data impacts that result from the information provided by the customer or their agent.

Results relate to supplied samples tested. This Certificate should not be reproduced except in full, without the written approval of the laboratory.

eDNA tests are used to confirm presence of eDNA in samples for the targeted species / species groups.

Collected eDNA samples will contain eDNA at various stages of degradation, being subject to environmental forces that breakdown DNA, including microbial activity, ultraviolet radiation, heat, hydrolysis, and enzymatic activity. eDNA is first evaluated for eDNA quality and presence of qPCR assay inhibitors using the IntegritE-DNA™ assay before testing for target species or genera to confirm that the eDNA is of sufficient quality for testing and to identify and address qPCR inhibition (if present) to avoid false negatives.

SAMPLE RETENTION: Samples and DNA extracts generated from the samples will be retained by Bureau Veritas Laboratories for a period of 90 days after which time they will be discarded unless prearrangement has been made by client with Bureau Veritas Laboratories for longer storage.

Attention: Véronique Furois
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: anguille
Site Location: N/A
C.O.C. #: 20240708-1
Quote #: N/A
PO#: N/A

Report Date: 2024/07/11
Report #: OB20240711
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20240708-1
Received: 2024/07/08, 10:14 A.M.

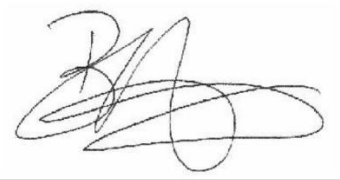
Methodology for Sample Analysis

Samples are logged in upon receipt. Samples were stored in freezer until processing in the laboratory. Sample analysis is completed within 10 business days (as indicated by the client on the COC) following receipt of samples by the testing laboratory.

eDNA isolation is completed using the DNeasy Blood & Tissue Kit™ (QIAGEN). A negative control is included as a blank filter sample with each batch of eDNA isolation to monitor for potential laboratory contamination during the eDNA isolation process.

Following eDNA isolation (150µL) from a quarter of filter, the IntegritE-DNA™ assay[1] is used to avoid the potential of a false negative (Type II error) during target species or genera testing. The IntegritE-DNA™ assay evaluates the integrity of eDNA for suitability for qPCR and for presence of qPCR inhibitors which may reduce the effectiveness of the qPCR assay for target species or genera. This assay evaluates the quality of eDNA to assess whether it is amplifiable using a qPCR assay that targets the chloroplast genome derived from plants/algae that are ubiquitously found in fresh water systems. Four technical replicates per eDNA sample, four technical replicates of negative control (Ultrapure water), and two technical replicates of positive control are used for the IntegritE-DNA™ assay. The cut-off Ct (qPCR cycle threshold) value for the IntegritE-DNA™ assay is 27 due to inhibition. If the IntegritE-DNA™ assay produces a positive detection frequency of ≥ 2 of the 4 technical replicates, this indicates that the eDNA for the target taxa is likely to be of sufficient quality to be detected (if present) with the target assay. If the IntegritE-DNA™ assay produces a positive detection frequency < 2 of the 4 technical replicates (eDNA is degraded or qPCR inhibitors are present), then sample cleanup is completed using the OneStep PCR Inhibitor Removal Kit™ (ZYMO Research) to remove potential qPCR assay inhibitors from the isolated eDNA. Subsequent to inhibitor removal, the IntegritE-DNA™ assay is repeated to re-assess whether the eDNA is of sufficient quality for qPCR. If a sample fails at the IntegritE-DNA™ assay (Ct Value over 30) for the second time the client will be informed that the quality of the sample is insufficient for the qPCR assay. eDNA indicator (IntegritE-DNA™) in the sample suggests that degradation has taken place and therefore the target species assay may be ineffective. Once a sample passes the IntegritE-DNA™ assay, then the target species or genera assay is performed. Eight technical replicates per eDNA sample, eight technical replicates of the negative control (Ultrapure water), and two technical replicates of positive control (total DNA or synthetic DNA) are used for the target species or genera assay to assess the detection or non-detection of DNA of the target species or genera. The cut-off Ct value for target species assay is 50.

[1] Hobbs J, Round JM, Allison MJ, Helbing CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849.



BECKY HENDERSON
Senior Customer Service Representative, Bureau Veritas Laboratories, DNA Services
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Phone #: (519) 836 2400 Ext. 7067714

Please direct all questions regarding this Certificate of Analysis to your Customer Service Representative above.

==========

For Service Group specific validation please refer to the Validation Signature Page.

Total Cover Pages: 2

BUREAU VERITAS

BV JOB #: E20240708-1
Report Date: 2024/07/11
Report #: OB20240711
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: N/A
Sampler Initials: N/A

RESULTS

Client Sample ID	BV Case ID	Sample Collection Date	IntegritE-DNA™ Positive detection (Ct≤27)[1]	QC Batch	Clean-up required	IntegritE-DNA™ Positive detection (Ct≤30)[1] after clean-up	QC Batch	Analytical Method (qPCR Primer/Probe set)	Target Species eDNA Positive detection (Ct≤50)[2]	QC Batch
GOUD_01	OB20240001	2024-07-03	4/4	240709Q1	No	N/A	N/A	eANRO1[3]	5/8	240710Q2
BLANC	OB20240002	2024-07-03	4/4	240709Q1	No	N/A	N/A	eANRO1	0/8	240710Q2

[1] **IntegritE-DNA™ Assay:** Four technical replicates were assayed for each eDNA sample. **Field samples:** The cut-off Ct value for IntegritE-DNA assay is 27 based on previous observation and due to presence of inhibitors. **Post clean-up and field blank samples:** The cut-off Ct value for IntegritE-DNA assay is 30. Results are reported as the number of positive detections (n) out of a total of 4 technical replicates, n/4.

[2] **Target Species Assay:** Eight technical replicates were assayed per eDNA sample. The cut-off Ct value for target species assay was 50. Results are reported as the number of positive detections (n) out of a total of 8 technical replicates, n/8.

[3] eANRO1: qPCR primer/probe assay to assess the presence of American Eel (*Anguilla rostrata*) eDNA.

GENERAL COMMENTS

eDNA is extracted (150 µL) from a quarter of filter, and 2 µL is used as a template for each technical replicate.

Results relate only to the items tested.

QUALITY ASSURANCE REPORT

			eDNA Isolation Negative Control[1]		qPCR Positive Controls[2]		qPCR Negative Controls[3]	
QC Batch	Parameter	Date	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail
240709Q1	IntegritE-DNA	2024-07-09	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
240710Q2	eANRO1	2024-07-10	eDNA Isolation Negative Control is assessed using IntegritE-DNA only once for each extraction batch.	N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass

[1] **eDNA Isolation Negative Control:** Blank filters were included for each batch of eDNA extraction to monitor for laboratory contamination during eDNA isolation. eDNA Isolation Negative Control is assessed using IntegritE-DNA™ only. QC results show no eDNA was isolated from the negative control, therefore there was no indication of sample contamination during handling. Acceptance criteria: 0 of 4 technical replicates

[2] **qPCR Positive Controls:** Two technical replicates of isolated eDNA from freshwater sample were used as positive controls for IntegritE-DNA™. Two technical replicates of total DNA or synthetic DNA from the target species were used as positive controls for eDNA assays. Results show that 100% of the technical replicates amplified the positive control eDNA as expected, therefore an observation of negative result in eDNA samples is not related to the qPCR performance. Acceptance criteria: 2 of 2 technical replicates

[3] **qPCR Negative Controls (Ultrapure water):** Four technical replicates for IntegritE-DNA™ and eight technical replicates for target species or genera were used to monitor for laboratory contamination. Results show that 0% of the technical replicates in the negative controls had amplified eDNA, indicating no contamination was detected. Acceptance criteria: 0 of 4 technical replicates for IntegritE-DNA™, and 0 of 8 technical replicates for other assays.

LABORATORY RESULTS VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Reporter: ALI MIRABZADEH, M.Sc.
Laboratory Supervisor, Bureau Veritas, Forensic and DNA Services

==========

BUREAU VERITAS

BV JOB #: E20240708-1
Report Date: 2024/07/11
Report #: OB20240711
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: N/A
Sampler Initials: N/A

American Eel (*Anguilla rostrata*) eDNA Assay Validation Information

eDNA assay Validation

All eDNA assays are validated through a rigorous multi-step evaluation protocol at University of Victoria that includes tests of DNA target specificity and amplification sensitivity. All eDNA tests available at Bureau Veritas Laboratories have been validated for performance using interlaboratory verification.

General eDNA Assay Information

Target Species: American Eel (*Anguilla rostrata*)
Species Code: te-ANRO
eDNA qPCR Tool: eANRO1
eDNA qPCR Format: TaqMan
Gene Target: MT-CYB
Published in: N/A

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA

LOD **0.4** 95% CI **0.3-0.7** Copies LOQ **1.5** 95% CI **1.1-2.6** Copies
LOB **0** hits/8

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].
When the LOQ < LOD, use the LOD for the LOQ.

