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Monitoring the pink salmon invasion in Tana using eDNA

Assessment of pink salmon, Atlantic salmon and European bullhead

Frode Fossøy, Jaakko Erkinaro, Panu Orell, Jan-Peter Pohjola, Hege Brandsegg, Ida Pernille Øystese Andersskog, Rolf Sivertsgård

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Ida Pernille Øystese Andersskog
Rolf Sivertsgård

Luke
NATURAL RESOURCES INSTITUTE FINLAND
NINAGEN
Centre for biodiversity genetics
NINA

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COVER PICTURE
Male pink salmon © Panu Orell

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CONTACT DETAILS

NINA head office
P.O.Box 5685 Torgarden
NO-7485 Trondheim
Norway
P: +47 73 80 14 00

NINA Oslo
Sognsveien 68
0855 Oslo
Norway
P: +47 73 80 14 00

NINA Tromsø
P.O.Box 6606 Langnes
NO-9296 Tromsø
Norway
P: +47 77 75 04 00

NINA Lillehammer
Vormstuguvegen 40
NO-2624 Lillehammer
Norway
P: +47 73 80 14 00

NINA Bergen:
Thormøhlens gate 55
NO-5006 Bergen.
Norway
P: +47 73 80 14 00

www.nina.no

Abstract

Fossøy, F., Erkinaro, J., Orell, P., Pohjola, J.-P., Brandsegg, H., Andersskog, I.P.Ø. & Sivertsgård, R. 2022. Monitoring the pink salmon invasion in Tana using eDNA. Assessment of pink salmon, Atlantic salmon and European bullhead. NINA Report 2213. Norwegian Institute for Nature Research.

Human introduction of non-native species is a major threat to biodiversity, and early detection is crucial for implementing conservation mitigations. The pink salmon (*Oncorhynchus gorbuscha*) is originally native to rivers draining to the Pacific ocean, but reports on occurrence of pink salmon in Norway have increased sharply in recent years. Pink salmon is an anadromous species with a two-year life cycle where both males and females die after spawning. In Norway, the odd-year spawners are dominating with large numbers of fish recorded in 2017, 2019 and 2021. Monitoring presence and abundance of pink salmon is crucial for implementing possible mitigation efforts. Analyses of environmental DNA (eDNA) is a new cost-efficient method for detecting rare and invasive species. Here we report the results from eDNA analyses of the river Tana, including 19 localities in 2019 and 24 localities in 2021. The Tana watercourse constitutes the border between northern Finland and Norway and is supporting the largest Atlantic salmon population in Norway. The eDNA analyses detected Atlantic salmon (*Salmo salar*) in almost all tributaries in both years, with a pronounced higher DNA-concentration in the middle of the watercourse. In 2019, we detected pink salmon in four different tributaries, representing more or less all parts of the watercourse. In 2021, we detected pink salmon in 15 localities, with somewhat higher DNA-concentrations than in 2019, reflecting the observed increase in pink salmon numbers. The eDNA analyses also included another alien species in the Tana system, the European bullhead (*Cottus gobio*), where detections were constrained to the lower parts of Tana. We conclude that analysis of eDNA water samples is a cost-efficient method for monitoring the invasion of pink salmon at many localities, with the potential of including analyses of multiple species. We recommend that future monitoring implements a standard design with resampling of the same localities at the same time each year to enable inference on long-term trends in eDNA-concentrations.

Frode Fossøy, Norwegian Institute for Nature Research (NINA), frode.fossoy@nina.no
Jaakko Erkinaro, Natural Resources Institute Finland (LUKE), jaakko.erkinaro@luke.fi
Panu Orell, Natural Resources Institute Finland (LUKE), panu.orell@luke.fi
Jan-Peter Pohjola, Natural Resources Institute Finland (LUKE), jan-peter.pohjola@luke.fi
Hege Brandsegg, Norwegian Institute for Nature Research (NINA), hege.brandsegg@nina.no
Ida Pernille Øystese Andersskog, Norwegian Institute for Nature Research (NINA), ida.andersskog@nina.no
Rolf Sivertsgård, Norwegian Institute for Nature Research (NINA), rolf.sivertsgard@nina.no

Contents

Foreword

This report is based on two projects commissioned by the County Governor of Troms and Finnmark for investigating pink salmon presence and distribution using eDNA in the Tana watercourse in 2019 and 2021. The Tana watercourse includes the longest anadromous river in Norway, and numerous tributaries feed into the main river. Whereas conventional monitoring is limited to some specific localities, this study implements eDNA for large-scale sampling of many tributaries covering 19 localities in 2019 and 24 localities in 2021. The results suggest that eDNA is a cost-effective method for mapping the geographical distribution of both pink and Atlantic salmon in the Tana watercourse and that it can be used for tracking changes for both species in the years to come. We are grateful to Eirik Frøiland, representing the County Governor of Troms and Finnmark, for supporting this project and fruitful discussion along the way.

29. November 2022
Frode Fossøy

1 Introduction

Human introduction of non-native species is a major threat to biodiversity globally, and early detection and response can be crucial for mitigating their effects (Dueñas et al. 2021). One such example is the spread of pink salmon (*Oncorhynchus gorbuscha*) in the Atlantic ocean, after human introduction to the Kola Peninsula in several stages since the late 1950s (Mo et al. 2018, Sandlund et al. 2019, VKM et al. 2020). The pink salmon is originally native to rivers draining to the Pacific ocean. Reports on occurrence of pink salmon in Norway have increased sharply in recent years, and in particular in northern Norway being close to the Kola peninsula (Berntsen et al. 2018, Berntsen et al. 2020). The reason for the sudden increase is still poorly understood, but rising sea temperatures could play an important role.

Pink salmon is an anadromous species with a two-year life cycle where both males and females die after spawning. The pink salmon young migrate to the ocean in the spring and spend one year feeding in the marine environment before returning to the rivers for spawning in August the following year. This creates two different populations, with even-year and odd-year spawners. In Norway, the odd-year spawners are dominating, with only a few even-year spawners in comparison (Berntsen et al. 2018, Mo et al. 2018, Berntsen et al. 2020).

