

# **Occurrence of the invasive pink salmon (***Oncorhynchus gorbuscha***, Walbaum 1792) in Greenland 2020 and 2021 as revealed using citizen science, snorkeling, and environmental DNA metabarcoding of fishes in the Kapisillit River**

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### **Abstract**

The occurrence of the invasive pink salmon (*Oncorhynchus gorbuscha*, Walbaum 1792) in Greenland was initially described in 2019 using social media to collect data on their presence. In this study we continue data collection through social media and initiate a monitoring program of the Kapisillit River in Southwest Greenland using snorkeling and environmental DNA (eDNA) metabarcoding in 2020 and 2021. The Kapisillit River is the only freshwater system in Greenland, where the red-listed Atlantic salmon (*Salmo salar*, Linnaeus 1758) is known to spawn. This genetically unique population of Atlantic salmon has been found to decline, wherefore there is general conservation concern that the occurrence of pink salmon at some point can become an additional stressor to the "Kapisillit salmon". In 2021, pink salmon were present near all larger populated areas in Greenland and likely more abundant than in 2019. From visual observations and using eDNA, the presence of pink salmon was documented in the Kapisillit River in 2021. From the number of individuals observed combined with the spatial distribution of eDNA detections in the river, we suggest that the pink salmon invasion in the Kapisillit River is at an early stage.

**Key words:** North Atlantic, Arctic char, three-spined stickleback, Atlantic salmon, American eel

### **1. Introduction**

The invasion of pink salmon (*Oncorhynchus gorbuscha*, Walbaum 1792) is evident across the North Atlantic [\(ICES 2022;](#page-7-0) [Lennox et al. 2023\)](#page-7-1). Their introduction in the region initially occurred in northwest Russia in the 1950s, but it was not until around the turn of the millennium that a population successfully established. From the Kola Peninsula in Russia, pink salmon spread to rivers in northern Norway, where they in recent years have been considered invasive [\(Mo et al. 2018;](#page-7-2) [Sandlund et al. 2019\)](#page-7-3). Due to the 2-year life cycle of pink salmon, their spawning run is observed in odd years in the North Atlantic [\(Mo et al. 2018\)](#page-7-2). In 2017, 12 000 pink salmon were registered in either rivers or coastal waters of Norway— a number which increased to 25 000 in 2019 and 208 000 in 2021 [\(Berntsen et al. 2022\)](#page-7-4). In 2023, ∼242 000 pink salmon were removed in Norwegian waters [\(Miljødirektoratet 2023\)](#page-7-5). Pink salmon have also been documented in smaller numbers in more southern parts of Scandinavia, the British Isles, Svalbard, Iceland, and Greenland among others [\(Millane](#page-7-6)

[et al. 2019;](#page-7-6) [Thorstad and Sandlund 2019](#page-7-7)[;](#page-7-8) Nielsen et al. 2020; [Bengtsson et al. 2023;](#page-7-9) [Skóra et al. 2023](#page-7-10)*a*, [2023](#page-7-11)*b*). This spread has created a transatlantic concern due to potential negative impacts on river systems and native salmonids, such as Atlantic salmon (*Salmo salar*, Linnaeus 1758), brown trout (*Salmo trutta*, Linnaeus 1758), and Arctic char (*Salvelinus alpinus*, Linnaeus 1758) due to expected interspecific [competition for example in relation to prey resources \(ICES](#page-7-0) 2022; [Bengtsson et al. 2023;](#page-7-9) [Lennox et al. 2023\)](#page-7-1). Since pink salmon die soon after spawning, a potentially large number of decomposing carcasses also poses a concern in terms of water quality and ecological impact on low-nutrient river systems [\(Lennox et al. 2023\)](#page-7-1). Currently, the biological consequences of the increased occurrence of pink salmon in the North Atlantic region are unknown. Nonetheless, this non-native species is considered a high-risk species with potential detrimental effects on freshwater ecosystems [\(Mo et al. 2018;](#page-7-2) [Bengtsson et al. 2023](#page-7-9)[;](#page-7-1) Lennox et al. 2023).



