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Detection of Non-Native Pink Salmon (*Oncorhynchus gorbuscha*) in Swedish Rivers Using eDNA

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Received: 9 October 2024 | **Revised:** 14 April 2025 | **Accepted:** 24 April 2025

Funding: This work was supported by Svenska Forskningsrådet Formas, 2022-01970.

Keywords: alien invasive species | Baltic Sea | fish | North Sea | salmonid | watercourse

ABSTRACT

Biological invasions are increasing on a worldwide scale and can have severe ecological and economic consequences. Pink salmon (*Oncorhynchus gorbuscha*) have a native range in the Pacific Ocean but have been introduced and are now spreading in the Arctic and North Atlantic Oceans, including Sweden. A comprehensive eDNA sampling scheme was conducted in 27 river systems in south-western Sweden in 2023, with the aim to detect adult spawning pink salmon. We applied qPCR and dPCR analysis on aquatic eDNA samples. Results indicated the presence of pink salmon at 24 sites across 11 river systems. Pink salmon were not detected in any of the sampled rivers that drain into the Baltic Sea. However, pink salmon were present in a river only 35 km from the entrance to the Baltic Sea, which means there is a high risk pink salmon will spread into the Baltic Sea in the coming years. Catch reporting is generally low and camera fish counters are few across these systems; therefore, the strength of incorporating eDNA methods is beneficial for the development of pink salmon monitoring programs and aquatic invasive species management.

1 | Introduction

Introductions of non-native fish are widespread across the globe (Gozlan et al. 2010). While many non-native species cause little or no harm to their new surroundings, some species have harmful impacts on the environment and pose a threat to biodiversity as they then become invasive (Britton et al. 2011; Pejchar and Mooney 2009). Subsequently, invasive species play a major role in altering ecosystem services and functions, and can lead to dire economic consequences (Bax et al. 2003). Difficulties often lie in detecting non-native, invasive species during the early stages of establishment in new areas, when they occur in low numbers.

Pink salmon (*Oncorhynchus gorbuscha* Walbaum) is native to the northern Pacific region, where it is the most widely distributed among numerous of the salmon species (Ruggerone et al. 2023). From the 1950s until the late 1990s, pink salmon were transplanted from the Pacific to the Kola Peninsula in Russia (Gordeeva and Salmenkova 2011) to establish self-recruiting fisheries. Since then, pink salmon have spread from northwest Russia and are now being reported in unprecedented numbers throughout many countries across the Barents Sea and North Atlantic region, from Europe to Greenland and eastern Canada (Armstrong et al. 2018; Mo et al. 2018; Millane et al. 2019; Nielsen et al. 2020; Eliasen and Johannesen 2021; Staveley and Ahlbeck Bergendahl 2022). Pink salmon have an almost exclusive 2-year lifecycle, with

spawning in rivers and long-distance ocean-feeding migrations. Distinct odd and even year spawning populations are genetically separate (Ruggerone et al. 2023) and the odd year population is known to be dominant in the North Atlantic and Barents Sea regions. In the Atlantic region, spawning of pink salmon generally occurs in August–September (Lennox et al. 2023).

Competition between non-native pink salmon and other aquatic species is largely unknown within the freshwater and marine environments in the North Atlantic Ocean and Barents Sea region (Lennox et al. 2023). Pink salmon often spawn relatively close to the river mouth but may also migrate far upstream to spawn if suitable spawning habitats are available (Dunmall et al. 2016). In their native range, pink salmon fry and adults feed relatively little in fresh water, and fry usually quickly migrate to the sea after emerging from the gravel (Scott and Crossman 1973). However, there is evidence of in-river feeding of juveniles in the Barents Sea region, which may pose competition with native fish species (Erkinaro et al. 2024; Veselov et al. 2016). There might be competition for food between pink salmon fry and juvenile native Atlantic salmon (*Salmo salar* L.) (Sandlund et al. 2019), but on the contrary, pink salmon eggs (Dunlop et al. 2021) and fry (Sandlund et al. 2019) may serve as a resource for other species. As pink salmon die after spawning in rivers, the decomposing adult carcasses provide an addition of nutrients to the system, affecting aquatic and riparian food webs and water quality (Sandlund et al. 2019; Thorstad et al. 2024).

In the marine environment, an increasing number of studies indicate that pink salmon, through their large abundance and intensive feeding, impact other species and food webs over vast regions of the North Pacific Ocean, mediated by competition for prey and top-down forcing (Ruggerone et al. 2023). In their non-native range in the North Atlantic Ocean, pink salmon have a varied diet that overlaps with Atlantic salmon and other native species but are, at present, not expected to have a large-scale effect on the ocean ecosystem due to their current relatively low abundance compared to other fish species (Diaz Pauli et al. 2023). However, non-native pink salmon may still have local effects from grazing on fish larvae and other prey in estuaries, fjords, and other coastal areas, particularly given that pink salmon abundance has, in general, constantly increased since 2015 (Diaz Pauli et al. 2023).

The Baltic Sea is a marginal sea of the Atlantic Ocean, with little exchange of water between the two basins through the narrow Danish Straits. We are not aware of any recordings of pink salmon in the Baltic Sea area in recent years. Many large rivers holding Atlantic salmon and sea trout (*Salmo trutta* L.) drain into the Baltic Sea, and should pink salmon enter and establish in this area, there is likely a large risk of ecological impacts not only in these rivers, but also on the marine ecosystem of the Baltic Sea. Monitoring the spread of pink salmon in the entrance area to the Baltic Sea, i.e., on each side of the Danish Straits, is crucial to predict further spread and ecological risks and to evaluate the need for mitigation measures in the Baltic Sea region.

Environmental DNA (eDNA) is a sensitive, non-invasive tool for rapid species detection, also when the target species occurs in low numbers, and eDNA methods are increasingly used for routine environmental monitoring and detection of invasive species (Bruce et al. 2021; Klymus et al. 2015; Morisette et al. 2021; Rishan et al. 2023; Takahara et al. 2013). In particular, these have become powerful methods to detect invasive species, for example, as part of fish community surveys in lake environments (Pukk et al. 2021), as early warning signals of invasive freshwater crustaceans (Mauvisseau, Tönges, et al. 2019) and specifically targeting pink salmon in a Greenland river (Nielsen et al. 2024). Therefore, eDNA tools can be used in management plans to detect early stages of invasion, thus giving potential for rapid mitigation action. eDNA methods are used for identifying taxonomic communities through multispecies analysis (Hänfling et al. 2016; Miya et al. 2020; Thomsen et al. 2012) or single target species analysis (Ficetola et al. 2008; Jerde et al. 2013; Mauvisseau, Burian, et al. 2019), such as pink salmon (Gargan et al. 2022).