Monitoring the presence and abundance of pink salmon in different rivers is crucial for implementing proper mitigations. Analyses of environmental DNA (eDNA) is a cost-efficient method for detecting single species and monitoring complex ecosystem using simple water samples collected in rivers and lakes (Taberlet et al. 2018). eDNA is the remains of genetic material shed by living organisms through saliva, faeces, scales, hair, etc. suspended in water or soil or adhered to particles. By filtering water, we can collect the eDNA and identify species living in the environment using genetic markers. Comparisons with conventional methods show that analyses of eDNA often are more sensitive in detecting rare species, and can return longer lists of species and biodiversity information across diverse taxa (Valentini et al. 2016). This method has also proven very effective in monitoring invasive species (Fossøy et al. 2019a, Sepulveda et al. 2020, Taugbøl et al. 2021). NINA has during the last few years developed both sampling equipment and genetic tools for analysing eDNA and implemented standard protocols for many aquatic organisms (Fossøy et al. 2017, Taugbøl et al. 2017, Fossøy et al. 2018, Taugbøl et al. 2018, Fossøy et al. 2019b, Wacker et al. 2019), and has recently developed a new genetic marker for detecting pink salmon (Gargan et al. 2021).

The large river Tana forms the border between northernmost Finland and Norway and is supporting the largest Atlantic salmon (*Salmo salar*) population complex and the highest salmon catches among Norwegian salmon rivers (VRL. 2022). Recently the Tana salmon stocks have declined (Anon. 2021) and salmon fishing have been ceased at least for the 2021 and 2022 seasons. At the same time numbers of non-native pink salmon have increased explosively. At the same time, numbers of non-native pink salmon has increased explosively. Occasional pink salmon have been caught in the Tana fisheries already since early 1960s, and catches have varied since, being higher in some odd years following large releases of pink salmon juveniles in Russia, but rather low in most years (Sandlund et al. 2019). The estimated numbers of pink salmon entering the river system in the 2000s have been some tens or hundreds. In 2017, close to 5000 pink salmon were estimated entering the river, and approximately the same amount was detected by a sonar in 2019. In 2021, estimated pink salmon run exceeded 50 000 individuals (Anon. 2021). Based on the Atlantic salmon monitoring programme extending to multiple tributaries of the large Tana system, expansion of pink salmon into the tributaries has been observed by video arrays, snorkelling and sonars. Until 2021, the monitoring programmes have revealed pink salmon only in large headwater branches and some other large or mid-size tributaries of the Tana system, in addition to the main stem of the river.

Here, we report the first results from eDNA analyses carried out in 2019 and 2021, where samples were collected in multiple tributaries of the Tana watercourse. The objective of this study

was to analyse the spatial distribution of pink salmon in different tributaries. In addition to pink salmon, a secondary focus was put on another introduced species in the Tana system, the European bullhead (*Cottus gobio*) which was accidentally introduced to the system in late 1970s and has expanded its spatial distribution since (Pihlaja et al. 1998, Pohjola et al. 2021).

2 Material and methods

2.1 Collection of samples

eDNA samples were collected by the Natural Resources Institute Finland (LUKE) in August 2019 and 2021 (**Figure 1**, **Appendix Table 1**). Water was filtrated in duplicates per station on a 2.0 µm glassfiber filter (Merck Millipore) in 2019 and on a 0.8 µm capsule filter (NatureMetrics) in 2021 by the help of a peristaltic pump (Bürkle Vampire). DNA was conserved by adding ATL-buffer (Qiagen) and the filters were stored at room temperature until further analyses at the Centre for Genetic Biodiversity (NINAGEN) in Trondheim. Positive field controls were sampled in the river Komag in 2019 and in the river Utsjoki in 2021. Negative field controls included filtering of bottled drinking water.