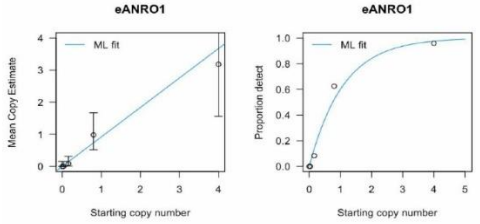
Enzyme: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (Species)	Detection	Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-ANRO	American Eel (*Anguilla rostrata*)	Yes	8	Prince Edward Island
te-COCO	Slimy Sculpin (*cottus cognatus*)	No	1	Yukon
te-ESLU	Northern Pike (*Esox lucius*)	No	1	British Columbia
te-MIDO	Smallmouth Bass (*Micropterus dolomieu*)	No	1	British Columbia
te-MISA	Largemouth Bass (*Micropterus salmoides*)	No	1	British Columbia
te-ONCL	Cutthroat Trout (*Oncorhynchus clarkii*)	No	1	British Columbia
te-ONGO	Pink Salmon (*Oncorhynchus gorbuscha*)	No	1	British Columbia
te-ONKE	Chum Salmon (*Oncorhynchus keta*)	No	1	British Columbia
te-ONKI	Coho Salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
te-ONMY	Rainbow Trout (*Oncorhynchus mykiss*)	No	1	British Columbia
te-ONNE	Sockeye Salmon (*Oncorhynchus nerka*)	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tschawytscha*)	No	1	British Columbia
te-PRCY	Round Whitefish (*prosopium cylindraceum*)	No	1	Yukon
te-SACO	Bull Trout (*Salvelinus confluentus*)	No	1	Alberta
te-SAMA	Dolly Varden (*Salvelinus malma*)	No	1	British Columbia
te-SASA	Atlantic Salmon (*Salmo Salar*)	No	1	Nova Scotia
te-THAR	Arctic Grayling (*Thymallus arcticus*)	No	1	Alberta
te-THPA	Eulachon (*Thaleichthys pacificus*)	No	1	British Columbia

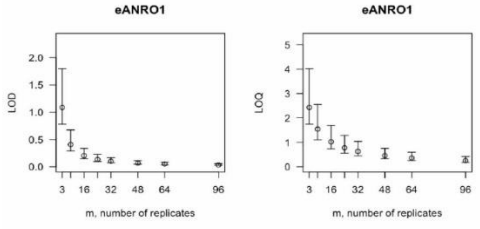
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



From 8 Technical Replicates

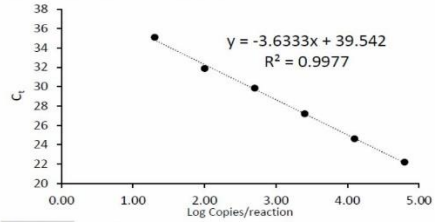
# Detects	# Copies	SE
0	0	0
1	0.15	0.14
2	0.31	0.23
3	0.51	0.32
4	0.76	0.41
5	1.07	0.54
6	1.51	0.73
7	2.27	1.12

Determined using eLowQuant R code[4].



Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 88%

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Guelph, ON N1G 4P7

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Toll Free: (877) 706-7678
Fax: (519) 836-4218
www.bvlabs.com

Field Sample Validation

Sample Type	Known presence	# Samples	Detected	Location
Water	Yes	30	Y	Prince Edward Island

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus* , in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. https://doi.org/10.1002/edn3.82
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
4. Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 00: 1-12. doi: 10.1002/edn3.220

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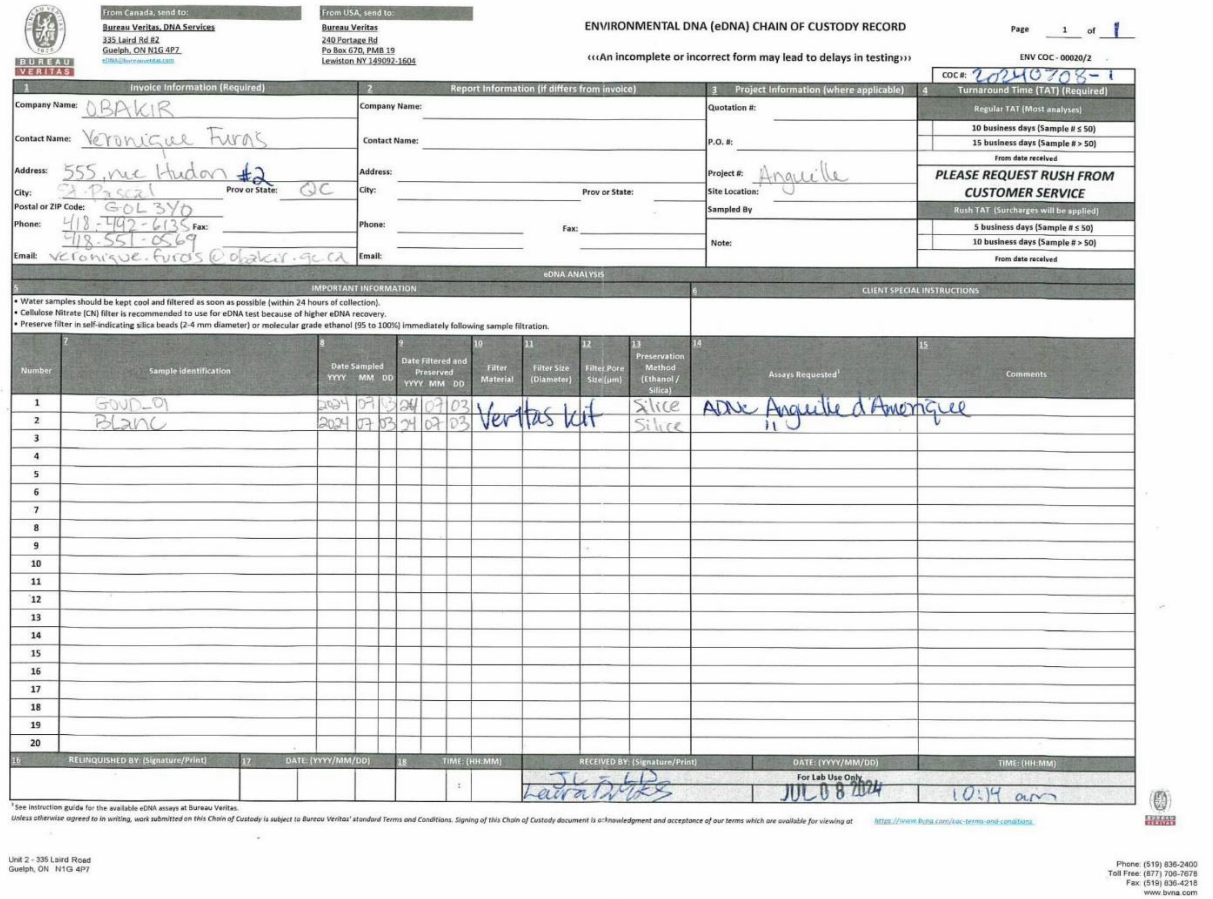
BV JOB #: E20240708-1
Report Date: 2024/07/11
Report #: OB20240711
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: N/A
Sampler Initials: N/A



From Canada, send to:
Bureau Veritas, DNA Services
335 Laird Rd #2
Guelph, ON N1G 4P7
eDNA@bureauveritas.com

From USA, send to:
Bureau Veritas
240 Portage Rd
Po Box 670, PMB 19
Lewiston NY 149092-1604

ENVIRONMENTAL DNA (eDNA) CHAIN OF CUSTODY RECORD

Page 1 of 1

‹‹‹An incomplete or incorrect form may lead to delays in testing›››

ENV COC - 00020/2

COC #: 20240708-1

1 Invoice Information (Required)	2 Report Information (if differs from invoice)	3 Project Information (where applicable)	4 Turnaround Time (TAT) (Required)
Company Name: OBAKIR	Company Name:	Quotation #:	Regular TAT (Most analyses)
Contact Name: Veronique Furas	Contact Name:	P.O. #:	10 business days (Sample # ≤ 50)
Address: 555, rue Hudon #2	Address:	Project #: Anguille	15 business days (Sample # > 50)
City: St-Pascal Prov or State: QC	City: Prov or State:	Site Location:	From date received
Postal or ZIP Code: G0L 3Y0		Sampled By	PLEASE REQUEST RUSH FROM CUSTOMER SERVICE
Phone: 418-492-6135 Fax:	Phone: Fax:	Note:	Rush TAT (Surcharges will be applied)
418-551-0569			5 business days (Sample # ≤ 50)
Email: veronique.furas@obakir.qc.ca	Email:		10 business days (Sample # > 50)
			From date received

eDNA ANALYSIS

5 IMPORTANT INFORMATION

- Water samples should be kept cool and filtered as soon as possible (within 24 hours of collection).
- Cellulose Nitrate (CN) filter is recommended to use for eDNA test because of higher eDNA recovery.
- Preserve filter in self-indicating silica beads (2-4 mm diameter) or molecular grade ethanol (95 to 100%) immediately following sample filtration.

6 CLIENT SPECIAL INSTRUCTIONS

Number	7 Sample Identification	8 Date Sampled YYYY MM DD	9 Date Filtered and Preserved YYYY MM DD	10 Filter Material	11 Filter Size (Diameter)	12 Filter Pore Size (µm)	13 Preservation Method (Ethanol / Silica)	14 Assays Requested[1]	15 Comments
1	SOUD_01	2024 07 03	24 07 03	Veritas kit			Silice	ADNe Anguille d'Amérique	
2	BLANC	2024 07 03	24 07 03				Silice	"	
3									
4									
5									
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20									

16 RELINQUISHED BY: (Signature/Print)	17 DATE: (YYYY/MM/DD)	18 TIME: (HH:MM)	RECEIVED BY: (Signature/Print)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)
		:	Laura Bilyea	For Lab Use Only JUL 08 2024	10:14 am

[1] See instruction guide for the available eDNA assays at Bureau Veritas.

Unless otherwise agreed to in writing, work submitted on this Chain of Custody is subject to Bureau Veritas' standard Terms and Conditions. Signing of this Chain of Custody document is acknowledgment and acceptance of our terms which are available for viewing at https://www.bvna.com/coc-terms-and-conditions

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Toll Free: (877) 706-7678
Fax: (519) 836-4218
www.bvna.com

Attention: **Véronique Furois**
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: anguille
Site Location: Rivière Cacouna
C.O.C. #: 20240725-1
Quote #: N/A
PO#: N/A

Report Date: 2024/07/29
Report #: OB20240729
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20240725-1
Received: 2024/07/25, 8:50 A.M.