Two eDNA methods to target single species include quantitative polymerase chain reaction (qPCR) and digital polymerase chain reaction (dPCR). These are both nucleic acid quantification techniques but differ in methodology. qPCR measures DNA amplification in real-time using fluorescence and requires a standard curve for quantification, while dPCR partitions the DNA sample into many individual reactions, each of which could contain a DNA molecule, thus providing an absolute count of DNA molecules (Zhang et al. 2024). qPCR as an assay has been on the market for several decades, which means that both reagents and protocols have been optimized over the years, whereas dPCR is relatively new with less established protocols. In regard to invasive species detections, both methods have strengths and limitations, which can affect sensitivity, accuracy, and resilience to environmental inhibitors.

Pink salmon have been observed and reported in the Skagerrak and Kattegat areas on the Swedish west coast from the 1970s, but most reports occurring from 2017 onwards (Staveley and Ahlbeck Bergendahl 2022). The majority of these reports have come from either a fish monitoring camera station in the river Ätran or reports from fishers and local authorities (Staveley and Ahlbeck Bergendahl 2022). The closest recordings to the Danish Straits and the Baltic Sea were from the River Ätran and the River Lagan. There is currently no national monitoring of pink salmon in Sweden and relatively small numbers have been reported; therefore, it has been difficult to determine the full extent of which rivers and how far upstream this non-native species has reached. Before any potential impacts can be further investigated, it is crucial to gain a thorough understanding of the current distribution of pink salmon in Swedish riverine systems. Therefore, this study aimed to:

1. Determine the current spatial distribution, over a season, of pink salmon during their upstream migration in 2023 along river systems on the west and south coasts of Sweden.
2. Investigate the effectiveness of using qPCR and additional dPCR analyses to detect pink salmon, providing valuable tools for monitoring and management.

2 | Methods

2.1 | Study Sites

During July and August 2023, 27 rivers were sampled for pink salmon DNA across western and southern Sweden (Figure 1; Table 1), when they begin their upstream river migration and

FIGURE 1 | The 27 rivers on the Swedish west and south coast that were sampled for pink salmon eDNA. Rivers where pink salmon were previously reported are italicized and with a red symbol. In 1975 and 1976, pink salmon were recorded in the rivers Nybroån and Örekilsälven, respectively. Except for these recordings, the southernmost observation of pink salmon before the present study was in the river Lagan.

spawning. Previous reporting (post 1975) showed the southernmost limits of pink salmon on the west coast were at the river Lagan, Kattegat; therefore, this study included rivers further south and those draining into the Baltic Sea. This was to detect the southernmost spread of pink salmon and to detect any evidence of pink salmon in the Baltic Sea region.

During the sampling period, three eDNA surveys were conducted; two small surveys on the 17th–18th July 2023 and again between the 14th and 16th of August 2023 in the rivers Örekilsälven, Göta älv, Säveån, Viskan, and Rönne å, and one large survey during 31st July—2nd August 2023, which incorporated 27 rivers (Figure 1; Table 1). During each small survey,

TABLE 1 | The 27 rivers and the number of sites per river included in the pink salmon eDNA small and large surveys 2023.

River	Small surveys	Large survey	Previous pink salmon reports (year; pre 2023)
Strömsån		2	—
Enningdalsälven		2	2021
Anråsälven		1	—
Örekilsälven	3	3	1976, 2017, 2021
Bäveån		1	—
Bratteforsån		1	—
Anråse å		1	—
Göta älv	2	3	2017, 2021
Säveån	1	2	2021
Rolfsån		2	—
Löftaån		2	—
Viskan	3	3	—
Himleån		2	—
Tvåkersån		1	—
Ätran		4	2017, 2019, 2021
Suseån		2	—
Nissan		3	—
Fylleån		2	2017
Genevadsån		1	—
Lagan		3	2021
Stensån		3	—
Rönne å	3	4	—
Råån		2	—
Kävlingeån		2	—
Nybroån		2	1975
Helge å		2	—
Mörrumsån		2	—

Note: Historical angling and camera reports of pink salmon before 2023 are also noted. Rivers are listed geographically around the coastline starting with the northernmost.

12 sites were sampled, and in the large survey, 58 sites, including those from the small surveys, were sampled (Table 1). Further site details, including coordinates, are outlined in the Appendix Tables A1–A3. The locations of the sampling sites in each river were carefully chosen based on available information on migration barriers (e.g., hydropower dams), known spawning sites of native salmonids based on results from electrofishing, and sites of previous reports (where data were available). In seven river systems, i.e., rivers Örekilsälven, Himleån, Ätran, Nissan, Lagan, Rönne å, and Helge å, both the main stems and tributaries were included.

2.2 | Field Sampling

Prior to fieldwork, all sampling equipment was sterilized and cleaned using 10% hypochlorite. The filtering equipment, including filters, syringes, gloves, and mouth protection were ordered in DNA-free one-unit disposable packages. Water collection methods and DNA capture on filters followed the European Committee for Standardization (CEN) standards for field collection of water for eDNA analyses (CEN 2023). Approximately, 10 liters of water were collected at each site using pooled subsamples. The subsamples were collected from five different points at each site, covering the shorelines and the mid-river. At the sites where the rivers were wide and deep, we made sure to have one subsample taken from the mid-river, using a Ruttner Water Sampler (Hydro-Bios DE) from bridges crossing the rivers. The collected water was mixed in five-liter sterile sampling bags (Whirl-Pak 5000 mL Stand-Up, Thermofisher Scientific, Sweden) and filtered through a single enclosed disc-filter capsule unit (NatureMetrics Ltd., UK) measuring 50 mm in diameter. Each single disc capsule unit contains two disc filters: a 5 μm GF filter on top of a 0.8 μm PES bottom filter. A peristaltic pump (Vampire sampler; Bürkle, Bad Bellingen) or a 60 mL sterile syringe (sterile Luer-Lock BD Plastipak) was used to push the water through the filters. Negative filter controls using bottled mineral water were processed in the field. Excess water from the filters was removed using a 60 mL syringe. The filters were fixated by injecting 1.5 mL 96% EtOH into the filter capsule and thereafter were sealed with sterile caps at both ends (Spens et al. 2017). At each site, the amount of water filtered, water temperature, and flow speed (using a Global water flow probe FP111) was recorded (Tables A1–A3).