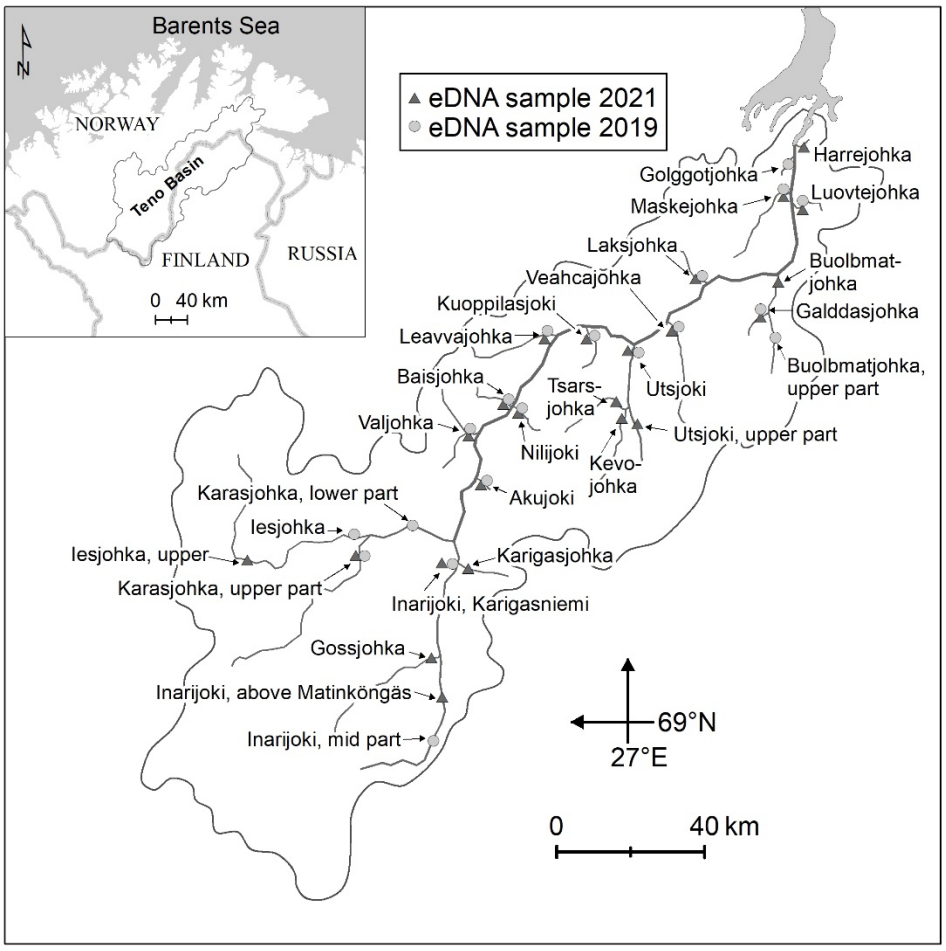

***Figure 1**. Map showing sampling localities for eDNA in Tana for both 2019 and 2021.*

2.2 Laboratory analyses

The glass fiber filters were stored in 5 mL tubes containing 4.05 mL ATL-buffer and DNA-extraction was initiated by adding 450 µL proteinase-K before incubation at 56°C overnight. The NatureMetrics capsule filters contained approx. 1.5 mL ATL-buffer and 130 µL proteinase-K (diluted 1:10) was added to the filters before incubation at 56°C overnight. DNA was extracted from both types of water filters using a combination of NucleoSpin Plant II (Machery-Nagel) spin columns, and Blood & Tissue buffers (Qiagen) for both filter types. DNA was eluted in 200 µL preheated AE-buffer and thereafter re-eluted for maximising the DNA-output.

Species-specific genetic markers for pink salmon (Gargan et al. 2021) and Atlantic salmon (Fossøy et al. 2019a) were analysed using digital droplet PCR (ddPCR). The ddPCRs consisted of 0.9 µM forward and reverse primers, 0.25 µM of the probes, ddPCR™ Supermix for Probes (No dUTP) (Bio-Rad Laboratories), dH2O, and 5 µl template-DNA. To generate droplets, an AutoDG™ Instrument (Bio-Rad Laboratories) was used, with subsequent PCR amplification in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems). The following thermal cycling conditions were used: an initial denaturation step at 95°C for 10 min, 40 cycles of denaturation at 95°C for 30 s, annealing and extension at 60°C for 1 min, a final step of denaturation at 98°C for 10 min, and a final hold at 4°C. PCR plates were transferred to a QX200™ Droplet Reader (Bio-Rad Laboratories) to automatically detect the fluorescent signal in the droplets. QuantaSoft software v.1.7.4 (Bio-Rad Laboratories) was used to separate positive from negative droplets, according to manufacturer's instructions. To prevent presence of false positives, we conservatively set a limit of minimum three positive droplets for assessing a sample as positive (Dobnik et al. 2015).

For European Bullhead, we developed a new genus-specific assay based on a part of the mitochondrial 16S gene. We used Primer Express 3.0.1 (Applied Biosystems) to design primers and TaqMan MGB probes (NINAcottus, **Table 1**). The new primer combination was tested for cross-species amplification of several related and un-related species, including the closely related Alpine bullhead (*Cottus poecilupus*) that was as expected amplified by the genus-specific assay. There was however no sign of cross-amplification when analysing Eurasian minnow (*Phoxonus phoxinus*), perch (*Perca fluviatilis*), pike (*Esox lucius*), burbot (*Lota lota*), sea lamprey (*Petromyzon marinus*), Arctic char (*Salvelinus alpinus*), trout (*Salmo trutta*), Atlantic salmon or pink salmon (data not shown). A standard curve of known DNA-concentrations revealed a high efficiency of the new assay (**Appendix Figure 1**). We also analysed the presence of pink salmon using quantitative PCR (qPCR) in both years. Each qPCR-reaction for both species had a total volume of 30 µL which included 15 µL TaqMan Fast Advanced Master Mix (ThermoFisher Scientific), 0.9 µM of forward, reverse primer and probe, 4.5 dH_20 and 5 µL DNA-template. PCR-conditions started with an onset of 50°C for 2 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec and 60°C for 90 sec, and were finalized with 72°C for 10 min. All samples were analysed using a QuantStudio 5 qPCR-machine (ThermoFisher Scientific), and all samples were analysed in triplicates, where only samples showing at least 2 out of 3 positive results were considered positive for the target species. A standard curve for the pink salmon assay showed a high efficiency (**Appendix Figure 2**).

***Table 1**. Details on the new genus-specific 16S qPCR-assay (NINAcottus) designed as part of this study for detecting European Bullhead (Cottus gobio) and other Cottus spp.*

Primer name	Primer sequence
Cottus1F_318	CCACGTGGAATGGGAACACT
Cottus1R_397	GCCGGATCTTGTTGGTCAGA
Cottus1P_340	TCCTACAACTAAGAGCTACAGC

3 Results and discussion

In total, we analysed 38 filters from 19 localities in 2019, and 48 filters from 24 localities in 2021, from the Tana watercourse. The results from both qPCR and ddPCR analyses showed that the positive field controls were positive, and that the negative field controls were negative. All extraction controls and PCR controls in the lab were also negative.

The eDNA analyses detected Atlantic salmon at almost all tributaries in both years, with a pronounced higher DNA-concentration in the middle of the watercourse (**Figure 2**). Because we switched filter types among years, we cannot directly compare changes in water concentration, but the results were relatively similar with ca. 3000 DNA-copies per litre water estimated in the ddPCR-analyses in the middle of the watercourse in both years.

Pink salmon was only detected in three localities in 2019 using ddPCR: in Maskejohka, in the lower part of Karasjohka and in the lower part of Inarijoki (**Figure 2**). Reanalysing the samples using qPCR confirmed these results, and moreover also revealed presence of pink salmon in the upper parts of both Karasjohka and Inarijoki (**Appendix Table 2**). The upper part of Inarijoki represents the most upstream detection of pink salmon recorded in Tana so far. In addition, the qPCR analyses detected pink salmon in the Leavvajohka tributary, in the middle of the Tana watercourse. Several samples showed amplification in 1 of 3 replicates, and we cannot exclude that these are undetectable concentrations of pink salmon. However, we treat these as negative results by definition.