Sample Type: Cellulose Nitrate (CN) filter, preserved in silica
Samples Received: 6

Analyses (eDNA Isolation - Species)	Test Requested	Test Performed	Date eDNA Extracted	Date Analyzed IntegritE-DNA™	Date Analyzed Target Species	Laboratory Method	Analytical Method (qPCR Primer/Probe set)
eDNA Isolation and IntegritE-DNA™	6	6	2024-07-25	2024-07-26	N/A	GUE SOP-00056	ePlant5
American Eel (*Anguilla rostrata*)	6	6	N/A	N/A	2024-07-26	GUE SOP-00056	eANRO1

Remarks:

Bureau Veritas Laboratories (Animal DNA Department, DNA Services) is accredited to ISO17025:2017 for eDNA testing.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by industry professionals using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Bureau Veritas Laboratories in writing). All data has met quality control and method performance criteria unless otherwise noted.

Bureau Veritas Laboratories' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Bureau Veritas Laboratories has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Bureau Veritas Laboratories unless otherwise agreed in writing. Bureau Veritas Laboratories is not responsible for the accuracy or any data impacts that result from the information provided by the customer or their agent.

Results relate to supplied samples tested. This Certificate should not be reproduced except in full, without the written approval of the laboratory.

eDNA tests are used to confirm presence of eDNA in samples for the targeted species / species groups.

Collected eDNA samples will contain eDNA at various stages of degradation, being subject to environmental forces that breakdown DNA, including microbial activity, ultraviolet radiation, heat, hydrolysis, and enzymatic activity. eDNA is first evaluated for eDNA quality and presence of qPCR assay inhibitors using the IntegritE-DNA™ assay before testing for target species or genera to confirm that the eDNA is of sufficient quality for testing and to identify and address qPCR inhibition (if present) to avoid false negatives.

SAMPLE RETENTION: Samples and DNA extracts generated from the samples will be retained by Bureau Veritas Laboratories for a period of 90 days after which time they will be discarded unless prearrangement has been made by client with Bureau Veritas Laboratories for longer storage.

Attention: **Véronique Furois**
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: **anguille**
Site Location: **Rivière Cacouna**
C.O.C. #: **20240725-1**
Quote #: **N/A**
PO#: **N/A**

Report Date: **2024/07/29**
Report #: **OB20240729**
Version: **1**

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: **E20240725-1**
Received: **2024/07/25, 8:50 A.M.**

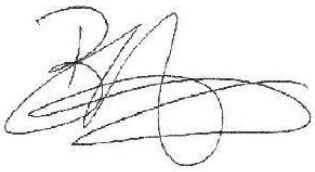
Methodology for Sample Analysis

Samples are logged in upon receipt. Samples were stored in freezer until processing in the laboratory. Sample analysis is completed within 10 business days (as indicated by the client on the COC) following receipt of samples by the testing laboratory.

eDNA isolation is completed using the DNeasy Blood & Tissue Kit™ (QIAGEN). A negative control is included as a blank filter sample with each batch of eDNA isolation to monitor for potential laboratory contamination during the eDNA isolation process.

Following eDNA isolation (150µL) from a quarter of filter, the IntegritE-DNA™ assay[1] is used to avoid the potential of a false negative (Type II error) during target species or genera testing. The IntegritE-DNA™ assay evaluates the integrity of eDNA for suitability for qPCR and for presence of qPCR inhibitors which may reduce the effectiveness of the qPCR assay for target species or genera. This assay evaluates the quality of eDNA to assess whether it is amplifiable using a qPCR assay that targets the chloroplast genome derived from plants/algae that are ubiquitously found in fresh water systems. Four technical replicates per eDNA sample, four technical replicates of negative control (Ultrapure water), and two technical replicates of positive control are used for the IntegritE-DNA™ assay. The cut-off Ct (qPCR cycle threshold) value for the IntegritE-DNA™ assay is 27 due to inhibition. If the IntegritE-DNA™ assay produces a positive detection frequency of ≥ 2 of the 4 technical replicates, this indicates that the eDNA for the target taxa is likely to be of sufficient quality to be detected (if present) with the target assay. If the IntegritE-DNA™ assay produces a positive detection frequency < 2 of the 4 technical replicates (eDNA is degraded or qPCR inhibitors are present), then sample cleanup is completed using the OneStep PCR Inhibitor Removal Kit™ (ZYMO Research) to remove potential qPCR assay inhibitors from the isolated eDNA. Subsequent to inhibitor removal, the IntegritE-DNA™ assay is repeated to re-assess whether the eDNA is of sufficient quality for qPCR. If a sample fails at the IntegritE-DNA™ assay (Ct Value over 30) for the second time the client will be informed that the quality of the sample is insufficient for the qPCR assay. eDNA indicator (IntegritE-DNA™) in the sample suggests that degradation has taken place and therefore the target species assay may be ineffective. Once a sample passes the IntegritE-DNA™ assay, then the target species or genera assay is performed. Eight technical replicates per eDNA sample, eight technical replicates of the negative control (Ultrapure water), and two technical replicates of positive control (total DNA or synthetic DNA) are used for the target species or genera assay to assess the detection or non-detection of DNA of the target species or genera. The cut-off Ct value for target species assay is 50.

[1] Hobbs J, Round JM, Allison MJ, Helbing CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849.



BECKY HENDERSON
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Phone #: (519) 836 2400 Ext. 7067714

Please direct all questions regarding this Certificate of Analysis to your Customer Service Representative above.
==========
For Service Group specific validation please refer to the Validation Signature Page.

Total Cover Pages: 2

BV JOB #: E20240725-1
Report Date: 2024/07/29
Report #: OB20240729
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

RESULTS

Client Sample ID	BV Case ID	Sample Collection Date	IntegritE-DNA™ Positive detection (Ct≤27)[1]	QC Batch	Clean-up required	IntegritE-DNA™ Positive detection (Ct≤30)[1] after clean-up	QC Batch	Analytical Method (qPCR Primer/Probe set)	Target Species eDNA Positive detection (Ct≤50)[2]	QC Batch
Cac_01	OB20240003	2024-07-22	4/4	240726Q1	No	N/A	N/A	eANRO1[3]	1/8	240726Q5
Cac_02	OB20240004	2024-07-22	4/4	240726Q1	No	N/A	N/A	eANRO1	0/8	240726Q5
Cac_03	OB20240005	2024-07-22	4/4	240726Q1	No	N/A	N/A	eANRO1	2/8	240726Q5
Cac_04	OB20240006	2024-07-22	4/4	240726Q1	No	N/A	N/A	eANRO1	1/8	240726Q5
Cac_05	OB20240007	2024-07-22	4/4	240726Q1	No	N/A	N/A	eANRO1	1/8	240726Q5
Cac_06	OB20240008	2024-07-22	4/4	240726Q1	No	N/A	N/A	eANRO1	1/8	240726Q5

[1] **IntegritE-DNA™ Assay:** Four technical replicates were assayed for each eDNA sample. **Field samples:** The cut-off Ct value for IntegritE-DNA assay is 27 based on previous observation and due to presence of inhibitors. **Post clean-up and field blank samples:** The cut-off Ct value for IntegritE-DNA assay is 30. Results are reported as the number of positive detections (n) out of a total of 4 technical replicates, n/4.

[2] **Target Species Assay:** Eight technical replicates were assayed per eDNA sample. The cut-off Ct value for target species assay was 50. Results are reported as the number of positive detections (n) out of a total of 8 technical replicates, n/8.

[3] eANRO1: qPCR primer/probe assay to assess the presence of American Eel (*Anguilla rostrata*) eDNA.

GENERAL COMMENTS

eDNA is extracted (150 µL) from a quarter of filter, and 2 µL is used as a template for each technical replicate.

Results relate only to the items tested.

QUALITY ASSURANCE REPORT

			eDNA Isolation Negative Control[1]		qPCR Positive Controls[2]		qPCR Negative Controls[3]	
QC Batch	Parameter	Date	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail
240726Q1	IntegritE-DNA	2024-07-26	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
240726Q5	eANRO1	2024-07-26	eDNA Isolation Negative Control is assessed using IntegritE-DNA only once for each extraction batch.	N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass

[1] **eDNA Isolation Negative Control:** Blank filters were included for each batch of eDNA extraction to monitor for laboratory contamination during eDNA isolation. eDNA Isolation Negative Control is assessed using IntegritE-DNA™ only. QC results show no eDNA was isolated from the negative control, therefore there was no indication of sample contamination during handling. Acceptance criteria: 0 of 4 technical replicates

[2] **qPCR Positive Controls:** Two technical replicates of isolated eDNA from freshwater sample were used as positive controls for IntegritE-DNA™. Two technical replicates of total DNA or synthetic DNA from the target species were used as positive controls for eDNA assays. Results show that 100% of the technical replicates amplified the positive control eDNA as expected, therefore an observation of negative result in eDNA samples is not related to the qPCR performance. Acceptance criteria: 2 of 2 technical replicates

[3] **qPCR Negative Controls (Ultrapure water):** Four technical replicates for IntegritE-DNA™ and eight technical replicates for target species or genera were used to monitor for laboratory contamination. Results show that 0% of the technical replicates in the negative controls had amplified eDNA, indicating no contamination was detected. Acceptance criteria: 0 of 4 technical replicates for IntegritE-DNA™, and 0 of 8 technical replicates for other assays.