2.3 | Molecular Laboratory Methods

2.3.1 | DNA Extractions

The eDNA samples, negative filter controls processed in the field, and extraction control samples (not containing DNA) were extracted in MIX Research Sweden's laboratories in Uppsala, in spaces dedicated to eDNA extractions only. All eDNA extractions took place in laminar flow hoods, which were UV-treated before and after extractions. The technicians wore protective suits, gloves, and facemasks to avoid contamination. Pipettes, tube racks, and working surfaces were disinfected using Virkon and decontaminated under UV treatment prior to use. The conditions in the laboratory followed recommendations for eDNA laboratories (Bruce et al. 2021).

Filtered water samples, negative field controls, and negative filter laborator extraction controls were extracted using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol with modifications for enclosed filters outlined in Spens et al. (2017). For each filter, 720 μL ATL and 80 μL Proteinase K (Qiagen) were added to the filter capsule and incubated at 56°C overnight. The post-lysis steps

followed the manufacturer's protocols. Samples were eluted twice in 75 μL (2 × 150 μL) 70°C TE buffer pH 8 (Thermofisher Scientific) and were incubated at RT for 10 min before centrifugation. The eDNA samples were analyzed on a Nanodrop for DNA concentrations (ng/μL). Purity was assessed by the ratio of wavelengths 260/280 nm emission (high-quality DNA ~1.8) and inhibition by measuring the 230/260 nm wavelength ratio (high quality DNA between 2.0 and 2.2) (Bruce et al. 2021). To check for the effect of possible inhibition in the extracted DNA, a set of subsamples was examined by comparing Ct of eDNA full concentration, a dilution of 1:10 eDNA: Tris (pH 8), and cleaned DNA samples (see below) in a separate qPCR assay. Dilutions of 1:10 and cleaned samples showed positive results compared to undiluted samples. A positive signal of the target species was assigned as detection, and no signal was assigned as no detection. Quantitative analyses were not performed due to the absence of IPC. All eDNA samples were cleaned in a volume of 70 μL of eDNA concentrated to 50 μL using the DNA Clean & Concentrator-5 kit (Zymo, NE) following the manufacturer's instructions.

2.3.2 | qPCR

The qPCR analyses were performed for all locations and time series, i.e., both small and large surveys, adding up to a total of 267 samples. Prior to the main qPCR analysis, species specificity for the primers and probes was tested on extracted DNA from *O. gorbuscha* and related non-target salmonid species (*Oncorhyncus mykiss*, *S. trutta* and *S. salar*). Tissue samples were preserved in absolute ethanol until they were extracted using the Qiagen DNeasy Blood and Tissue Kit following manufacturers' guidelines. The samples were eluted at 70°C 200 μL TE pH 8 (Thermofisher Scientific) and stored at −20°C until analysis.

For the qPCR assays (e.g., Thalinger et al. 2021; Wilcox et al. 2013), a species-specific TaqMan primers/probe set was used targeting 89 base pair fragments of the mitochondrial cytochrome COI gene (Gargan et al. 2022, PinkF 3′ FCACCGCCMTAAGCCTACTAA, PinkR 3′ AGGCATGGGCTGTAACGATT, Pinkprobe 3′ 6-FAM-CGCTCTTCTAGGGAATGACCA-BHQ-1). The test assay (following the PCR conditions outlined) amplified pink salmon but did not generate any amplification signals for the non-target species.

We established calibration standard curves by analyzing a 1:10 dilution series of the DNA from the tissue samples of pink salmon (1 ng/μL). This dilution series ranged from 10^{-1} to 10^{-5}. We ran four technical replicates for each dilution step to assess the lowest Ct (threshold) value for detection. The results were interpreted as detection/no detection of the target species.

The qPCR assays were performed in quadruplicates. Each assay had a final volume of 15 μL using 7.5 μL TaqMan Environmental Master Mix 2.0 (Applied Biosystems), 0.2 μL forward primer (10 μm), 0.2 μL reverse primer (10 μm) and 0.2 μL probe (2·5 μm), 0.2 μL BSA (Neo Biotechnologies) 3 μL template DNA, and 2.7 μL UV-treated molecular grade water. The TaqMan qPCRs were performed on a CFX384 Touch Real-Time PCR Detection System (Bio-Rad) using thermal cycling parameters of 50°C (2 min), 95°C (10 min) followed by 45 cycles of 95°C (15 s) and 60°C (1 min). For each plate, no-template controls (NTCs) and positive control tissue extracts (POCs) were run alongside the samples. All filtering and extraction negatives were included in the qPCR assays.

To make sure that the PCR samples were not contaminated by positive controls or by qPCR standards, a subset of the samples was run on a separate qPCR plate, without controls or standards, on another date, to detect possible contamination.

2.3.3 | dPCR

To test dPCR for future applicability of pink salmon monitoring, a subset of 40 samples was analyzed using dPCR with the same primers and probe as for qPCR. Each sample was measured twice using the QIAGEN QIAcuity One Digital PCR System and QIAGEN QIAcuity Nanoplate 26,000-partition 24-well microfluidic dPCR plate. Each plate contained NTC and a POC (extracted DNA from tissue diluted to 0.05 ng/μL). The dPCR assays were performed in duplicates. The reagents in each well had a final volume of 40 μL as follows: 10 μL QIAGEN QIAcuity Probe (4×) Master Mix, 3.2 μL of each primer (concentration 10 μM), 1.6 μL probe (concentration 10 μM), 5 μL of eDNA template, topped up with 17 nuclease-free water to reach a final volume of 40 μL. The PCR cycling consisted of an initial denaturation phase of 95°C for 2 min, followed by 40 × (95°C for 30 s, and annealing and extension at 60°C for 1 min). After PCR, images of the plate were obtained for the green color channel for the FAM dye, using 500 ms of exposure with a gain of six.