In 2021, we detected pink salmon in 15 localities, with somewhat higher DNA water concentrations than in 2019 (**Figure 3**). Again, we cannot directly compare the two years since we switched filter types. However, whereas ca. 5000 pink salmon were estimated to enter the river in 2019, the pink salmon run in 2021 likely exceeded 50 000 individuals, i.e. a ten-fold increase (Anon. 2021). An increase in the number of detections as well as DNA-concentration is therefore expected. The detections were distributed across all parts of the Tana watercourse and shows the large-scale geographical span of the alien species in this river. The negative results were all accompanied by other samples showing positive detections in all tributaries, and most tributaries showed positive detection in the most upstream region (**Figure 3**, **Appendix Table 3**). The upmost sample in Iesjohka was however negative, and only sampled in 2021. Hence, we can only document pink salmon as far up as Karasjohka in this part of the watercourse.

The DNA-quantities of Atlantic salmon appeared to be higher than for pink salmon. There is likely species-specific differences in shedding and excretion of DNA that could affect these estimates. However, although the pink salmon run was ca. twice of that for Atlantic salmon (Anon. 2021), there is only one age class of fish. For Atlantic salmon, there are up to six different age classes of juveniles, plus adult spawners returning from the sea. This fact also makes it hard to assess the relative abundance of pink vs. Atlantic salmon using eDNA, as we cannot classify either number of individuals or age. However, an increasing or decreasing eDNA-concentration between years would suggest an increase or decrease in biomass for each species. Finally, the picture we see is only a snapshot in time, and represents the distribution of fish the day, or perhaps a couple of days before sampling. Hence time of sampling becomes very important for the result and needs to be standardized among years.

Detections of European bullhead were constrained to the lower parts of Tana (**Figure 4**), mainly below the River Utsjoki system where its spreading started in 1970s (Pihlaja et al. 1998). There was however positive detection in the river Valjohka, which is quite far up in the main Tana river. The new genetic marker designed in this study seems to give reliable results but still needs to be confirmed in future studies.

False positives can occur in eDNA analyses, but we try to avoid such results by including strict criteria. However, we cannot completely exclude the presence of false positives in our analyses. The confidence of a negative result is not known. The failure to detect a species can be caused

by many factors, such as water quality, temperature, sample volume and the number of individuals of the target species as well as lab protocols and procedures. A negative eDNA results should therefore not be considered as final evidence for the target species not occurring in the sample location.

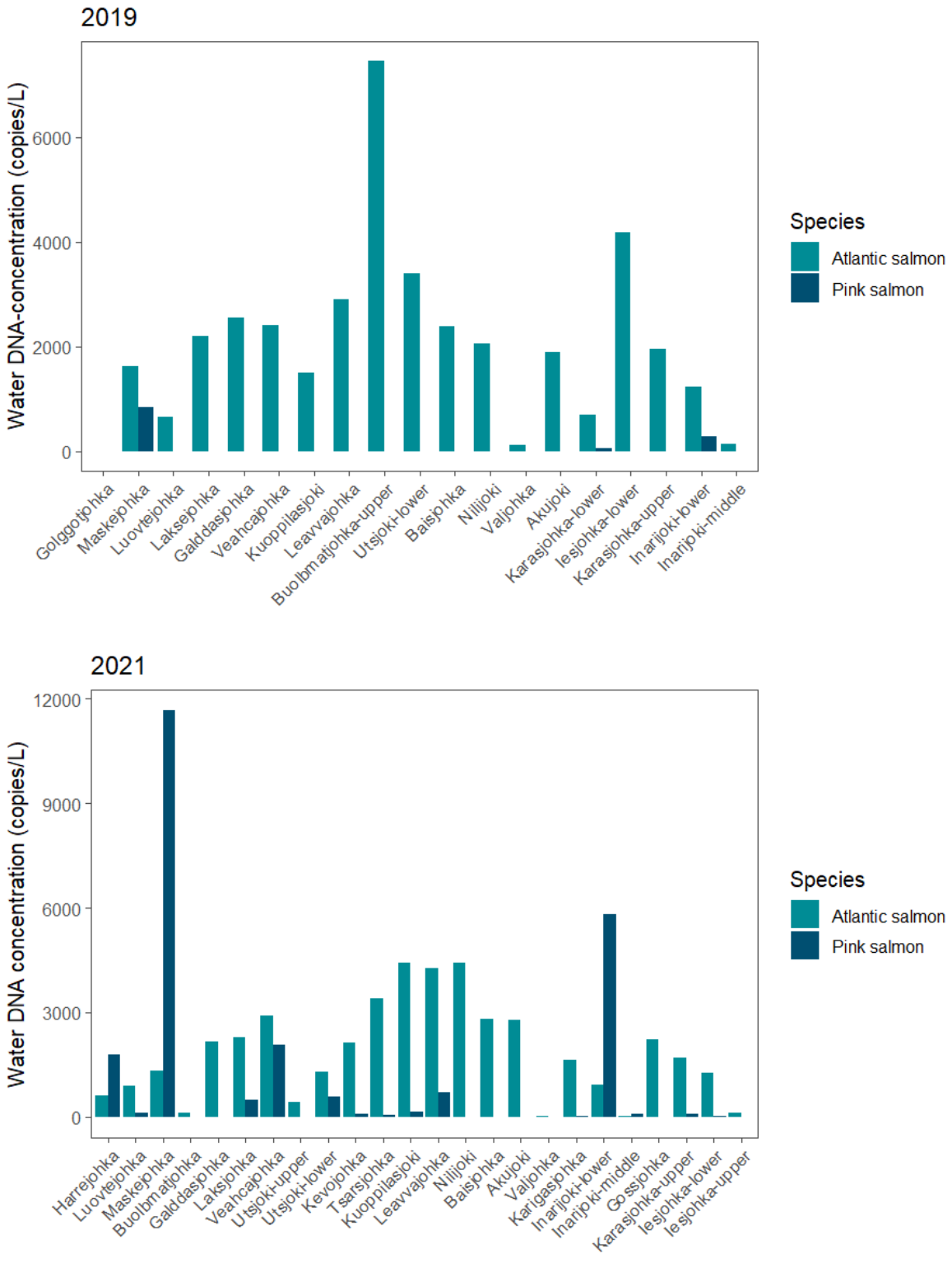

***Figure 2**. Water DNA-concentrations from analyses digital droplet PCR (ddPCR) for Atlantic and pink salmon in 2019 (upper panel) and 2021 (lower panel). Localities are sorted by longitude with downstream localities to the left and upstream to the right.*