LABORATORY RESULTS VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Reporter: ALI MIRABZADEH, M.Sc.
Laboratory Supervisor, Bureau Veritas, Forensic and DNA Services

==========

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Guelph, ON N1G 4P7

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Toll Free: (877) 706-7678
Fax: (519) 836-4218
www.bvlabs.com

BV JOB #: E20240725-1
Report Date: 2024/07/29
Report #: OB20240729
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

American Eel (*Anguilla rostrata*) eDNA Assay Validation Information

eDNA assay Validation

All eDNA assays are validated through a rigorous multi-step evaluation protocol at University of Victoria that includes tests of DNA target specificity and amplification sensitivity. All eDNA tests available at Bureau Veritas Laboratories have been validated for performance using interlaboratory verification.

General eDNA Assay Information

Target Species: American Eel (*Anguilla rostrata*)
Species Code: te-ANRO
eDNA qPCR Tool: eANRO1
eDNA qPCR Format: TaqMan
Gene Target: MT-CYB
Published in: N/A

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA

LOD 0.4 (95% CI 0.3-0.7 Copies)
LOB 0 hits/8
LOQ 1.5 (95% CI 1.1-2.6 Copies)

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].
When the LOQ < LOD, use the LOD for the LOQ.

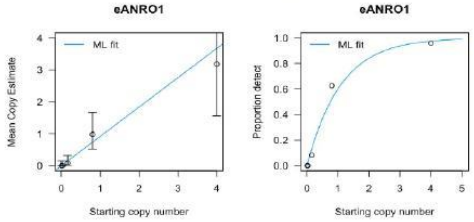
Enzyme: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (Species)	Detection	Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-ANRO	American Eel (*Anguilla rostrata*)	Yes	8	Prince Edward Island
te-COCO	Slimy Sculpin (*cottus cognatus*)	No	1	Yukon
te-ESLU	Northern Pike (*Esox lucius*)	No	1	British Columbia
te-MIDO	Smallmouth Bass (*Micropterus dolomieu*)	No	1	British Columbia
te-MISA	Largemouth Bass (*Micropterus salmoides*)	No	1	British Columbia
te-ONCL	Cutthroat Trout (*Oncorhynchus clarkii*)	No	1	British Columbia
te-ONGO	Pink Salmon (*Oncorhynchus gorbuscha*)	No	1	British Columbia
te-ONKE	Chum Salmon (*Oncorhynchus keta*)	No	1	British Columbia
te-ONKI	Coho Salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
te-ONMY	Rainbow Trout (*Oncorhynchus mykiss*)	No	1	British Columbia
te-ONNE	Sockeye Salmon (*Oncorhynchus nerka*)	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tschawytscha*)	No	1	British Columbia
te-PRCY	Round Whitefish (*prosopium cylindraceum*)	No	1	Yukon
te-SACO	Bull Trout (*Salvelinus confluentus*)	No	1	Alberta
te-SAMA	Dolly Varden (*Salvelinus malma*)	No	1	British Columbia
te-SASA	Atlantic Salmon (*Salmo Salar*)	No	1	Nova Scotia
te-THAR	Arctic Grayling (*Thymallus arcticus*)	No	1	Alberta
te-THPA	Eulachon (*Thaleichthys pacificus*)	No	1	British Columbia

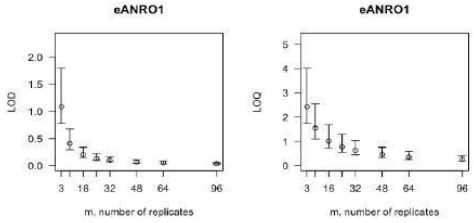
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



From 8 Technical Replicates

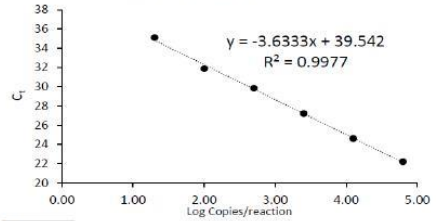
# Detects	# Copies	SE
0	0	0
1	0.15	0.14
2	0.31	0.23
3	0.51	0.32
4	0.76	0.41
5	1.07	0.54
6	1.51	0.73
7	2.27	1.12

Determined using eLowQuant R code[4].



Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µL DNA input in a total 15 µL reaction

Applied to reactions with 100% positive hits



Efficiency 88%

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BV JOB #: E20240725-1
Report Date: 2024/07/29
Report #: OB20240729
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

American Eel (*Anguilla rostrata*) eDNA Assay Validation Information

Field Sample Validation

Sample Type	Known presence	# Samples	Detected	Location
Water	Yes	30	Y	Prince Edward Island

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus* , in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. https://doi.org/10.1002/edn3.82
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
4. Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 00: 1-12. doi: 10.1002/edn3.220

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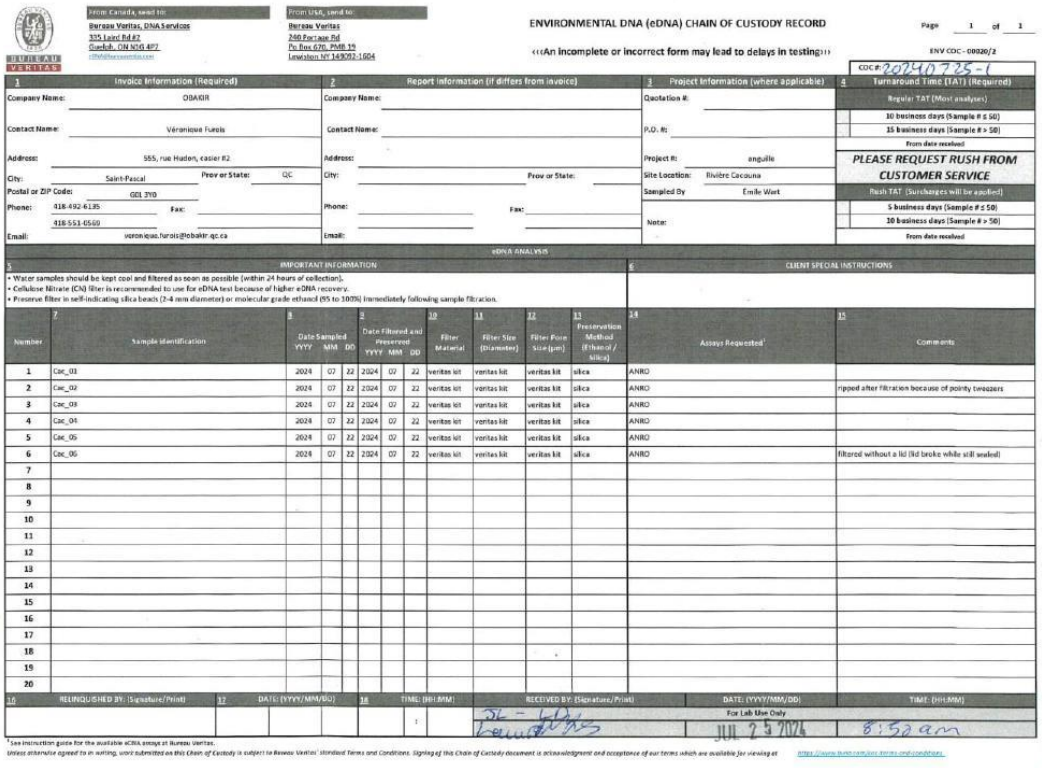
BV JOB #: E20240725-1
Report Date: 2024/07/29
Report #: OB20240729
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW



From Canada, send to:
Bureau Veritas, DNA Services
335 Laird Rd #2
Guelph, ON N1G 4P7

From USA, send to:
Bureau Veritas
240 Pacrage Rd
Po Box 670, PMB 19
Lewiston NY 14092-1604

ENVIRONMENTAL DNA (eDNA) CHAIN OF CUSTODY RECORD

«An incomplete or incorrect form may lead to delays in testing»

Page 1 of 1

ENV COC - 00020/2

COC #: 20240725-1

1 Invoice Information (Required)		2 Report Information (if differs from invoice)		3 Project Information (where applicable)		4 Turnaround Time (TAT) (Required)
Company Name:	OBAKIR	Company Name:		Quotation #:		Regular TAT (Most analyses)
Contact Name:	Véronique Furois	Contact Name:		P.O. #:		10 business days (Sample # ≤ 50)
Address:	555, rue Haden, easier #2	Address:		Project #:	anguille	15 business days (Sample # > 50)
City:	Saint-Pascal	Prov or State:	QC	Site Location:	Rivière Cacouna	From date received
Postal or ZIP Code:	G0L 3Y0	City:		Sampled By	Emile Wert	PLEASE REQUEST RUSH FROM CUSTOMER SERVICE
Phone:	418-492-6135	Prov or State:		Note:		Rush TAT (Surcharges will be applied)
	418-551-0569	Phone:				5 business days (Sample # ≤ 50)
Fax:		Fax:				10 business days (Sample # > 50)
Email:	veronique.furois@obakir.qc.ca	Email:				From date received

eDNA ANALYSIS

5 IMPORTANT INFORMATION	6 CLIENT SPECIAL INSTRUCTIONS
• Water samples should be kept cool and filtered as soon as possible (within 24 hours of collection). • Cellulose Nitrate (CN) filter is recommended to use for eDNA test because of higher eDNA recovery. • Preserve filter in self-indicating silica beads (2-4 mm diameter) or molecular grade ethanol (95 to 100%) immediately following sample filtration.	