2.4 | Other Reporting and Monitoring of Pink Salmon

In 2023, pink salmon observations from the camera-equipped automatic Vaki-counter (Fiskevårdsteknik AB) in Herting, river Ätran, as well as any angling reports of pink salmon throughout the region, were collected.

3 | Results

Positive detection for pink salmon occurred in altogether 11 rivers across 24 sites.

3.1 | qPCR—Pink Salmon Species Detection

Based on the results from the qPCR analysis, pink salmon were detected in 10 rivers across 22 sites in one or more of the three surveys that were conducted in July and August 2023 (Figure 2; Table 2). All the positive controls were positive, and negative controls were negative. The river Örekilsälven was the northernmost river on the Swedish west coast to detect pink salmon, whereas the southernmost limit was the river Rönne å. No pink salmon DNA was detected in any of the rivers in the Öresund (one of the Danish Straits) or the Baltic Proper region of the Baltic Sea.

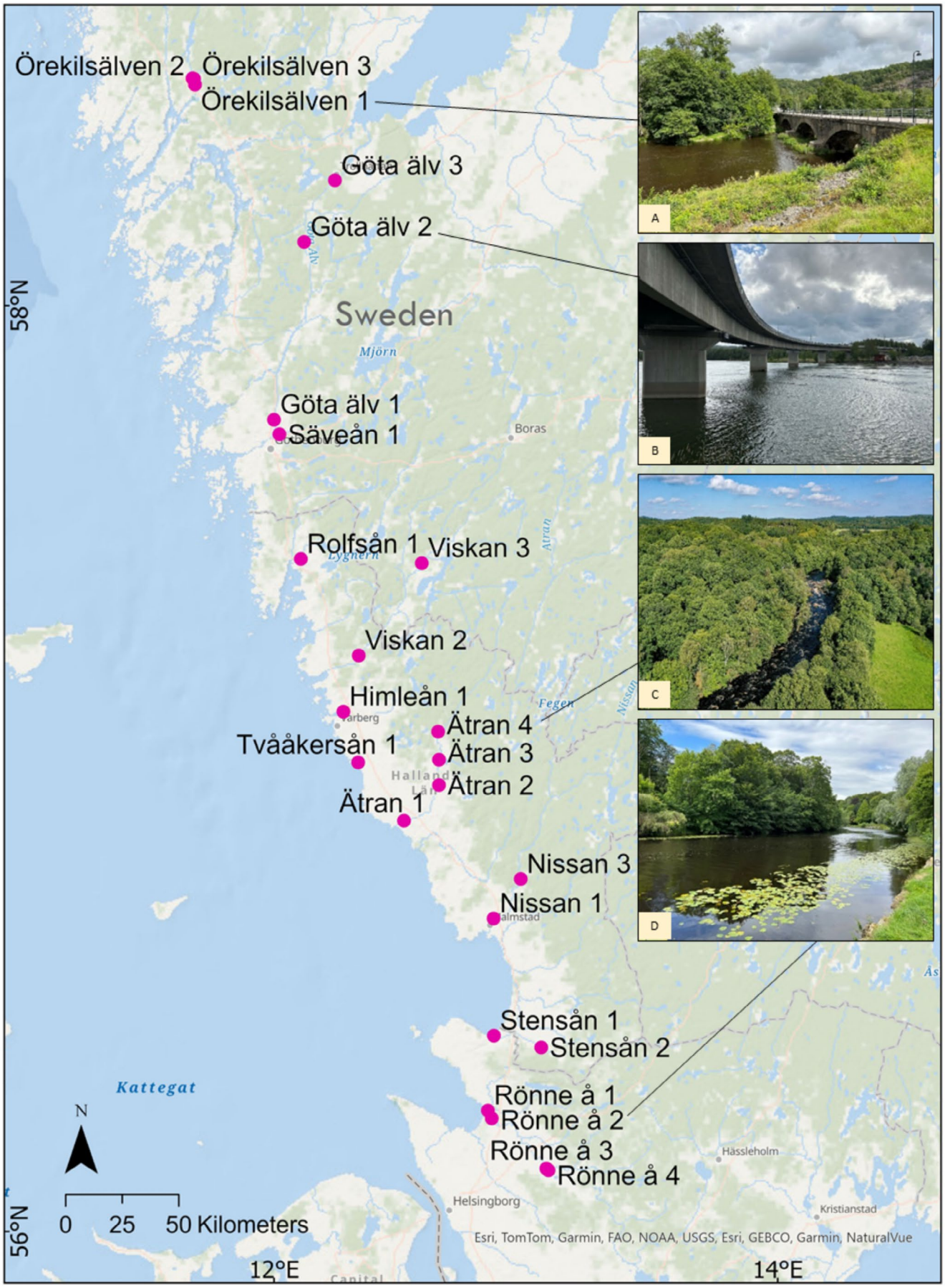

FIGURE 2 | Legend on next page.

FIGURE 2 | Sites where positive pink salmon eDNA detections were found from all surveys from mid-July to mid-August 2023. Note: See Figure 1 for a better geographic overview of the region. Insets: Locations of some of the sites where pink salmon were detected (a) Örekilsälven 1, (b) Lilla Edet; Göta älv 2, (c) Sumpafallen, tributary Högvadsån to the river Ätran; Ätran 4 and (d) Rönne å 2. Photo credits: A, B & D—Patrick Hernvall, C—Duncan Philpott.

TABLE 2 | Results of qPCR and dPCR analyses from the 24 sites in eleven rivers where pink salmon was detected in one or more of the three surveys.

	qPCR			dPCR		
Site	**Small survey 1**	**Large survey**	**Small survey 2**	**Small survey 1**	**Large survey**	**Small survey 2**
Örekilsälven 1	0	0	X (1)	0		0
Örekilsälven 2	0	0	X (2)	0		0
Örekilsälven 3	X* (1)	0	0	0		0
Göta älv 1	X (1)	0	0	X	X	0
Göta älv 2	X (4)	0	X (2)	0	0	0
Göta älv 3		0			X	
Säveån 1	0	0	0	0	X	0
Rolfsån 1		X (1)			0	
Viskan 2	0	X (2)	X (2)	0	X	0
Viskan 3	0	0	X (1)	0	0	X
Himleån 1		X (2)			0	
Tvåkersån 1		X (4)				
Ätran 1		X (4)			X	
Ätran 2		X (2)			0	
Ätran 3		X (4)			0	
Ätran 4		X (4)				
Nissan 1		X (4)				
Nissan 3		X (4)				
Stensån 1		X* (2)				
Stensån 2		X (1)				
Rönne å 1	X (4)	X (4)	0	0	X	
Rönne å 2	X (4)	X (4)	0	X	0	
Rönne å 3	X (1)	0	X* (2)	0	0	
Rönne å 4	0	X (4)	0			