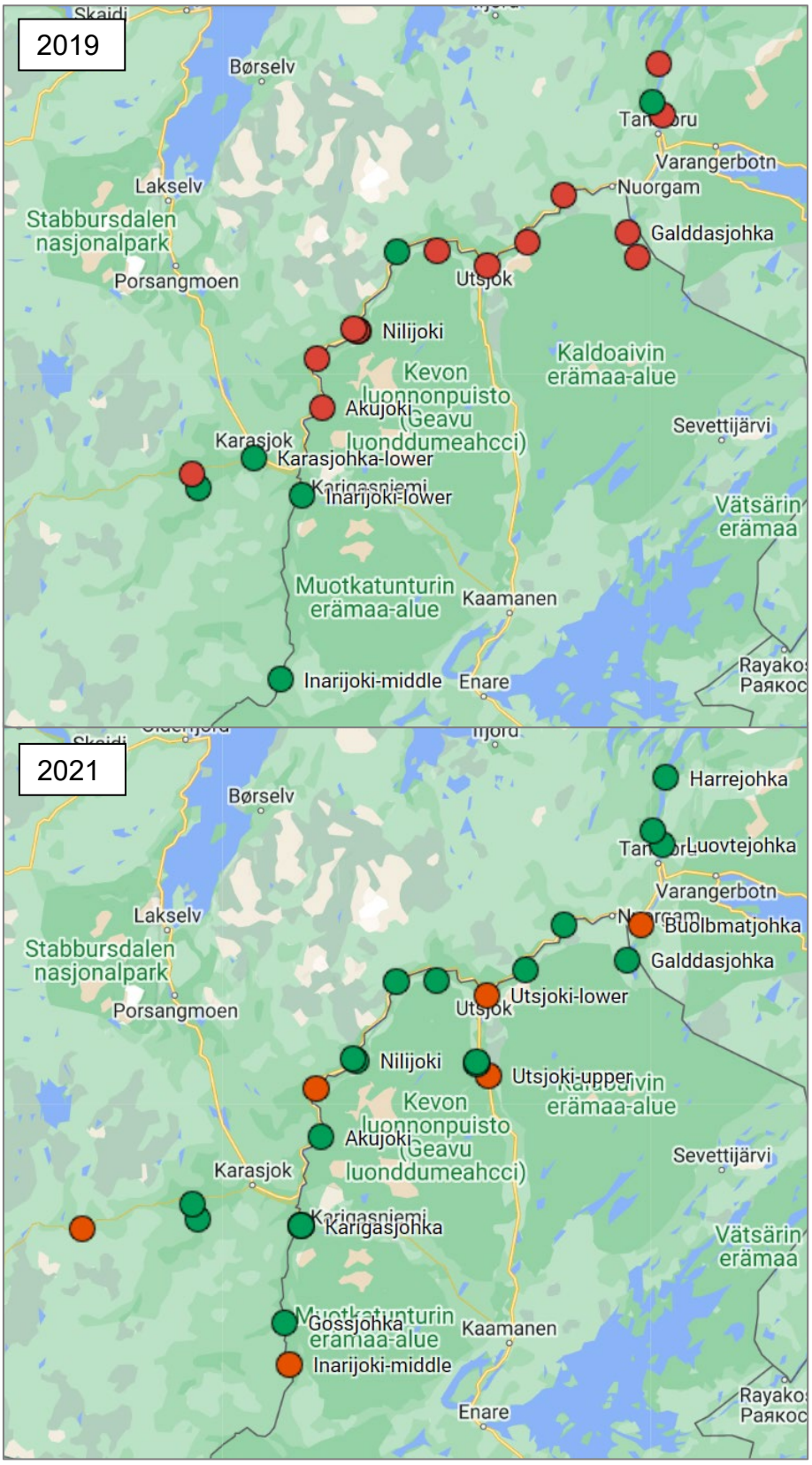

***Figure 3**. Map showing positive (green) and negative (red) results in eDNA qPCR or ddPCR analyses of pink salmon in 2019 (upper panel) and 2021 (lower panel). See Appendix Tables for details on sampling localities and qPCR results.*