Number	7 Sample Identification	8 Date Sampled YYYY	MM	DD	9 Date Filtered and Preserved YYYY	MM	DD	10 Filter Material	11 Filter Size (Diameter)	12 Filter Pore Size (µm)	13 Preservation Method (Ethanol / Silica)	14 Assays Requested*	15 Comments
1	Cac_01	2024	07	22	2024	07	22	veritas kit	veritas kit	veritas kit	silica	ANRO	
2	Cac_02	2024	07	22	2024	07	22	veritas kit	veritas kit	veritas kit	silica	ANRO	ripped after filtration because of pointy tweezers
3	Cac_03	2024	07	22	2024	07	22	veritas kit	veritas kit	veritas kit	silica	ANRO	
4	Cac_04	2024	07	22	2024	07	22	veritas kit	veritas kit	veritas kit	silica	ANRO	
5	Cac_05	2024	07	22	2024	07	22	veritas kit	veritas kit	veritas kit	silica	ANRO	
6	Cac_06	2024	07	22	2024	07	22	veritas kit	veritas kit	veritas kit	silica	ANRO	filtered without a lid (lid broke while still sealed)
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19													
20													

16 RELINQUISHED BY: (Signature/Print)	17 DATE: (YYYY/MM/DD)	18 TIME: (HH:MM)	RECEIVED BY: (Signature/Print)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)
		:		For Lab Use Only JUL 25 2024	8:52 am

*See instruction guide for the available eDNA assays at Bureau Veritas.

Unless otherwise agreed to in writing, work submitted on this Chain of Custody is subject to Bureau Veritas' Standard Terms and Conditions. Signing of this Chain of Custody document is acknowledgment and acceptance of our terms which are available for viewing at https://www.bvnet.com/doc/terms-and-conditions

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Attention: Véronique Furois
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: anguille
Site Location: Rivière Cacouna
C.O.C. #: 20240806-1
Quote #: N/A
PO#: N/A

Report Date: 2024/08/16
Report #: OB20240816
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20240806-1
Received: 2024/08/06, 11:12 A.M.

Sample Type: Cellulose Nitrate (CN) filter, preserved in silica
Samples Received: 6

Analyses (eDNA Isolation - Species)	Test Requested	Test Performed	Date eDNA Extracted	Date Analyzed IntegritE-DNA™	Date Analyzed Target Species	Laboratory Method	Analytical Method (qPCR Primer/Probe set)
eDNA Isolation and IntegritE-DNA™	6	6	2024-08-12	2024-08-13	N/A	GUE SOP-00056	ePlant5
American Eel (*Anguilla rostrata*)	6	6	N/A	N/A	2024-08-14	GUE SOP-00056	eANRO1

Remarks:

Bureau Veritas Laboratories (Animal DNA Department, DNA Services) is accredited to ISO17025:2017 for eDNA testing.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by industry professionals using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Bureau Veritas Laboratories in writing). All data has met quality control and method performance criteria unless otherwise noted.

Bureau Veritas Laboratories' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Bureau Veritas Laboratories has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Bureau Veritas Laboratories unless otherwise agreed in writing. Bureau Veritas Laboratories is not responsible for the accuracy or any data impacts that result from the information provided by the customer or their agent.

Results relate to supplied samples tested. This Certificate should not be reproduced except in full, without the written approval of the laboratory.

eDNA tests are used to confirm presence of eDNA in samples for the targeted species / species groups.

Collected eDNA samples will contain eDNA at various stages of degradation, being subject to environmental forces that breakdown DNA, including microbial activity, ultraviolet radiation, heat, hydrolysis, and enzymatic activity. eDNA is first evaluated for eDNA quality and presence of qPCR assay inhibitors using the IntegritE-DNA™ assay before testing for target species or genera to confirm that the eDNA is of sufficient quality for testing and to identify and address qPCR inhibition (if present) to avoid false negatives.

SAMPLE RETENTION: Samples and DNA extracts generated from the samples will be retained by Bureau Veritas Laboratories for a period of 90 days after which time they will be discarded unless prearrangement has been made by client with Bureau Veritas Laboratories for longer storage.

Attention: Véronique Furois
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: anguille
Site Location: Rivière Cacouna
C.O.C. #: 20240806-1
Quote #: N/A
PO#: N/A

Report Date: 2024/08/16
Report #: OB20240816
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20240806-1
Received: 2024/08/06, 11:12 A.M.

Methodology for Sample Analysis

Samples are logged in upon receipt. Samples were stored in freezer until processing in the laboratory. Sample analysis is completed within 10 business days (as indicated by the client on the COC) following receipt of samples by the testing laboratory.

eDNA isolation is completed using the DNeasy Blood & Tissue Kit™ (QIAGEN). A negative control is included as a blank filter sample with each batch of eDNA isolation to monitor for potential laboratory contamination during the eDNA isolation process.

Following eDNA isolation (150µL) from a quarter of filter, the IntegritE-DNA™ assay[1] is used to avoid the potential of a false negative (Type II error) during target species or genera testing. The IntegritE-DNA™ assay evaluates the integrity of eDNA for suitability for qPCR and for presence of qPCR inhibitors which may reduce the effectiveness of the qPCR assay for target species or genera. This assay evaluates the quality of eDNA to assess whether it is amplifiable using a qPCR assay that targets the chloroplast genome derived from plants/algae that are ubiquitously found in fresh water systems. Four technical replicates per eDNA sample, four technical replicates of negative control (Ultrapure water), and two technical replicates of positive control are used for the IntegritE-DNA™ assay. The cut-off Ct (qPCR cycle threshold) value for the IntegritE-DNA™ assay is 27 due to inhibition. If the IntegritE-DNA™ assay produces a positive detection frequency of ≥ 2 of the 4 technical replicates, this indicates that the eDNA for the target taxa is likely to be of sufficient quality to be detected (if present) with the target assay. If the IntegritE-DNA™ assay produces a positive detection frequency < 2 of the 4 technical replicates (eDNA is degraded or qPCR inhibitors are present), then sample cleanup is completed using the OneStep PCR Inhibitor Removal Kit™ (ZYMO Research) to remove potential qPCR assay inhibitors from the isolated eDNA. Subsequent to inhibitor removal, the IntegritE-DNA™ assay is repeated to re-assess whether the eDNA is of sufficient quality for qPCR. If a sample fails at the IntegritE-DNA™ assay (Ct Value over 30) for the second time the client will be informed that the quality of the sample is insufficient for the qPCR assay. eDNA indicator (IntegritE-DNA™) in the sample suggests that degradation has taken place and therefore the target species assay may be ineffective. Once a sample passes the IntegritE-DNA™ assay, then the target species or genera assay is performed. Eight technical replicates per eDNA sample, eight technical replicates of the negative control (Ultrapure water), and two technical replicates of positive control (total DNA or synthetic DNA) are used for the target species or genera assay to assess the detection or non-detection of DNA of the target species or genera. The cut-off Ct value for target species assay is 50.

[1] Hobbs J, Round JM, Allison MJ, Helbing CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849.

BECKY HENDERSON
Senior Customer Service Representative, Bureau Veritas Laboratories, DNA Services
Email: becky-a.henderson@bureauveritas.com
Phone #: (519) 836 2400 Ext. 7067714

Please direct all questions regarding this Certificate of Analysis to your Customer Service Representative above.
==========
For Service Group specific validation please refer to the Validation Signature Page.

Total Cover Pages: 2

BV JOB #: E20240806-1
Report Date: 2024/08/16
Report #: OB20240816
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

RESULTS

Client Sample ID	BV Case ID	Sample Collection Date	IntegritE-DNA™ Positive detection (Ct≤27)[1]	QC Batch	Clean-up required	IntegritE-DNA™ Positive detection (Ct≤30)[1] after clean-up	QC Batch	Analytical Method (qPCR Primer/Probe set)	Target Species eDNA Positive detection (Ct≤50)[2]	QC Batch
Cac_01	OB20240009	2024-07-31	4/4	240813Q1	No	N/A	N/A	eANRO1[3]	0/8	240814Q2
Cac_02	OB20240010	2024-07-31	4/4	240813Q1	No	N/A	N/A	eANRO1	0/8	240814Q2
Cac_03	OB20240011	2024-07-31	4/4	240813Q1	No	N/A	N/A	eANRO1	8/8	240814Q2
Cac_04	OB20240012	2024-07-31	4/4	240813Q1	No	N/A	N/A	eANRO1	7/8	240814Q2
Cac_05	OB20240013	2024-07-31	4/4	240813Q1	No	N/A	N/A	eANRO1	0/8	240814Q2
Cac_06	OB20240014	2024-07-31	4/4	240813Q1	No	N/A	N/A	eANRO1	1/8	240814Q2

[1] **IntegritE-DNA™ Assay:** Four technical replicates were assayed for each eDNA sample. **Field samples:** The cut-off Ct value for IntegritE-DNA assay is 27 based on previous observation and due to presence of inhibitors. **Post clean-up and field blank samples:** The cut-off Ct value for IntegritE-DNA assay is 30. Results are reported as the number of positive detections (n) out of a total of 4 technical replicates, n/4.

[2] **Target Species Assay:** Eight technical replicates were assayed per eDNA sample. The cut-off Ct value for target species assay was 50. Results are reported as the number of positive detections (n) out of a total of 8 technical replicates, n/8.

[3] eANRO1: qPCR primer/probe assay to assess the presence of American Eel (*Anguilla rostrata*) eDNA.

GENERAL COMMENTS

eDNA is extracted (150 µL) from a quarter of filter, and 2 µL is used as a template for each technical replicate.

Results relate only to the items tested.

QUALITY ASSURANCE REPORT

			eDNA Isolation Negative Control[1]		qPCR Positive Controls[2]		qPCR Negative Controls[3]	
QC Batch	Parameter	Date	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail
240813Q1	IntegritE-DNA	2024-08-13	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
240814Q2	eANRO1	2024-08-14	eDNA Isolation Negative Control is assessed using IntegritE-DNA only once for each extraction batch.	N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass

[1] **eDNA Isolation Negative Control:** Blank filters were included for each batch of eDNA extraction to monitor for laboratory contamination during eDNA isolation. eDNA Isolation Negative Control is assessed using IntegritE-DNA™ only. QC results show no eDNA was isolated from the negative control, therefore there was no indication of sample contamination during handling. Acceptance criteria: 0 of 4 technical replicates

[2] **qPCR Positive Controls:** Two technical replicates of isolated eDNA from freshwater sample were used as positive controls for IntegritE-DNA™. Two technical replicates of total DNA or synthetic DNA from the target species were used as positive controls for eDNA assays. Results show that 100% of the technical replicates amplified the positive control eDNA as expected, therefore an observation of negative result in eDNA samples is not related to the qPCR performance. Acceptance criteria: 2 of 2 technical replicates

[3] **qPCR Negative Controls (Ultrapure water):** Four technical replicates for IntegritE-DNA™ and eight technical replicates for target species or genera were used to monitor for laboratory contamination. Results show that 0% of the technical replicates in the negative controls had amplified eDNA, indicating no contamination was detected. Acceptance criteria: 0 of 4 technical replicates for IntegritE-DNA™, and 0 of 8 technical replicates for other assays.