Note: X shows detections, O denotes sampled sites with no detection of the target species. Detection rate based on qPCR (curves and Ct (threshold) value) shows the number of samples (n/4) with a positive qPCR signal. Note that three of the samples (indicated by an *) showed high Ct values exceeding the values of the standard curve and are therefore less trustworthy. The highest Ct value was 42.9 cycles (Göta älv 1) and corresponds to $1 \times 10\text{-}4$ ng/μL DNA, but are included in the analysis. Positive detection for the dPCR analyses denotes any partitions for the given sample. Rivers are listed in geographical order from north to south. Further detailed information can be found in Tables A4 and A5.

3.2 | dPCR—Pink Salmon Species Detection

The results from the 38 dPCR analyses showed positive signals on eight sites. Of these, six were positive for qPCR as well. These sites were Göta älv 1, Viskan 2, Viskan 3, Ätran 1, Rönne å 1, and Rönne å 2. Göta Älv 3 and Säveån 1 were the only sites where pink salmon was detected using only dPCR (Table 2).

Out of the 38 samples examined using dPCR, nine samples over eight sites displayed two partitions, which are regarded as below the recommended threshold of three for a positive sample (Table A5). The positive controls displayed partitions between 44 and 88 (out of 26,000 possible). The NTC controls were negative on four out of five plates. The samples that were analyzed on the same plate as the negative PCR control, which

displayed one partition, were discarded from the analysis. Table A4 shows in detail the results of the samples that were analyzed with both dPCR and qPCR.

3.3 | Other Reporting and Monitoring of Pink Salmon From 2023

During 2023, reports of pink salmon from anglers and video monitoring were low in Sweden. Only one catch report of a male pink salmon (1.7 kg; 57 cm) from Lilla Edet on the river Göta älv was reported on the 17th of July 2023. A further 12 pink salmon were recorded by the camera-equipped automatic Vaki-counter in Herting, river Ätran (4.3 km upstream of the river mouth). Most of these pink salmon passed through the camera system during July 2023 and were between 37 and 50 cm in length (mean length 43 cm; Fiskevårdsteknik AB).

4 | Discussion

This study demonstrated, by use of eDNA methods, that the distribution of pink salmon in Sweden is more widespread and includes more rivers than previously known. No pink salmon detections were found in any of the rivers that drain into the Baltic Sea, but the southernmost recording was in Rönne å only 35 km from the entrance to the Baltic Sea through the narrow strait of Öresund between Sweden and Denmark. Hence, the distribution of pink salmon is prevalent along the whole West Coast, and there is a high risk that pink salmon will spread into the Baltic Sea in the coming years and is certainly something to be diligent about in future monitoring programs. Only a few angling catches of pink salmon have been recorded in the study area, showing the need for more powerful tools such as eDNA to detect the spread and document the distribution of non-native pink salmon.

4.1 | Applicability of eDNA Methods

This study conducted environmental DNA analyses to detect pink salmon in Swedish freshwater systems using two different types of assays: single-species analyses by qPCR and dPCR on a subset of samples. While the positive dPCR samples showed partitions below the detection threshold, they correlated with positive qPCR signals in six out of eight sites. The dPCR protocol adhered to the manufacturer's guidelines, though using larger amounts of template DNA is recommended for improved results. Overall, qPCR detected more positive samples than dPCR, which aligns with similar findings from Norway when comparing qPCR and dPCR for other species at an early stage of invasion (Frode Frossøy, pers. comm.). Despite this, dPCR remains a highly sensitive method for detecting low-copy DNA, and the observed discrepancies in detection may stem from differences in the protocols used. Manufacturers state that dPCR is less susceptible to sample inhibition compared to other methods; however, in this study, DNA inhibition was observed, which required sample cleaning for accurate results.

The discrepancies between qPCR and dPCR can likely be explained by the inherent sensitivities and limitations of each method (Zhang et al. 2024). qPCR is well known for amplifying even small amounts of DNA by detecting the accumulation of fluorescent signals during amplification. While this makes qPCR highly sensitive, it is also more vulnerable to inhibitors in the sample, which can lead to false negatives or reduced amplification efficiency. As a result, qPCR may detect positive signals in samples where dPCR fails, as dPCR is less affected by inhibitors (Kuypers and Jerome 2017). However, this heightened sensitivity in qPCR can also lead to false positives, especially if the DNA is degraded or if background noise from other genetic material is amplified.

In contrast, dPCR works by dividing the sample into thousands of partitions and detecting the presence or absence of the target DNA in each partition. This method's resistance to inhibitors makes it valuable for detecting low-copy targets, but its sensitivity to low DNA concentrations can be problematic. In cases of very low DNA concentrations—common in the early stages of species invasion—the target DNA may be too diluted to be detected, explaining why dPCR sometimes fails to detect pink salmon when qPCR does. Furthermore, discrepancies between qPCR and dPCR may also arise from differences in assay design, such as primer specificity, amplification conditions, and the volume of template DNA used.

A key challenge in detecting newly introduced species is their low abundance in the environment, which increases the likelihood of false negatives. During early invasion stages, some sites that may harbor the target species remain undetected. Even if detection rates are low, these sites should still be considered as potentially containing the target species, and revisiting them in future surveys is recommended. This approach is critical for identifying invasive species early on, as they can easily be overlooked. One effective strategy to reduce false negatives is using two primers targeting different genes of the same species. This method has proven to increase detection rates for newly invasive species (Brys et al. 2023). Additionally, well-designed droplet digital PCR (ddPCR) analyses have been shown to be more efficient than qPCR in certain contexts (Campomenosi et al. 2016). Although dPCR was not applied to all samples in this study, a clear trend emerged: dPCR generally failed to detect pink salmon compared to qPCR. Based on these results, we recommend using qPCR for future pink salmon surveys, though further development of both existing and newer assays would be beneficial.