In Greenland, four fish species naturally occur in freshwater. These are three-spined stickleback (*Gasterosteus aculatus*, Linnaeus 1758), Arctic char and Atlantic salmon as well as American eel (*Anguilla rostrata*, Lesueur 1817) (Møller [et al. 2010\). In 2019, an unprecedented high occurrence of](#page-7-12) pink salmon was documented using social media outlets of the Greenland Institute of Natural Resources (GINR). Although the number of reports was much smaller in Greenland than, e.g., Norway, it was clear that pink salmon also had become abundant throughout Greenland waters (Nielsen [et al. 2020\). One observation of pink salmon, near the out](#page-7-8)let of the Kapisillit River in the bottom of the Nuuk Fjord (Nuup Kangerlua, Southwest Greenland), was highlighted by [Nielsen et al. \(2020\)](#page-7-8), because this particular river is the only spawning location for Atlantic salmon in Greenland-the "Kapisillit salmon" [\(Nielsen 1961;](#page-7-13) [Krohn 2013;](#page-7-14) Hedeholm et [al. 2018\). The "Kapisillit salmon" is classified on the national](#page-7-15) Red List of Greenland as "Vulnerable" (Boertmann and Bay [2018\) and the observation of a single pink salmon near this](#page-7-16) particular river raised conservation concerns for the genetically unique population of Atlantic salmon [\(Krohn 2013;](#page-7-14) [Arnekleiv et al. 2019\)](#page-7-17). GINR therefore initiated a monitoring program for 2020 and 2021 to detect the possible presence of pink salmon in the Kapisillit River system from which the findings are reported here.

The current study had two main aims: (1) to present the findings from a citizen science effort to evaluate the distribution of pink salmon across Greenland in 2020 and 2021 (similar to what was done in 2019); (2) to assess the spatial and temporal distribution of pink salmon in the Kapisillit River system in 2020 and 2021 using snorkeling and environmental DNA (eDNA) metabarcoding of fishes in the Kapisillit River system.

### **2. Methods**

2.1. Pink salmon in Greenland—citizen science

To evaluate the presence of pink salmon across Greenland, public requests were posted on GINR's Facebook page in August 2020 and 2021. The request contained pictures of male and female pink salmon specimens plus close-ups of the dark-spotted tail fin (see [Appendix A\)](#page-7-18). The text, made in Greenlandic and Danish, called for people to report their catches of pink salmon (either by telephone or messenger). Similarly to 2019, data on sex, length, capture posi[tion, fishing gear, and date were requested \(see](#page-7-8) Nielsen et al. 2020).

### 2.2. Monitoring the Kapisillit River—eDNA metabarcoding

The Kapisillit River is a system made up by multiple freshwater bodies encompassing river stretches and lakes [\(Fig. 1\)](#page-2-0). The occurrence of pink salmon in the river was monitored with eDNA in 2020 and 2021 from water samples collected approximately every second week from July to October. Sampling of surface water for eDNA was done from four sites/sampling stations (St.). These were St.  $1 =$  the fjord; St.  $2 =$  first river stretch (closest to the fjord); St. 3 = second

river stretch; and St.  $4 =$  third river stretch [\(Fig. 1\)](#page-2-0). Each of the riverine stations was in the lowermost part of the river stretch. In 2020, only St. 1–2 were sampled and in 2021, St. 1–2, St. 1–3, or St. 1–4 were sampled depending on weather and logistics. In 2020, seven sampling trips were made from 14th July to 6th October and in 2021, 10 sampling trips from 11th June to 14th October were made. See overview of sampling trips and number of stations taken per trip in [Table 1.](#page-3-0)

In September and October, the occurrence of pink salmon was also evaluated visually during snorkeling in the first (lowermost) and third river stretch, as well in the two lowermost lakes of the river system [\(Fig. 1\)](#page-2-0). Standardized transects were neither made in the river nor in the lakes but the deepest pools with high concentrations of fish (Atlantic salmon and Arctic char) were always inspected. All relevant laws and guidelines were followed with permission from the relevant Greenland authorities to catch pink salmon from the Kapisillit River (Case number/Akt-id:2021-16687/17956614).

#### **2.2.1. Sampling of environmental DNA**

Surface water was always collected starting with the fjord (St. 1) and ending with the stations highest up in the river (St. 2, St. 3, or St. 4). All samples were analyzed for eDNA from marine and freshwater fishes, but we focused on reporting results on pink salmon, three-spined stickleback, Arctic char, Atlantic salmon, and American eel. For each station, 51 plastic containers were used to collect sample water. Before sampling, the containers were decontaminated using a 10% bleach solution, and subsequently flushed in sample water before the actual sample was collected. Three replicates were obtained by filtering  $3 \times 1.0$  l sample water through Sterivex filters (0.22 μm, SVGPL10RC, Merck Millipore), simultaneously, using a custom made 12 V battery driven peristaltic pump system (contact K. Præbel for further details on the 12 V pump).