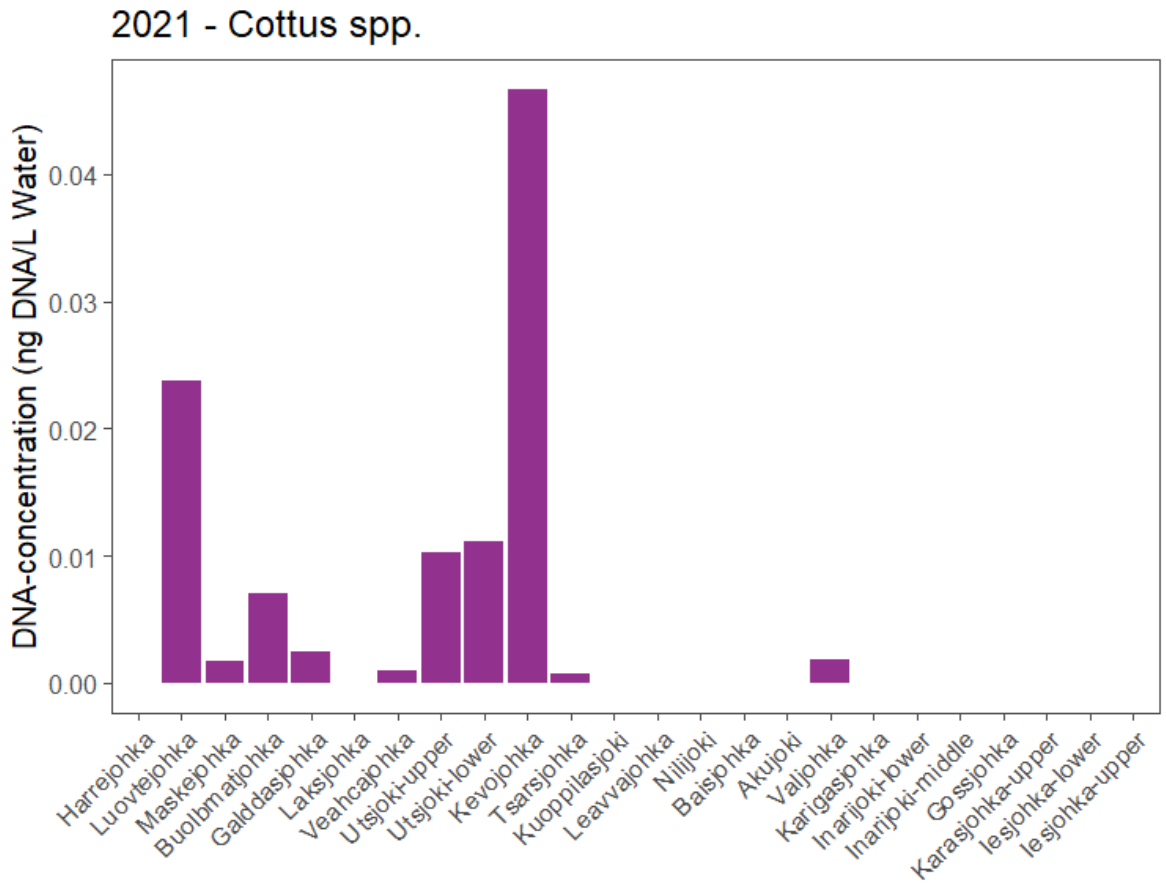

***Figure 4**. eDNA-quantity as measured by qPCR for Cottus spp., likely reflecting only European bullhead in Tana, at different localities, sorted by longitude with downstream localities to the left and upstream to the right.*

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5 Appendix

***Appendix Table 1**. List of samples collected in 2019 and 2021 showing localities, dates and GPS-coordinates in UTM35 format. For each locality, two samples were collected.*

	2021			2019		
Locality	Date	Latitude	Longitude	Date	Latitude	Longitude
Akujoki	09.08.2021	7719993	459594	14.08.2019	7720052	459591
Baisjohka	12.08.2021	7739779	468002	14.08.2019	7739753	467980
Buolbmatjohka-lower	10.08.2021	7773539	540221			
Buolbmatjohka-upper				12.08.2019	7758193	539723
Galddasjohka	10.08.2021	7764503	537239	12.08.2019	7764465	537247
Golggotjohka				13.08.2019	7806403	544066
Gossjohka	11.08.2021	7672134	449047			
Harrejohka	10.08.2021	7810098	545782			
Iesjohka-lower	12.08.2021	7703322	425915	13.08.2019	7703388	425848
Iesjohka-upper	12.08.2021	7698177	397270			
Inarijoki-middle	11.08.2021	7661056	450118	14.08.2019	7649191	447534
Inarijoki-lower	11.08.2021	7697386	453990	13.08.2019	7697416	454006
Karasjohka-lower				13.08.2019	7707458	441508
Karasjohka-upper	11.08.2021	7699783	427122	13.08.2019	7699824	427160
Karigasjohka	11.08.2021	7697339	454081			
Kevojohka	12.08.2021	7738194	499378			
Kuoppilasjoki	09.08.2021	7759587	489209	13.08.2019	7759592	489182
Laksjohka	10.08.2021	7773194	521021	13.08.2019	7773196	521020
Leavvajohka	13.08.2021	7759075	479153	14.08.2019	7759040	479177
Luovtejohka	10.08.2021	7793790	545258	13.08.2019	7793787	545272
Maskejohka	10.08.2021	7796940	542983	13.08.2019	7796940	542983
Nilijoki	09.08.2021	7739506	468860	13.08.2019	7739471	469322
Utsjoki-lower	23.07.2021	7755708	501789	12.08.2019	7755708	501789
Utsjoki-upper	13.08.2021	7735183	502137			
Tsarsjohka	12.08.2021	7738804	499302			
Valjohka	12.08.2021	7732083	458531	14.08.2019	7732154	458611
Veahcajohka	09.08.2021	7761705	511547	12.08.2019	7761579	512038
Komag				16.08.2019		

***Appendix Table 2**. Results from qPCR analyses of samples collected in 2019. All samples were analysed in triplicates where only samples showing at least 2 out of 3 positive replicates were considered positive for pink salmon. The number of temperature cycles in the PCR before a positive signal was recorded (Ct Mean) indicates the concentration of target DNA in the sample, where a lower Ct indicates higher concentrations.*