These eDNA methods cannot yet quantify the biomass of fish, which makes it challenging to estimate how many pink salmon may have been in a site or system at any one time. Albeit this information is still pivotal to understand the spread of alien invasive species, and together with the application of other monitoring methods (e.g., nets, cameras) further quantitative data collection could be conducted. Additionally, the use and development of eDNA methods for detecting pink salmon is increasing across North Atlantic countries, for example, through collaborative projects such as PINKTrack, coordinated by the North Atlantic Salmon Conservation Organization (Staveley 2024).

4.2 | No Evidence of Pink Salmon in the Baltic Sea

Pink salmon were not detected in rivers draining into the Baltic Sea but in rivers close by, and there is a high risk pink salmon

will spread into the Baltic Sea in the coming years. To date, the effects and impacts that this migratory fish may have on the Baltic Sea ecosystems are still unknown, but pink salmon are known from the Pacific area as numerous species that can impact other species and entire ecosystems (Ruggerone et al. 2023). Another alien species, the round goby (*Neogobius melanostomus*), has in recent times entered the Baltic Sea and has been found to compete for food resources and could potentially impact trophic levels in this brackish water sea (Wallin Kihlberg et al. 2023). The feeding areas of pink salmon in their native range in the Pacific Ocean include vast ocean areas with full-strength seawater, and the same is true for their non-native range in the Barents Sea and North Atlantic Ocean. The Baltic Sea is different from these ocean areas by being a large brackish water inland sea. However, the introduced pink salmon in rivers draining into the Great Lakes in North America have shown that they were able to adapt from an anadromous to a full freshwater life history very fast and perform feeding migrations to the Great Lakes instead of the ocean habitats (Bagdovitz et al. 1986; Sparks et al. 2024). The history of the introduction to the Great Lakes demonstrates that the Baltic Sea may be a suitable habitat for pink salmon and that this is a species that can rapidly adapt to new environments.

4.3 | Pink Salmon Detected in Swedish West Coast Rivers

Eleven of the sampled rivers in this study had positive eDNA detection for pink salmon, and in six of these rivers, pink salmon had previously not been recorded. In these six rivers, 2/4 to 4/4 replicates detected pink salmon. As this is the first time in Sweden that eDNA methods have been specifically targeting pink salmon, it may be that pink salmon previously have occurred unnoticed in many river systems or that 2023 was the first migration year for pink salmon in some or all of these systems. Based on the few angling catch reports of pink salmon in Swedish rivers, and the wide distribution of pink salmon among and within river systems documented using eDNA, we warn against using river catches as the only monitoring method, as angling catches seem to greatly underestimate the spread and occurrence.

The southernmost detection of pink salmon on the Swedish west coast (since the 1970s) has now been confirmed in the river Rönne å, in the county of Skåne, which was further south than the southernmost observation by Staveley and Ahlbeck Bergendahl (2022). Pink salmon were detected at all four sampling sites throughout the Rönne å system at some time point over the three surveys (i.e., mid-July to mid-August) and displayed detections in four out of four PCR replicates at three out of four sites. Not only were pink salmon detected in the main stem and close to the river mouth, but also in the tributaries Rössjöholmsån and Bäljane å, and as far upstream as 35 km. Just a few more kilometers further upstream, there are three hydropower dams where there is a complete barrier for migrating fish, such as the Atlantic salmon and sea trout. In an effort to restore connectivity in the system, plans are in place to make these barriers open to fish migration all the way to the source of the river at the lakes Ringsjöarna (ca. 88 km from the sea; https://www.lifeconnects.se/ronne-a). The benefits of free-flowing rivers by barrier removal have shown to be beneficial for migratory fish species by, for example, creating accessibility to spawning grounds (Boardman and Foster 2023). Although, in this case, it will also most likely open new areas for non-native migratory species, like the pink salmon. Thus, bringing the accompanying increase in nutrients and other yet unknown potential impacts, such as disease, parasites, and inter-specific competition for spawning areas (Lennox et al. 2023) into the system.

As well as Rönne å, pink salmon were found relatively far upstream in some other monitored systems, such as the rivers Ätran, Viskan, and Göta älv; 37, 63, and 74 km from the river mouths, respectively. Generally, this is not a huge distance compared to other river systems in the Pacific and elsewhere where pink salmon can swim hundreds of kilometers upstream (Heard 1991). Nonetheless, from these results, it highlights that they can migrate as far as they can before stopping at a barrier, thus indicating that given the chance, pink salmon have the potential to migrate much further upstream if the conditions and connectivity allow.

5 | Conclusions & Future Recommendations

Utilizing appropriate eDNA methods, as shown in this study, as well as including other forms of monitoring such as citizen science and camera traps, is recommended for monitoring non-native species, particularly while they are in low numbers or becoming newly established. Future migrations of pink salmon need to be monitored carefully by authorities and appropriate management actions taken so that Sweden can safeguard existing native species and habitats from any potential impacts from pink salmon.

Author Contributions

All authors: conception and design of the study; M.H., V.B., and P.H.: field sampling and lab work; T.A.B.S., M.H., E.B.T., and I.A.B.: writing – original draft; all authors: writing – review and editing.

Acknowledgments

This study was funded by the Swedish Research Council FORMAS (2022-01970). We would like to thank Rafael Augusto and Griffin Heicher for assisting with water collections in the field. We are grateful to Bernd Hänfling's laboratory at the University of Highland and Islands, Inverness, UK, for providing facilities for dPCR analysis, especially Nathan Griffiths, Barbara Morrisey, Dasha Svobodova, and Victoria Pritchard. We are also grateful to Rein Brys and Cuong Tang for advice on qPCR and markers.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data for this study are available on Figshare https://doi.org/10.6084/m9.figshare.27194520.

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Appendix

TABLE A1 | Location of sites, date, water volume, water temperature, and water flow where water samples were taken for the first small survey, July 2023.