After water filtration was completed, the remaining water drops inside the filter were removed by pumping air through the filter. Each filter was then sealed with an end cap (BRAU04495152, VWR) and transferred to sterile 50-mL Falcon centrifuge tubes. In 2020, water filtration was done in the field and samples stored in a cooling box (4–5 h) before being stored at −18 ◦C until extraction. In 2021, the 5 L water samples were brought to GINR in Nuuk by boat before filtration. In the laboratory, 1.0 L of sample water was filtered (4–5 h after being sampled). Filters were then immediately stored at −18 ◦C. All sampling equipment was sterilized with 10% bleach after usage and sterilized nitrile gloves were used for all procedures during sampling and filtration, to prevent risk of contamination between samples, or from outside sources. Negative controls (blanks) in triplicate were made from distilled water on each sampling day to control for possible ineffective decontamination procedures or potential contamination from the environment [\(Turon et al. 2022\)](#page-7-19). The filters were subsequently shipped, on dry ice, to UiT The Arctic University of Norway (UiT), Tromsø, Norway. Upon arrival the plastic bags where filters were stored were decon-

**Fig. 1.** (*a* + *b*) Observations of pink salmon (*Oncorhynchus gorbuscha*, PS) throughout Greenland (*a*) and in the Nuup Kangerlua/Nuuk Fjord (*b*) in 2019 (red) and in 2021 (yellow). The Kapisillit River is marked with an open star. (*c*) The Kapisillit River system with river stretches and lakes, where eDNA sampling stations (St. 1–4) are marked with filled white stars. (*d* + *e*) Male and female pink salmon from the Kapisillit River observed/caught during snorkeling, September 2021. Picture credit is a: QGIS 3.20.3; *b* + *c*: Google Earth 10.52.00 ©2024; *d* + *e*: Julius Nielsen.

<span id="page-2-0"></span>

<span id="page-3-0"></span>**Table 1.** Overview of stations taken on each sampling trip 2020 and 2021 in the Kapisillit River.

	Station			
	$\mathbf{1}$	$\overline{2}$	3	4
2020				
July 14th	x	$\mathbf x$		
Aug. 7th	X	$\mathbf x$		
Aug. 26th	$\mathbf x$	$\mathbf x$		
Sep. 2nd	$\mathbf x$	$\mathbf x$		
Sep. 16th	X	$\mathbf x$		
Sep. 29th	X	X		
Oct. 6th	X	X		
2021				
June 11th	X	X		
June 18th	X	X		
July 6th	X	$\mathbf x$	$\mathbf x$	$\mathbf x$
July 20th	X	$\mathbf x$	$\mathbf x$	$\mathbf X$
Aug. 19th	$\mathbf x$	$\mathbf x$	$\mathbf x$	$\mathbf X$
Aug. 27th	X	X	$\mathbf x$	
Sep. 6th	X	X	$\mathbf x$	
Sep. 10th	X	X	$\mathbf x$	X
Sep. 24th	X	X	$\mathbf x$	$\mathbf X$
Oct. 14th	X	$\mathbf x$	X	$\mathbf x$

taminated using 10% bleach and transferred to a dedicated −80 ◦C eDNA freezer for storage until extraction.

#### **2.2.2. Extraction, amplification, and sequencing of environmental DNA**

eDNA was extracted in over-pressured eDNA clean-labs at the Norwegian College of Fishery Science, UiT. Trace eDNA work routines, specifically designed to prevent contamination from all airborne DNA present within UiTs facilities or present on the lab user's skin, hair, or breath, were enforced following [Turon et al. \(2022\)](#page-7-19). The eDNA was extracted using a modified protocol of the DNeasy Blood and Tissue® kit (Qiagen, Hilden, Germany), as described in detail in [Turon et al. \(2022\)](#page-7-19). Each sample was eluted in 75 μL elution buffer, of which 20 μL was aliquoted for library preparation and sequencing, and the remaining 55 μL stock transferred to a monitored −80 ◦C freezer for long-term storage.