LABORATORY RESULTS VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Reporter: ALI MIRABZADEH, M.Sc.
Laboratory Supervisor, Bureau Veritas, Forensic and DNA Services

==========

BV JOB #: E20240806-1
Report Date: 2024/08/16
Report #: OB20240816
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

American Eel (*Anguilla rostrata*) eDNA Assay Validation Information

eDNA assay Validation

All eDNA assays are validated through a rigorous multi-step evaluation protocol at University of Victoria that includes tests of DNA target specificity and amplification sensitivity. All eDNA tests available at Bureau Veritas Laboratories have been validated for performance using interlaboratory verification.

General eDNA Assay Information

Target Species: American Eel (*Anguilla rostrata*)
Species Code: te-ANRO
eDNA qPCR Tool: eANRO1
eDNA qPCR Format: TaqMan
Gene Target: MT-CYB
Published in: N/A

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA

LOD 0.4 95% CI 0.3-0.7 Copies LOQ 1.5 95% CI 1.1-2.6 Copies
LOB 0 hits/8

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].
When the LOQ < LOD, use the LOD for the LOQ.

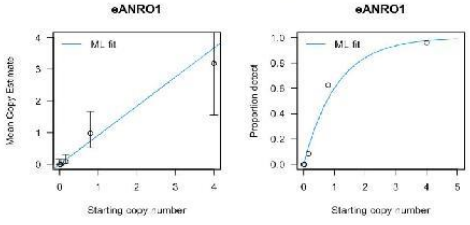
Enzyme: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (Species)	Detection	Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
te-ANRO	American Eel (*Anguilla rostrata*)	Yes	8	Prince Edward Island
te-COCO	Slimy Sculpin (*cottus cognatus*)	No	1	Yukon
te-ESLU	Northern Pike (*Esox lucius*)	No	1	British Columbia
te-MIDO	Smallmouth Bass (*Micropterus dolomieu*)	No	1	British Columbia
te-MISA	Largemouth Bass (*Micropterus salmoides*)	No	1	British Columbia
te-ONCL	Cutthroat Trout (*Oncorhynchus clarkii*)	No	1	British Columbia
te-ONGO	Pink Salmon (*Oncorhynchus gorbuscha*)	No	1	British Columbia
te-ONKE	Chum Salmon (*Oncorhynchus keta*)	No	1	British Columbia
te-ONKI	Coho Salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
te-ONMY	Rainbow Trout (*Oncorhynchus mykiss*)	No	1	British Columbia
te-ONNE	Sockeye Salmon (*Oncorhynchus nerka*)	No	1	British Columbia
te-ONTS	Chinook Salmon (*Oncorhynchus tschawytscha*)	No	1	British Columbia
te-PRCY	Round Whitefish (*prosopium cylindraceum*)	No	1	Yukon
te-SACO	Bull Trout (*Salvelinus confluentus*)	No	1	Alberta
te-SAMA	Dolly Varden (*Salvelinus malma*)	No	1	British Columbia
te-SASA	Atlantic Salmon (*Salmo Salar*)	No	1	Nova Scotia
te-THAR	Arctic Grayling (*Thymallus arcticus*)	No	1	Alberta
te-THPA	Eulachon (*Thaleichthys pacificus*)	No	1	British Columbia

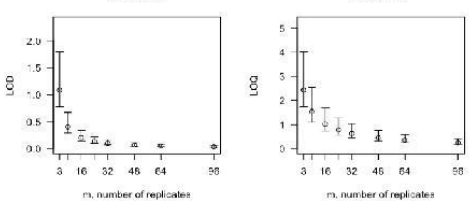
eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



From 8 Technical Replicates

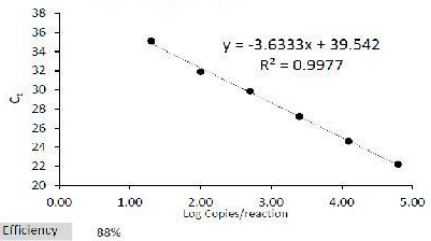
# Detects	# Copies	SE
0	0	0
1	0.15	0.14
2	0.31	0.23
3	0.51	0.32
4	0.76	0.41
5	1.07	0.54
6	1.51	0.73
7	2.27	1.12

Determined using eLowQuant R code[4].



Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µl DNA input in a total 15 µl reaction

Applied to reactions with 100% positive hits



Efficiency 88%

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American Eel (*Anguilla rostrata*) eDNA Assay Validation Information

Field Sample Validation

Sample Type	Known presence	# Samples	Detected	Location
Water	Yes	30	Y	Prince Edward Island

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus* , in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. https://doi.org/10.1002/edn3.82
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
4. Lesperance, M, Allison, MJ, Bergman, LC, Hocking, MD, and Helbing, CC (2021) A statistical model for calibration and computation of detection and quantification limits for low copy number environmental DNA samples. Environmental DNA, 00: 1-12. doi: 10.1002/edn3.220

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BV JOB #: E20240806-1
Report Date: 2024/08/16
Report #: OB20240816
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

From Canada, send to:
Bureau Veritas, DNA Services
335 Laird Rd #2
Guelph, ON N1G 4P7
rdnd@bureauveritas.com

From USA, send to:
Bureau Veritas
240 Portage Rd
Po Box 670, PMB 19
Lewiston NY 149092-1604

ENVIRONMENTAL DNA (eDNA) CHAIN OF CUSTODY RECORD

›››An incomplete or incorrect form may lead to delays in testing‹‹‹

ENV COC - 00020/2

COC #: 20240806-1

1 Invoice Information (Required)		2 Report Information (if differs from invoice)		3 Project Information (where applicable)		4 Turnaround Time (TAT) (Required)	
Company Name:	OBAKIR	Company Name:		Quotation #:		Regular TAT (Most analyses)	
Contact Name:	Véronique Furois	Contact Name:		P.O. #:		10 business days (Sample # ≤ 50)	
Address:	555, rue Hudon, casier #2	Address:		Project #:	anguille	15 business days (Sample # > 50)	
City:	Saint-Pascal	Prov or State:	QC	Site Location:	Rivière Cacouna	From date received	
Postal or ZIP Code:	G0L 3Y0	City:		Sampled By	Émile Wart	PLEASE REQUEST RUSH FROM CUSTOMER SERVICE	
Phone:	418-492-6135	Fax:		Note:		Rush TAT (Surcharges will be applied)	
	418-551-0569	Phone:				5 business days (Sample # ≤ 50)	
Email:	veronique.furois@obakir.qc.ca	Email:				10 business days (Sample # > 50)	
						From date received	

eDNA ANALYSIS

5 IMPORTANT INFORMATION	6 CLIENT SPECIAL INSTRUCTIONS
• Water samples should be kept cool and filtered as soon as possible (within 24 hours of collection). • Cellulose Nitrate (CN) filter is recommended to use for eDNA test because of higher eDNA recovery. • Preserve filter in self-indicating silica beads (2-4 mm diameter) or molecular grade ethanol (95 to 100%) immediately following sample filtration.	

Number	7 Sample identification	8 Date Sampled YYYY	MM	DD	9 Date Filtered and Preserved YYYY	MM	DD	10 Filter Material	11 Filter Size (Diameter)	12 Filter Pore Size (µm)	13 Preservation Method (Ethanol / Silica)	14 Assays Requested[1]	15 Comments
1	Cac_01	2024	07	31	2024	08	01	veritas kit	veritas kit	veritas kit	silica	ANRO	
2	Cac_02	2024	07	31	2024	08	01	veritas kit	veritas kit	veritas kit	silica	ANRO	
3	Cac_03	2024	07	31	2024	08	01	veritas kit	veritas kit	veritas kit	silica	ANRO	
4	Cac_04	2024	07	31	2024	08	01	veritas kit	veritas kit	veritas kit	silica	ANRO	
5	Cac_05	2024	07	31	2024	08	01	veritas kit	veritas kit	veritas kit	silica	ANRO	
6	Cac_06	2024	07	31	2024	08	01	veritas kit	veritas kit	veritas kit	silica	ANRO	
7					10731								
8													
9													
10													
11													
12													
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20													

16 RELINQUISHED BY: (Signature/Print)	17 DATE: (YYYY/MM/DD)	18 TIME: (HH:MM)	RECEIVED BY: (Signature/Print)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)
			TW – BV Laura Dupuis	For Lab Use Only 2024/08/06	11:12 am
			2024/08/07		

[1] See Instruction guide for the available eDNA assays at Bureau Veritas.

2024/08/06

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Fax: (519) 836-4218
www.bvlabs.com

Attention: Antoine Plourde-Rouleau
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: anguille
Site Location: Rivière Cacouna
C.O.C. #: 20240822-1
Quote #: N/A
PO#: N/A

Report Date: 2024/08/23
Report #: OB20240823
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20240822-1
Received: 2024/08/22, 10:12 A.M.