Site	Date	WGS 84 (N)	WGS 84 (E)	Water volume (ml)	Water temperature (°C)	Water flow (m/s)
Örekilsälven 1	17/07/2023	58.4614539328	11.6852966655	1300	17	0.3
Örekilsälven 2	17/07/2023	58.4747469824	11.6759524177	2000	15.5	0.4
Örekilsälven 3	17/07/2023	58.4734906287	11.6825578451	1800	17	1.1–1.2
Göta älv 1	17/07/2023	57.7594777571	11.9995481723	3000	15.4	0.1–0.2
Göta älv 2	17/07/2023	58.1334950043	12.1193650201	3000	14.7	0.3
Säveån 1	18/07/2023	57.7286565144	12.0206932303	3000	16.5	0.1
Viskan 1	18/07/2023	57.2299584431	12.2319603891	2000	17.6	0.1–0.2
Viskan 2	18/07/2023	57.2569592337	12.3362965925	2000	17.4	0.5
Viskan 3	18/07/2023	57.4544070943	12.5870267269	2000	17.3	0.4–0.5
Rönne å 1	18/07/2023	56.2670695227	12.8494270545	2000	17	0.1–0.2
Rönne å 2	18/07/2023	56.2498615804	12.8650795538	1800	20	0.1–0.2
Rönne å 3	18/07/2023	56.139444249	13.0818262144	800	16.4	0.2–0.3

TABLE A2 | Location of sites, date, water volume, water temperature, and water flow where water samples were taken for the large survey, July–August 2023.

Site	Date	WGS 84 (N)	WGS 84 (E)	Water volume (ml)	Water temperature (°C)	Water flow (m/s)
Strömsån 1	31/07/2023	58.9398170304	11.1773490589	4000	19	0.8–0.9
Strömsån 1	31/07/2023	58.8927112726	11.2955625198	1000	19	0.7–0.8
Enningdalsälven 1	31/07/2023	58.8770630327	11.5371181658	3000	22	0.1–0.2
Enningdalsälven 2	31/07/2023	58.796524984	11.5696660591	4000	22	0.1–0.2
Örekilsälven 1	31/07/2023	58.4614539328	11.6852966655	2000	19	0.6–0.7
Örekilsälven 2	31/07/2023	58.4747469824	11.6759524177	2000	16	0.4–0.5
Örekilsälven 3	31/07/2023	58.4734906287	11.6825578451	2000	17	0.7–0.8
Bäveån 1	01/08/2023	58.3487160735	11.9358024181	1100	17	0.3–0.4
Bratteforsån 1	01/08/2023	58.216325607	11.9118256343	1700	15	0.2–0.3
Anråsälven 1	01/08/2023	58.0010419694	11.817098741	1400	16	0.4–0.5
Anråse å 1	31/07/2023	58.6424767485	11.3369789634	600	16	0.5–0.6
Göta älv 1	01/08/2023	57.7594777571	11.9995481723	3500	17	0.1–0.2
Göta älv 2	01/08/2023	58.1334950043	12.1193650201	4000	15	0.2–0.3
Göta älv 3	01/08/2023	58.2662102875	12.2519496348	4500	16	0.2–0.3
Säveån 1	01/08/2023	57.7286565144	12.0206932303	2500	16	0.1–0.2
Säveån 2	01/08/2023	57.741601385	12.1266688246	3500	16	0.2–0.3
Rolfsån 1	02/08/2023	57.4632422572	12.1068820552	2000	17.7	0.4–0.5
Rolfsån 2	02/08/2023	57.4931395901	12.1243748879	4000	17	0.3–0.4
Löftaån 1	02/08/2023	57.3116775396	12.1812694431	500	15.9	0.6–0.7
Löftaån 2	02/08/2023	57.3299333196	12.2452114875	500	14.5	0.6–0.7
Viskan 1	02/08/2023	57.2299584431	12.2319603891	2000	15.9	0.4–0.5
Viskan 2	02/08/2023	57.2569592337	12.3362965925	2000	17.7	0.3–0.4
Viskan 3	02/08/2023	57.4544070943	12.5870267269	4000	18.2	0.3–0.4
Himleån 1	02/08/2023	57.1360116968	12.2748930423	600	18	0.5–0.6
Himleån 2	02/08/2023	57.1520969921	12.4797278494	1500	16.5	0.6–0.7
Tvåkersån 1	02/08/2023	57.0265927413	12.3337915759	1500	18.1	1.4–1.5
Ätran 1	02/08/2023	56.9005024899	12.5163953873	1550	18	1–1.2
Ätran 2	02/08/2023	56.9779449554	12.655037938	1900	18.3	0.7–0.8
Ätran 3	02/08/2023	57.0323830645	12.6545109447	1000	17.3	0.6
Ätran 4	02/08/2023	57.0927779881	12.651829789	1000	17.6	0.7–0.8
Suseån 1	02/08/2023	56.8647080091	12.5921359769	1000	16	0.6
Suseån 1	02/08/2023	56.8366903997	12.7139844901	1000	16.6	0.1–0.2
Nissan 1	02/08/2023	56.6883423139	12.8721789044	900	17.1	0.2–0.3
Nissan 2	02/08/2023	56.774581274	12.9771229036	900	16.7	0.8–0.9
Nissan 3	02/08/2023	56.7739994046	12.9800005154	1000	15	0.7–0.8
Fylleån 1	02/08/2023	56.6415599571	12.9086354123	900	16.5	0.1–0.2
Fylleån 2	02/08/2023	56.6874493681	12.9800709043	1000	16.8	0.5–0.6
Genevadsån 1	02/08/2023	56.5714433361	12.977617576	700	16.1	0.6–0.7

(Continues)

TABLE A2 | (Continued)

Site	Date	WGS 84 (N)	WGS 84 (E)	Water volume (ml)	Water temperature (°C)	Water flow (m/s)
Lagan 1	01/08/2023	56.5185638223	12.9712303879	1200	18.7	0.1
Lagan 2	01/08/2023	56.476917724	12.9916134487	1000	15.3	0.2–0.3
Lagan 3	01/08/2023	56.4497772407	12.987585122	1600	16.8	0.1
Stensån 1	01/08/2023	56.4319749557	12.8729509194	1900	16.5	0.3–0.4
Stensån 2	01/08/2023	56.406258266	13.0605662108	1000	15.6	0.1–0.2
Stensån 3	01/08/2023	56.3830023282	13.150447261	1000	15.5	0.1–0.2
Rönne å 1	01/08/2023	56.2670695227	12.8494270545	1000	16.5	0.1–0.2
Rönne å 2	01/08/2023	56.2498615804	12.8650795538	2500	18.1	0.1–0.2
Rönne å 3	01/08/2023	56.139444249	13.0818262144	1400	16.1	0.2
Rönne å 4	01/08/2023	56.1352938064	13.089384378	4000	18.3	0.4–0.6
Råån 1	01/08/2023	55.9979723149	12.7440572871	3000	16	0–0.1
Råån 2	01/08/2023	55.9639635271	12.8653956133	2500	15.4	0.3–0.4
Kävlingeån 1	01/08/2023	55.7566417838	13.0189671649	3100	18.5	0.1
Kävlingeån 2	01/08/2023	55.7888037359	13.1999902982	4000	18.6	0.5
Nybroån 1	31/07/2023	55.4349668271	13.9151655435	3800	17.2	0–0.1
Nybroån 2	31/07/2023	55.504931757	13.9045609683	3000	17.4	0.3–0.4
Helge å 1	31/07/2023	55.8749145318	14.2233644229	3000	16.6	0.1
Helge å 2	31/07/2023	55.9335008021	14.1801293129	3500	17.3	0.4–0.5
Mörrumsån 1	31/07/2023	56.1786955324	14.7498129778	4000	16	0.8
Mörrumsån 2	31/07/2023	56.2204995257	14.7577421667	3000	16.3	0.6–0.7