A hypervariable region in the mitochondrial 12S rRNA gene (163–185 bp), that specifically amplifies fish and other vertebrate DNA, was amplified in all sample replicates, including field and extraction blanks, using the MiFish-U primer set [\(Miya et al. 2015\)](#page-7-20). We used a corrected version of the MiFishforward primer, which differs from the original in the second base (5 -GCCGGTAAAACTCGTGCCAGC-3 ), designed to better match 12S sequences of an alignment of Atlantic species. This primer set has proven useful to amplify eDNA samples with high specificity and adequate levels of species-specific resolution for fishes (e.g., [Miya et al. 2015;](#page-7-20) [Turon et al. 2020\)](#page-7-21). Amplification of the 12S fragment was achieved in a single-step

PCR protocol by using a set of 7-bp indexed primers (twin indices in both ends). The PCR included 10 μl AmpliTaq Gold 360 Master mix (Applied Biosystems), 3 μg Bovine Serum Albumin (Thermo Scientific), 1  $\mu$ l of each of the 5  $\mu$ mol/L forward and reverse tagged-primers (including 2–4 leading Ns and 7-bp sample tags), 5.84  $\mu$ l H2O, and 2  $\mu$ l extracted DNA template. The PCR profile included an initial denaturing step of 95 ◦C for 10 min, 40 cycles of 95 ◦C for 30 s, 60 ◦C for 30 s, and 72 ◦C for 30 s and a final extension step of 72 ◦C for 5 min. Five technical PCR replicates were produced for each biological replicate sample or negative control. PCR products were pooled into five multiplexed sample pools (one pool per PCR replicate). The pools were purified and concentrated using MinElute columns (Qiagen). Illumina libraries were prepared using the NextFlex PCR-free library preparation kit (BIOO Scientific) and quantified using the NEBNext qPCR quantification kit (New England Biolabs). Libraries were pooled in an equimolar concentration ratio and sequenced at a commercial sequencing platform (NOVOGENE) on a Novaseq6000 using  $2 \times 150$  bp paired-end chemistry. Setting up PCRs, PCR amplification, and library preparation were all performed following strict clean lab routines in separate dedicated overpressured rooms at the Norwegian College of Fishery Science, UiT.

#### **2.2.3. Pipeline and statistics**

eDNA libraries were demultiplexed by Novogene and the multiplexed sample data was processed using MJOLNIR pipeline [\(https://github.com/uit-metabarcoding/MJOLNIR\)](https://github.com/uit-metabarcoding/MJOLNIR). During the FREYJA step of the pipeline length filtering is set to Lmin  $= 140$  and Lmax  $= 150$ . After quality filtering HELA removes chimeric sequences, and then ODIN clusters reads into molecular operational taxonomical units (MOTUs). Here, we used the standard parameter for our 12S MiFish primers [\(Miya et al. 2015\)](#page-7-20),  $d = 1$  and we did not generate exact sequence variants (ESVs). In order for Targeted High-throughput Ortholog Reconstructor (THOR) to perform taxonomic assignment we used a locally curated database of [Miya et al. \(2015\)](#page-7-20) 12S sequences. After our initial MOTU table was generated at the end of the Mjolnir pipeline, MOTUs assigned to bacteria, species of terrestrial origin, or present in the negative control samples with more than 10% sequence reads of the total read abundance, were removed from the study. The output read numbers were then normalized (counts of reads per MOTU divided with total sample read number multiplied by 100). The read count data for freshwater species was plotted in a presence/absence heatmap to visually show occurrence of key fish species over the sampling period. The retained marine fish species in the MOTU table were not included for further analysis (see Table S1 for a complete list of detected MOTUs).

#### **3. Results**

The Novaseq sequencing run of 12S MiFish metabarcoding amplicons yielded 254 153 918 paired reads across the 48 samples. After the filtering (Freya) and quality control (Hela) steps of the pipeline there were 75 126 870 reads remaining and 347 397 unique reads. These sequences were clustered by SWARM into 3525 initial MOTUs. The Mjolnir initial MOTU table output consisted of 408 MOTUs. After all subsequent filters, particularly the elimination of non-eukaryotes, and MOTUs from contamination, the final 12S dataset consisted of 328 MOTUs and 73 950 389 reads, of which 70 219 442 reads and 10 MOTUs corresponded to five freshwater fish species. The blank samples contained  $6221.5 \pm 2230.6$  reads and the average number of reads per sample was  $169\,680.1 \pm 6095.1$ . MOTU tables with taxonomic assignments, count data, and sequences are found in supplementary material (Table S1).