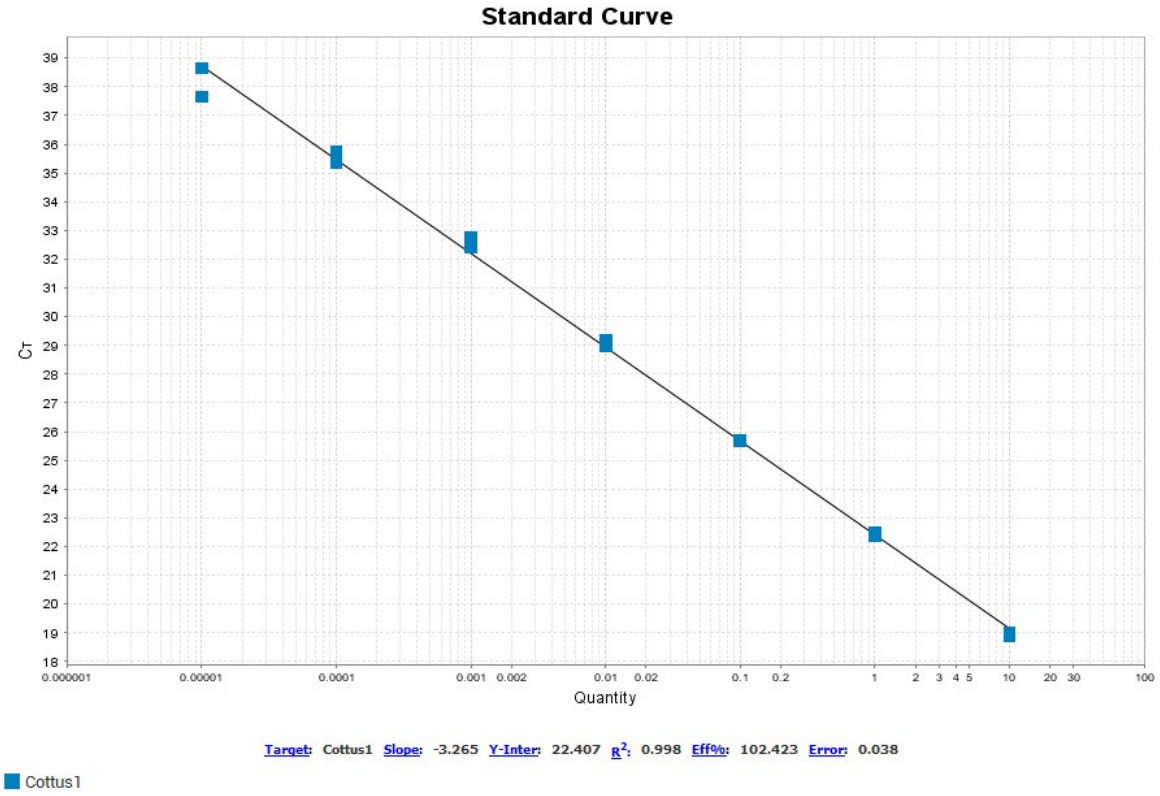
Sample no.	Locality	Water temperature (C)	Water volume (L)	No. PCR replicates	No. positive PCR replicates	Ct Mean	Ct SD	Conclusion
1	Utsjoki	12.1	5	3	0			NEG
2	Utsjoki	12.1	5	3	1	38.96		NEG
3	Veahcajohka	11.0	5	3	0			NEG
4	Veahcajohka	11.0	5	3	1	38.34		NEG
5	Galddasjohka	10.7	5	3	1	40.10		NEG
6	Galddasjohka	10.7	5	3	0			NEG
7	Buolbmatjohka-upper	11.2	4	3	0			NEG
8	Buolbmatjohka-upper	11.2	5	3	0			NEG
9	Luovtejohka	9.3	5	3	1	40.02		NEG
10	Luovtejohka	9.3	5	3	0			NEG
11	Golggotjohka	13.8	3.5	3	0			NEG
12	Golggotjohka	13.8	3.5	3	0			NEG
13	Maskejohka	10.1	5	3	3	31.16	0.19	POS
14	Maskejohka	10.1	5	3	3	31.65	0.16	POS
15	Laksejohka	10.3	5	3	0			NEG
16	Laksejohka	10.3	5	3	0			NEG
17	Kuoppilasjoki	10.0	5	3	0			NEG
18	Kuoppilasjoki	10.0	5	3	0			NEG
19	Nilijoki	10.7	5	3	0			NEG
20	Nilijoki	10.7	5	3	0			NEG
21	Inarijoki-lower	12.2	5	3	3	36.95	0.67	POS
22	Inarijoki-lower	12.2	5	3	3	34.58	0.58	POS
23	Karasjohka-upper	12.3	4	3	1	39.03		NEG
24	Karasjohka-upper	12.3	5	3	3	37.38	0.64	POS
25	Iesjohka-lower	12.5	5	3	0			NEG
26	Iesjohka-lower	12.5		3	0			NEG
27	Karasjohka-lower	12.8	5	3	3	36.94	0.74	POS
28	Karasjohka-lower	12.8	5	3	3	38.16	1.18	POS
29	Leavvajohka	8.6	5	3	2	39.43	0.74	POS
30	Leavvajohka	8.6	5	3	0			NEG
31	Baisjohka	10.0	5	3	0			NEG
32	Baisjohka	10.0	5	3	0			NEG
33	Valjohka	12.0	5	3	1	39.67		NEG
34	Valjohka	12.0	5	3	0			NEG

35	Akujoki	10.0	5	3	0			NEG
36	Akujoki	10.0	5	3	0			NEG
37	Inarijoki-middle	12.7	5	3	2	39.14	0.47	POS
38	Inarijoki-middle	12.7	5	3	0			NEG
K1	Komag (positive control)	10.9	5	3	3	28.04	0.15	POS
K2	Komag (positive control)	10.9	5	3	3	27.47	0.15	POS
K3	Komag (positive control)	10.9	5	3	3	28.34	0.37	POS
K4	Komag (positive control)	10.9	5	3	3	28.27	0.11	POS

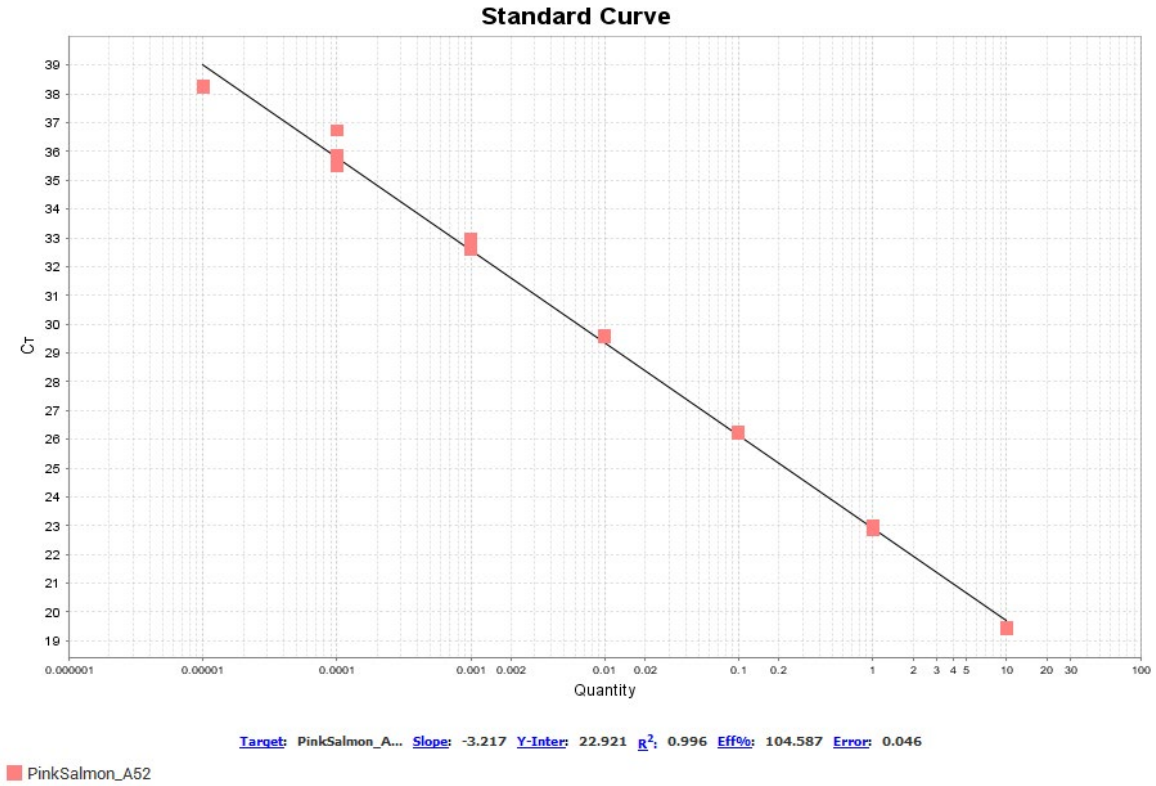
***Appendix Table 3**. Results from qPCR analyses of samples collected in 2021. All samples were analysed in triplicates where only samples showing at least 2 out of 3 positive replicates were considered positive for pink salmon. The number of temperature cycles in the PCR before a positive signal was recorded (Ct Mean) indicates the concentration of target DNA in the sample, where a lower Ct indicates higher concentrations.*