TABLE A3 | Location of sites, date, water volume, water temperature, and water flow where water samples were taken for the second small survey, August 2023.

Site	Date	WGS 84 (N)	WGS 84 (E)	Water volume (ml)	Water temperature (°C)	Water flow (m/s)
Örekilsälven 1	14/08/2023	58.4614539328	11.6852966655	1300	18.4	1.1–1.2
Örekilsälven 2	14/08/2023	58.4747469824	11.6759524177	1500	17.6	0.5–0.8
Örekilsälven 3	14/08/2023	58.4734906287	11.6825578451	1200	18.7	1.1–1.2
Göta älv 1	14/08/2023	57.7594777571	11.9995481723	2000	17.7	0.1–0.2
Göta älv 2	14/08/2023	58.1334950043	12.1193650201	4000	17.5	0.6–0.7
Säveån 1	14/08/2023	57.7286565144	12.0206932303	3000	18.1	0.3–0.4
Viskan 1	15/08/2023	57.2299584431	12.2319603891	1900	17.7	0.6
Viskan 2	15/08/2023	57.2569592337	12.3362965925	2000	18.1	0.6–0.7
Viskan 3	16/08/2023	57.4544070943	12.5870267269	2000	16.5	0.6–0.7
Rönne å 1	15/08/2023	56.2670695227	12.8494270545	1300	19	0.3
Rönne å 2	15/08/2023	56.2498615804	12.8650795538	1600	19	0.2–0.3
Rönne å 3	15/08/2023	56.139444249	13.0818262144	700	17	0.4–0.5

TABLE A4 | Results of the qPCR analyses from the 24 sites in eleven rivers where pink salmon was detected in one or more of the three surveys. The detection rate based on qPCR (curves and Ct (threshold) value) shows the number of samples (n/4) with a positive qPCR signal. Note that three of the samples (indicated by an *) showed high Ct values exceeding the values of the standard curve and are therefore less trustworthy. The highest Ct value is 42.9 cycles and corresponds to 1 × 10-4 ng/μl DNA, but are included in the analysis. Rivers are listed in geographical order from north to south.

	qPCR Ct (n/4)		
Site	**Small survey 1**	**Large survey**	**Small survey 2**
Örekilsälven 1	0	0	37.6 (1)
Örekilsälven 2	0	0	38.2 (2)
Örekilsälven 3	44.8 (1)*	0	0
Göta älv 1	42.9 (1)	0	0
Göta älv 2	40.5 (4)	0	41.1 (2)
Rolfsån 1		36.8 (1)	
Viskan 2	0	37.1 (2)	38.8 (2)
Viskan 3	0	0	39.0 (1)
Himleån 1		37.2 (2)	
Tvåkersån 1		38.9 (4)	
Ätran 1		39.7 (4)	
Ätran 2		42.1 (2)	
Ätran 3		36.7 (4)	
Ätran 4		41.6 (4)	
Nissan 1		36.3 (4)	
Nissan 3		35.6 (4)	
Stensån 1		43.7 (2)*	
Stensån 2		40.7 (1)	
Rönne å 1	37.4 (4)	38.2 (4)	0
Rönne å 2	37.2 (4)	38.2 (4)	0
Rönne å 3	42.1 (1)	0	44.1 (2)*
Rönne å 4	0	41.8 (4)	0

TABLE A5 | Results from the subset of samples where both qPCR and dPCR were performed.

Site	Survey	dPCR number of partitions	qPCR detection
Örekilsälven 1	Small 1	0	No
Örekilsälven 1	Small 2	0	Yes
Örekilsälven 2	Small 1	0	No
Örekilsälven 2	Small 2	0	Yes
Örekilsälven 3	Small 1	0	Yes
Örekilsälven 3	Small 2	0	No
Göta älv 1	Small 1	2	Yes
Göta älv 1	Large	2	No
Göta älv 1	Small 2	0	No
Göta älv 2	Small 1	0	Yes
Göta älv 2	Large	0	No
Göta älv 2	Small 2	0	Yes
Göta älv 3	Large	2	No
Sävån 1	Small 1	0	No
Sävån 1	Large	2	No
Sävån 1	Small 2	0	No
Rolfsån 1	Large	0	Yes
Rolfsån 2	Large	0	No
Viskan 1	Small 1	0	No
Viskan 1	Small 2	0	No
Viskan 2	Small 1	0	No
Viskan 2	Large	2	Yes
Viskan 2	Small 2	0	Yes
Viskan 3	Small 1	0	No
Viskan 3	Large	0	No
Viskan 3	Small 2	2	Yes
Himleån 1	Large	0	Yes
Himleån 2	Large	0	No
Ätran 1	Large	2	Yes
Ätran 2	Large	0	Yes
Ätran 3	Large	0	Yes
Rönne å 1	Small 1	0	Yes
Rönne å 1	Large	2	Yes
Rönne å 2	Small 1	2	Yes
Rönne å 2	Large	0	Yes
Rönne å 3	Large	0	Yes
Rönne å 3	Small 1	0	Yes
Rönne å 3	Small 1	0	Yes
Positive lab control 0.05 ng/μL	—	44 to 88	Yes
Lab negative	Small 1	0	—
Lab negative	Small 2	0	—
Lab negative	Large	0	—

(Continues)