#### 3.1. Pink salmon in Greenland

From the social media request, no observations were made in 2020, whereas a total of 146 reports on pink salmon from 23 different locations were made in 2021. Exact capture positions were often not possible to collect; therefore, the plotted positions in [Fig. 1](#page-2-0)*a* represent the home city/settlement of the fisherman reporting the respective catches. However, positions in the Nuuk Fjord system represent exact capture locations [\(Fig. 1](#page-2-0)*b*). The highest number of reports in 2021 were in the Tasiilaq area  $(N = 58)$  in Southeast Greenland, the Nuuk Fjord ( $N = 25$ ), and on the southside of Disko Island ( $N = 16$ ) in West Greenland [\(Fig. 1](#page-2-0)*a*).

#### 3.2. Pink salmon in Kapisillit

In 2020, seven sampling trips were made from July 14th to October 6th to the Kapisillit River, where samples were taken only from two stations each time  $(N = 14, St. 1-2, Fig. 1*c*).$  $(N = 14, St. 1-2, Fig. 1*c*).$  $(N = 14, St. 1-2, Fig. 1*c*).$  In 2021, 10 sampling trips from June 11th to October 14th were done, where between two and four stations were visited per trip (*N* = 34, St. 1–2, St. 1–3, and St. 1–4, [Fig. 1](#page-2-0)*c*).

eDNA from pink salmon was not detected in 2020, but in 2021 pink salmon was detected in seven eDNA samples. The first positive sample was 6th September 6 (St. 1), followed by 10th September (St. 1 and 2), 24th September (St. 1), and 14th October (St., 1, 3, and 4). Pink salmon were visually spotted in the first river stretch (corresponding to St. 2) on September 6th  $(N = 2)$  and September 10th  $(N = 5)$  but not on September 24th and October 14th. eDNA from three-spined stickleback was detected in all 48 samples, respectively, of 2020 and 2021. Atlantic salmon and Arctic char were both detected in 37 of the 48 samples [\(Fig. 2\)](#page-5-0). The absence of Atlantic salmon was distributed randomly over both years and all months and stations, whereas Arctic char appeared absent from the river system especially in August during both sampling years [\(Fig. 2\)](#page-5-0). Three spined stickleback, Arctic char and Atlantic salmon were visually spotted during all snorkeling events (September 6th, 10th, 24th, and October 14th). American eel was neither detected by eDNA nor spotted visually during snorkeling.

### **4. Discussion**

The invasion of pink salmon in Greenland waters was first documented in 2019 [\(Nielsen et al. 2020\)](#page-7-8). Although no genetic

investigations have yet established their origin, the presence of pink salmon in Greenland is presumably linked to the high occurrence in Norway, and thus originating from Russian stocking programs [\(Berntsen et al. 2022\)](#page-7-4). In addition to Russia and Norway, successful spawning in the Atlantic region have only been verified in Scotland and Iceland in 2021 [\(Skóra et al. 2023](#page-7-10)*a* and [2023](#page-7-11)*b*). However, given the widespread and continuously expanding distribution of this species-in absolute numbers and geographically—pink salmon likely have multiple undocumented spawning locations in rivers across the northeastern Atlantic. Greenland appears to be the northwesternmost frontier of the invasion [\(ICES 2022\)](#page-7-0) but from the currently available data, it is not possible to establish if, and in which rivers, spawning has yet occurred in Greenland. The Tasiilaq region in Southeast Greenland stands out however, as most pink salmon observations in both 2019 and 2021 are from there [\(Fig. 1](#page-2-0) and [Nielsen et al. 2020\)](#page-7-8). Furthermore, the authors are aware of a high number of additional unreported catches in the Tasiilaq area, as well as unreported visual encounters of pink salmon in unnamed streams and rivers during 2021. All combined, the Tasiilaq area appears to be a hot spot for the pink salmon in Greenland although we emphasize, that it is difficult to evaluate spatial and temporal differences in abundance from the available data. This difficulty is partially due to what we consider as a "fatigue-phenomenon", where public reports are expected less likely to be made when the news value of the subject has diminished. Although the number of reported pink salmon increased (as expected) from 74 in 2019 to 146 in 2021, we believe the 2021 report did not accurately reflect the true relative abundance of pink salmon compared to the previous pink salmon spawning season. The reason for this suspicion is that 99 of the 146 reports were from only three different regions: Tasiilaq, Disko Island, and the Nuuk Fjord [\(Fig. 1\)](#page-2-0). From the two first regions, the majority of reported individuals were from the same fishermen making us suspect, that many other catches were not reported because pink salmon were common and hence people did not report them. In the Nuuk area, there was a higher number of individual reports from all over the fjord in 2021 compared to 2019. However, many of the reports came from people acquainted to the scientific project through either friends, colleagues, or family at GINR. In all, we do not consider pink salmon necessarily to be more plentiful in the Nuuk fjord than elsewhere in West Greenland in 2021. Despite the overall uncertainties associated with this kind of data collection, we think the invasion in Greenland was stronger in 2021 compared to 2019.