Sample Type: Cellulose Nitrate (CN) filter, preserved in silica
Samples Received: 6

Analyses (eDNA Isolation - Species)	Test Requested	Test Performed	Date eDNA Extracted	Date Analyzed IntegritE-DNA™	Date Analyzed Target Species	Laboratory Method	Analytical Method (qPCR Primer/Probe set)
eDNA Isolation and IntegritE-DNA™	6	6	2024-08-22	2024-08-23	N/A	GUE SOP-00056	ePlant5
American Eel (*Anguilla rostrata*)	6	6	N/A	N/A	2024-08-23	GUE SOP-00056	eANRO1

Remarks:

Bureau Veritas Laboratories (Animal DNA Department, DNA Services) is accredited to ISO17025:2017 for eDNA testing.

All work recorded herein has been done in accordance with procedures and practices ordinarily exercised by industry professionals using accepted testing methodologies, quality assurance and quality control procedures (except where otherwise agreed by the client and Bureau Veritas Laboratories in writing). All data has met quality control and method performance criteria unless otherwise noted.

Bureau Veritas Laboratories' liability is limited to the actual cost of the requested analyses, unless otherwise agreed in writing. There is no other warranty expressed or implied. Bureau Veritas Laboratories has been retained to provide analysis of samples provided by the Client using the testing methodology referenced in this report. Interpretation and use of test results are the sole responsibility of the Client and are not within the scope of services provided by Bureau Veritas Laboratories unless otherwise agreed in writing. Bureau Veritas Laboratories is not responsible for the accuracy or any data impacts that result from the information provided by the customer or their agent.

Results relate to supplied samples tested. This Certificate should not be reproduced except in full, without the written approval of the laboratory.

eDNA tests are used to confirm presence of eDNA in samples for the targeted species / species groups.

Collected eDNA samples will contain eDNA at various stages of degradation, being subject to environmental forces that breakdown DNA, including microbial activity, ultraviolet radiation, heat, hydrolysis, and enzymatic activity. eDNA is first evaluated for eDNA quality and presence of qPCR assay inhibitors using the IntegritE-DNA™ assay before testing for target species or genera to confirm that the eDNA is of sufficient quality for testing and to identify and address qPCR inhibition (if present) to avoid false negatives.

SAMPLE RETENTION: Samples and DNA extracts generated from the samples will be retained by Bureau Veritas Laboratories for a period of 90 days after which time they will be discarded unless prearrangement has been made by client with Bureau Veritas Laboratories for longer storage.

Attention: Antoine Plourde-Rouleau
OBAKIR
555, rue Hudon, casier #2
Saint-Pascal, QC
G0L 3Y0, Canada

Client Project #: anguille
Site Location: Rivière Cacouna
C.O.C. #: 20240822-1
Quote #: N/A
PO#: N/A

Report Date: 2024/08/23
Report #: OB20240823
Version: 1

ENVIRONMENTAL DNA - CERTIFICATE OF ANALYSIS

BV JOB #: E20240822-1
Received: 2024/08/22, 10:12 A.M.

Methodology for Sample Analysis

Samples are logged in upon receipt. Samples were stored in freezer until processing in the laboratory. Sample analysis is completed within 10 business days (as indicated by the client on the COC) following receipt of samples by the testing laboratory.

eDNA isolation is completed using the DNeasy Blood & Tissue Kit™ (QIAGEN). A negative control is included as a blank filter sample with each batch of eDNA isolation to monitor for potential laboratory contamination during the eDNA isolation process.

Following eDNA isolation (150µL) from a quarter of filter, the IntegritE-DNA™ assay[1] is used to avoid the potential of a false negative (Type II error) during target species or genera testing. The IntegritE-DNA™ assay evaluates the integrity of eDNA for suitability for qPCR and for presence of qPCR inhibitors which may reduce the effectiveness of the qPCR assay for target species or genera. This assay evaluates the quality of eDNA to assess whether it is amplifiable using a qPCR assay that targets the chloroplast genome derived from plants/algae that are ubiquitously found in fresh water systems. Four technical replicates per eDNA sample, four technical replicates of negative control (Ultrapure water), and two technical replicates of positive control are used for the IntegritE-DNA™ assay. The cut-off Ct (qPCR cycle threshold) value for the IntegritE-DNA™ assay is 27 due to inhibition. If the IntegritE-DNA™ assay produces a positive detection frequency of ≥ 2 of the 4 technical replicates, this indicates that the eDNA for the target taxa is likely to be of sufficient quality to be detected (if present) with the target assay. If the IntegritE-DNA™ assay produces a positive detection frequency < 2 of the 4 technical replicates (eDNA is degraded or qPCR inhibitors are present), then sample cleanup is completed using the OneStep PCR Inhibitor Removal Kit™ (ZYMO Research) to remove potential qPCR assay inhibitors from the isolated eDNA. Subsequent to inhibitor removal, the IntegritE-DNA™ assay is repeated to re-assess whether the eDNA is of sufficient quality for qPCR. If a sample fails at the IntegritE-DNA™ assay (Ct Value over 30) for the second time the client will be informed that the quality of the sample is insufficient for the qPCR assay. eDNA indicator (IntegritE-DNA™) in the sample suggests that degradation has taken place and therefore the target species assay may be ineffective. Once a sample passes the IntegritE-DNA™ assay, then the target species or genera assay is performed. Eight technical replicates per eDNA sample, eight technical replicates of the negative control (Ultrapure water), and two technical replicates of positive control (total DNA or synthetic DNA) are used for the target species or genera assay to assess the detection or non-detection of DNA of the target species or genera. The cut-off Ct value for target species assay is 50.

[1] Hobbs J, Round JM, Allison MJ, Helbing CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849.

BECKY HENDERSON
Senior Customer Service Representative, Bureau Veritas Laboratories, DNA Services
Email: becky-a.henderson@bureauveritas.com
Phone #: (519) 836 2400 Ext. 7067714

Please direct all questions regarding this Certificate of Analysis to your Customer Service Representative above.
==========
For Service Group specific validation please refer to the Validation Signature Page.

Total Cover Pages: 2

BV JOB #: E20240822-1
Report Date: 2024/08/23
Report #: OB20240823
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

RESULTS

Client Sample ID	BV Case ID	Sample Collection Date	IntegritE-DNA™ Positive detection (Ct≤27)[1]	QC Batch	Clean-up required	IntegritE-DNA™ Positive detection (Ct≤30)[1] after clean-up	QC Batch	Analytical Method (qPCR Primer/Probe set)	Target Species eDNA Positive detection (Ct≤50)[2]	QC Batch
Cac_01	OB20240015	2024-08-19	4/4	240823Q1	No	N/A	N/A	eANRO1[3]	0/8	240823Q4
Cac_02	OB20240016	2024-08-19	4/4	240823Q1	No	N/A	N/A	eANRO1	0/8	240823Q4
Cac_03	OB20240017	2024-08-19	4/4	240823Q1	No	N/A	N/A	eANRO1	4/8	240823Q4
Cac_04	OB20240018	2024-08-19	4/4	240823Q1	No	N/A	N/A	eANRO1	4/8	240823Q4
Cac_05	OB20240019	2024-08-19	4/4	240823Q1	No	N/A	N/A	eANRO1	1/8	240823Q4
Cac_06	OB20240020	2024-08-19	4/4	240823Q1	No	N/A	N/A	eANRO1	4/8	240823Q4

[1] **IntegritE-DNA™ Assay:** Four technical replicates were assayed for each eDNA sample. **Field samples:** The cut-off Ct value for IntegritE-DNA assay is 27 based on previous observation and due to presence of inhibitors. **Post clean-up and field blank samples:** The cut-off Ct value for IntegritE-DNA assay is 30. Results are reported as the number of positive detections (n) out of a total of 4 technical replicates, n/4.

[2] **Target Species Assay:** Eight technical replicates were assayed per eDNA sample. The cut-off Ct value for target species assay was 50. Results are reported as the number of positive detections (n) out of a total of 8 technical replicates, n/8.

[3] eANRO1: qPCR primer/probe assay to assess the presence of American Eel (*Anguilla rostrata*) eDNA.

GENERAL COMMENTS

eDNA is extracted (150 µL) from a quarter of filter, and 2 µL is used as a template for each technical replicate.

Results relate only to the items tested.

QUALITY ASSURANCE REPORT

			eDNA Isolation Negative Control[1]		qPCR Positive Controls[2]		qPCR Negative Controls[3]	
QC Batch	Parameter	Date	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail	Detection at: Ct 27 (IntegritE-DNA™) Ct 50 (other assays)	Pass / Fail
240823Q1	IntegritE-DNA	2024-08-23	0 of 4 technical replicates	Pass	2 of 2 technical replicates	Pass	0 of 4 technical replicates	Pass
240823Q4	eANRO1	2024-08-23	eDNA Isolation Negative Control is assessed using IntegritE-DNA only once for each extraction batch.	N/A	2 of 2 technical replicates	Pass	0 of 8 technical replicates	Pass

[1] **eDNA Isolation Negative Control:** Blank filters were included for each batch of eDNA extraction to monitor for laboratory contamination during eDNA isolation. eDNA Isolation Negative Control is assessed using IntegritE-DNA™ only. QC results show no eDNA was isolated from the negative control, therefore there was no indication of sample contamination during handling. Acceptance criteria: 0 of 4 technical replicates

[2] **qPCR Positive Controls:** Two technical replicates of isolated eDNA from freshwater sample were used as positive controls for IntegritE-DNA™. Two technical replicates of total DNA or synthetic DNA from the target species were used as positive controls for eDNA assays. Results show that 100% of the technical replicates amplified the positive control eDNA as expected, therefore an observation of negative result in eDNA samples is not related to the qPCR performance. Acceptance criteria: 2 of 2 technical replicates

[3] **qPCR Negative Controls (Ultrapure water):** Four technical replicates for IntegritE-DNA™ and eight technical replicates for target species or genera were used to monitor for laboratory contamination. Results show that 0% of the technical replicates in the negative controls had amplified eDNA, indicating no contamination was detected. Acceptance criteria: 0 of 4 technical replicates for IntegritE-DNA™, and 0 of 8 technical replicates for other assays.