Sample no.	Verbatim locality	Water temperature (C)	Water volume (L)	No. PCR replicatess	No. positive PCR-repliacates	Ct_Mean	Ct_SD	Conclusion
1	Harrejohka	11.5	5	3	3	30.15	0.17	POS
2	Harrejohka	11.5	5	3	3	29.19	0.00	POS
3	Maskejohka	15.7	5	3	3	26.72	0.18	POS
4	Maskejohka	15.7	5	3	3	26.76	0.11	POS
5	Luovtejohka	12.3	5	3	3	33.20	0.23	POS
6	Luovtejohka	12.3	5	3	3	33.46	0.31	POS
7	Buolbmatjohka-lower	14.5	5	3	0			NEG
8	Buolbmatjohka-lower	14.5	5	3	0			NEG
9	Galddasjohka	15.4	5	3	3	35.99	0.93	POS
10	Galddasjohka	15.4	5	3	0			NEG
11	Laksjohka	15	5	3	3	30.88	0.12	POS
12	Laksjohka	15	5	3	3	35.54	1.43	POS
13	Veahcajohka	15.6	5	3	3	29.09	0.20	POS
14	Veahcajohka	15.6	5	3	3	29.69	0.10	POS
15	Utsjoki-upper	14.8	5	3	0			NEG
16	Utsjoki-upper	14.8	5	3	0			NEG
17	Kevojohka	15.5	5	3	3	33.86	0.35	POS
18	Kevojohka	15.5	5	3	3	33.44	0.46	POS
19	Tsarsjohka	15.5	5	3	3	34.52	0.44	POS
20	Tsarsjohka	15.5	5	3	3	34.60	0.79	POS
21	Kuoppilasjoki	15.1	5	3	3	32.92	0.07	POS
22	Kuoppilasjoki	15.1	5	3	3	33.06	0.34	POS
23	Leavvajohka	13.4	5	3	3	31.11	0.28	POS
24	Leavvajohka	13.4	5	3	3	30.50	0.09	POS
25	Baisjohka	15.5	5	3	0			NEG
26	Baisjohka	15.5	5	3	0			NEG
27	Nilijoki	15.4	5	3	0			NEG
28	Nilijoki	15.4	5	3	1	41.30		NEG
29	Valjohka	15.9	5	3	2	38.72	1.57	POS
30	Valjohka	15.9	5	3	1	37.09		NEG
31	Akujoki	14.8	5	3	1	37.32		NEG
32	Akujoki	14.8	5	3	1	37.47		NEG
33	Karasjohka-upper	17.1	5	3	3	36.23	0.40	POS
34	Karasjohka-upper	17.1	5	3	3	35.49	0.37	POS
35	Iesjohka-lower	15.7	5	3	3	39.10	2.52	POS

36	Iesjohka-lower	15.7	5	3	3	38.06	0.32	POS
37	Iesjohka-upper	16.1	5	3	0			NEG
38	Iesjohka-upper	16.1	5	3	0			NEG
39	Inarijoki-lower	15.5	5	3	3	28.60	0.03	POS
40	Inarijoki-lower	15.5	5	3	3	28.90	0.52	POS
41	Karigasjohka	14.4	5	3	3	35.83	0.60	POS
42	Karigasjohka	14.4	5	3	0			NEG
43	Gossjohka	16.6	5	3	2	39.52	0.99	POS
44	Gossjohka	16.6	5	3	0			NEG
45	Inarijoki-middle	15.8	5	3	0			NEG
46	Inarijoki-middle	15.8	5	3	0			NEG
47	Negative field control	17.7	5	3	0			NEG
48	Negative field control	17.7	5	3	1	38.21		NEG
49	Utsjoki-lower	14.5	5	3	0			NEG
50	Utsjoki-lower	14.5	5	3	3	39.66	0.62	POS



Appendix Figure 1. *Standard curve for the qPCR analyses of the genus specific Cottus spp. genetic marker used in this study showing the relationship between DNA-quantity in a dilution series on the x-axis and the resulting Ct on the y-axis.*



***Appendix Figure 2**. Standard curve for the qPCR analyses of the pink salmon genetic marker used in this study showing the relationship between DNA-quantity in a dilution series on the x-axis and the resulting Ct on the y-axis.*

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NINA Report

The Norwegian Institute for Nature Research, NINA,
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Norwegian Institute for Nature Research
NINA head office
Postal address: P.O. Box 5685 Torgarden,
NO-7485 Trondheim, NORWAY
Visiting address: Høgskoleringen 9, 7034 Trondheim
Phone: +47 73 80 14 00
E-mail: firmapost@nina.no
Organization Number: 9500 37 687
http://www.nina.no

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