LABORATORY RESULTS VALIDATION SIGNATURE PAGE

The analytical data and all QC contained in this report were reviewed and validated by the following individual(s).

Ali Mirabzadeh

Reporter: ALI MIRABZADEH, M.Sc.
Laboratory Supervisor, Bureau Veritas, Forensic and DNA Services

==========

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BV JOB #: E20240822-1
Report Date: 2024/08/23
Report #: OB20240823
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

American Eel (*Anguilla rostrata*) eDNA Assay Validation Information

eDNA assay Validation

All eDNA assays are validated through a rigorous multi-step evaluation protocol at University of Victoria that includes tests of DNA target specificity and amplification sensitivity. All eDNA tests available at Bureau Veritas Laboratories have been validated for performance using interlaboratory verification.

General eDNA Assay Information

Target Species: American Eel (*Anguilla rostrata*)
Species Code: te-ANRO
eDNA qPCR Tool: eANRO1
eDNA qPCR Format: TaqMan
Gene Target: MT-CYB
Published in: N/A

eDNA Assay Sensitivity Test using gBlocks™ synthetic DNA

LOD 0.4 (95% CI 0.3-0.7 Copies)
LOB 0 hits/8
LOQ 1.5 (95% CI 1.1-2.6 Copies)

Binomial-Poisson model for 8 technical replicates determined using eLowQuant R code[4].
When the LOQ < LOD, use the LOD for the LOQ.

Enzyme: Immolase

eDNA Assay Specificity Test Information

Each qPCR reaction in the specificity assay contained 10 picograms of voucher target gDNA (n=25 technical replicates)

Species	Common Name (Species)	Detection	Specimens	Sample Sources/Locations
ma-HOSA	Human (*Homo sapiens*)	No	1	Netherlands
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te-ONKI	Coho Salmon (*Oncorhynchus kisutch*)	No	1	British Columbia
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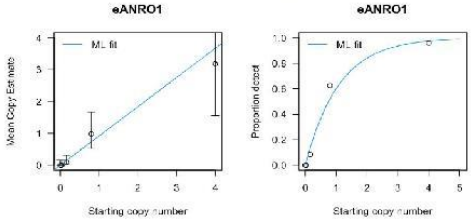
te-PRCY	Round Whitefish (*prosopium cylindraceum*)	No	1	Yukon
te-SACO	Bull Trout (*Salvelinus confluentus*)	No	1	Alberta
te-SAMA	Dolly Varden (*Salvelinus malma*)	No	1	British Columbia
te-SASA	Atlantic Salmon (*Salmo Salar*)	No	1	Nova Scotia
te-THAR	Arctic Grayling (*Thymallus arcticus*)	No	1	Alberta
te-THPA	Eulachon (*Thaleichthys pacificus*)	No	1	British Columbia

eDNA Assay Sensitivity Test Details using gBlocks™ synthetic DNA



From 8 Technical Replicates

# Detects	# Copies	SE
0	0	0
1	0.15	0.14
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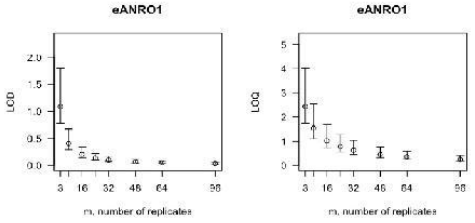
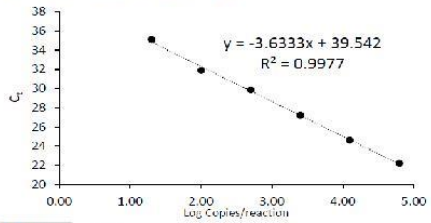
7	2.27	1.12

Determined using eLowQuant R code[4].



Binomial-Poisson model: No intercept
Determined using eLowQuant R code[4].
Based on a 2 µl DNA input in a total 15 µl reaction

Applied to reactions with 100% positive hits



Efficiency 88%

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BV JOB #: E20240822-1
Report Date: 2024/08/23
Report #: OB20240823
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

American Eel (*Anguilla rostrata*) eDNA Assay Validation Information

Field Sample Validation

Sample Type	Known presence	# Samples	Detected	Location
Water	Yes	30	Y	Prince Edward Island

Abbreviations

95% CI	95% Confidence interval	LOQ	Limit of quantification
eDNA	Environmental DNA	MT-CYB	Mitochondrial cytochrome B gene
gDNA	Total genomic DNA extracted from voucher specimen	NTC	qPCR no template control
LOB	Limit of blank	qPCR	Quantitative real-time polymerase chain reaction
LOD	Limit of detection	SE	Standard error

References

1. Hobbs, J, Adams, IT, Round, JM, Goldberg, CS, Allison, MJ, Bergman, LC, Mirabzadeh, A, Allen, H, Helbing, CC (2020) Revising the range of Rocky Mountain tailed frog, *Ascaphus montanus* , in British Columbia, Canada, using environmental DNA methods. Environmental DNA. 2020; 2: 350-361. https://doi.org/10.1002/edn3.82
2. Hobbs, J, Round, JM, Allison, MJ, Helbing, CC (2019) Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei* , in British Columbia, Canada, using robust eDNA detection methods. PLOS ONE 14(3): e0213849. https://doi.org/10.1371/journal.pone.0213849
3. Langlois, VS, Allison, MJ, Bergman, LC, To, TA, and Helbing, CC (2020) The need for robust qPCR-based eDNA detection assays in environmental monitoring and risk assessments. Environmental DNA, 3: 519-527. doi: 10.1002/edn3.164
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BV JOB #: E20240822-1
Report Date: 2024/08/23
Report #: OB20240823
Version #: 1

Client Name: OBAKIR
Client Project #: anguille
Site Location: Rivière Cacouna
Sampler Initials: EW

From Canada, send to:
Bureau Veritas, DNA Services
335 Laird Rd #2
Guelph, ON N1G 4P7
eDNA@bureauveritas.com

From USA, send to:
Bureau Veritas
240 Portage Rd
Po Box 670, PMB 19
Lewiston NY 149092-1604

ENVIRONMENTAL DNA (eDNA) CHAIN OF CUSTODY RECORD

ENV COC - 00020/2

‹‹‹An incomplete or incorrect form may lead to delays in testing›››

COC #: 20240822-1

1 Invoice Information (Required)		2 Report Information (if differs from invoice)		3 Project Information (where applicable)		4 Turnaround Time (TAT) (Required)
Company Name:	OBAKIR	Company Name:		Quotation #:		Regular TAT (Most analyses)
Contact Name:	Antoine Plourde-Rouleau	Contact Name:		P.O. #:		☐ 10 business days (Sample # ≤ 50) ☐ 15 business days (Sample # > 50) From date received
Address:	555, rue Hudon, casier #2	Address:		Project #:	anguille	PLEASE REQUEST RUSH FROM CUSTOMER SERVICE
City:	Saint-Pascal Prov or State: QC	City:	Prov or State:	Site Location:	Rivière Cacouna	
Postal or ZIP Code:	G0L 3Y0			Sampled By	Émile Wart	Rush TAT (Surcharges will be applied)
Phone:	418-492-6135 Fax:	Phone:	Fax:	Note:		☐ 5 business days (Sample # ≤ 50) ☐ 10 business days (Sample # > 50) From date received
Email:	direction@obakir.qc.ca	Email:				

eDNA ANALYSIS

5 IMPORTANT INFORMATION

- Water samples should be kept cool and filtered as soon as possible (within 24 hours of collection).
- Cellulose Nitrate (CN) filter is recommended to use for eDNA test because of higher eDNA recovery.
- Preserve filter in self-indicating silica beads (2-4 mm diameter) or molecular grade ethanol (95 to 100%) immediately following sample filtration.

6 CLIENT SPECIAL INSTRUCTIONS

Number	7 Sample Identification	8 Date Sampled YYYY	MM	DD	9 Date Filtered and Preserved YYYY	MM	DD	10 Filter Material	11 Filter Size (Diameter)	12 Filter Pore Size (µm)	13 Preservation Method (Ethanol / Silica)	14 Assays Requested[1]	15 Comments
1	Cac_01	2024	08	19	2024	08	19	veritas kit	veritas kit	veritas kit	silica	ANRO	
2	Cac_02	2024	08	19	2024	08	19	veritas kit	veritas kit	veritas kit	silica	ANRO	
3	Cac_03	2024	08	19	2024	08	19	veritas kit	veritas kit	veritas kit	silica	ANRO	
4	Cac_04	2024	08	19	2024	08	19	veritas kit	veritas kit	veritas kit	silica	ANRO	
5	Cac_05	2024	08	19	2024	08	19	veritas kit	veritas kit	veritas kit	silica	ANRO	
6	Cac_06	2024	08	19	2024	08	19	veritas kit	veritas kit	veritas kit	silica	ANRO	
7													
8													
9													
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20													

16 RELINQUISHED BY: (Signature/Print)	17 DATE: (YYYY/MM/DD)	18 TIME: (HH:MM)	RECEIVED BY: (Signature/Print)	DATE: (YYYY/MM/DD)	TIME: (HH:MM)
			JL-AM Mirabzadeh	For Lab Use Only AUG 22 2024	10:17am

[1] See instruction guide for the available eDNA assays at Bureau Veritas.

Unless otherwise agreed to in writing, work submitted on this Chain of Custody is subject to Bureau Veritas' standard Terms and Conditions. Signing of this Chain of Custody document is acknowledgment and acceptance of our terms which are available for viewing at https://www.bvna.com/coc-terms-and-conditions

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