The Kapisillit River was monitored for the presence of pink salmon for two reasons: (1) It is the only river where Atlantic salmon are reproducing in Greenland waters and [hence the river has a high conservational value \(Nielsen](#page-7-13) 1961; [Hedeholm et al. 2018\)](#page-7-15); (2) the conditions in terms of temperature and substrate making the river suitable for Atlantic salmon, also makes us expect it to be a strong candidate for a location in West Greenland, where pink salmon could successfully establish. In spite of the fact, that there are thousands of small and large rivers in both East and West Greenland, these are unsuitable for Atlantic salmon as they

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**Fig. 2.** Heatmap of freshwater species occurrence through time, across four stations in the Kapsillit River, Greenland, identified with environmental DNA (eDNA) over two sampling campaigns in 2020 and 2021. eDNA read numbers (%) are shown in the key and displayed as colors ranging from white to either purple (pink salmon/*Oncorhynchus gorbuscha*), blue (Atlantic salmon/*Salmo salar*), green (three-spined stickleback/*Gasterosteus aculeatus*), or red (Arctic char/*Salvelinus alpinus*). Note that in the key, the scale differs among species for the eDNA read numbers. Each vertical bar represents a sampling station, where St. 1 is the fjord and St. 2–4 is in the river.

<span id="page-5-0"></span>

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are typically short, steep, and water temperatures are low due to their source being melting snow or glacial water (Jonas [1974\). In these typical Greenland rivers, Arctic char and](#page-7-22) three-spined stickleback are commonly distributed, whereas American eel, have only been found in a few freshwater systems in Southwest Greenland [\(Møller et al. 2010\)](#page-7-12). As expected, we did not detect American eel in water samples from the Kapisillit River, but we found Arctic char, three-spined stickleback, and Atlantic salmon from eDNA samples in both sampling years in the river and the fjord. This was in alignment with a previous eDNA study in the river (Jacobsen et [al. 2023\). We also find lower levels of Arctic char eDNA from](#page-7-23) the end of July to the beginning of September in both 2020 and 2021. This is also expected as Arctic char are commonly known to undertake feeding migrations from rivers into the sea during summer months and then return to rivers and lakes during autumn to overwinter [\(Born and Böcher 1999\)](#page-7-24).

The presence of a population of Atlantic salmon in the Kapisillit River is due to a unique combination of (relatively) high water temperatures and suitable gravel material for spawning at the lower-most river stretches [\(Nielsen 1961;](#page-7-13) [Krohn 2013;](#page-7-14) [Broberg 2017\)](#page-7-25). These features are the result of the river's topography which is characterized by a large low-altitude drainage area and multiple low-land lakes that are joined by the slowly running river [\(Nielsen 1961;](#page-7-13) Jonas 1974; [Broberg 2017\). The "Kapisillit salmon" is a genetically](#page-7-22) unique population of Atlantic salmon isolated from the nearest salmon river populations by thousands of kilometers, and resides mainly in the Nuuk Fjord [\(Nielsen 1961;](#page-7-13) [Krohn 2013;](#page-7-14) [Broberg 2017;](#page-7-25) [Arnekleiv et al. 2019\)](#page-7-17). Elsewhere in Greenland fjords and coastal waters, Atlantic salmon are migratory in[dividuals from North American and European rivers \(Nielsen](#page-7-13) 1961; [Krohn 2013;](#page-7-14) [Broberg 2017\)](#page-7-25). Estimates from 2017 are that around ∼5900 smolt of the "Kapisillit salmon" are present in the river, and the spawning population counted between 254 and 318 individuals [\(Hedeholm et al. 2018\)](#page-7-15). That estimate suggests that the population has declined 52% since 1959, supposedly due to human disturbances from targeted local fisheries. It is therefore of even further concern in relation to conservation of "Kapisillit salmon" since the presence of the invasive pink salmon has been confirmed in the Kapisillit river both from eDNA and from visual observations from early September to mid-October 2021. The total number of individuals observed is only seven suggesting the pink salmon invasion is at an early stage in the Kapisillit River in 2021.However, on October 14th, pink salmon were detected in eDNA samples collected from three of four river stretches and although no pink salmon were visually spotted, the eDNA reveals that all stretches where suitable spawning grounds are most dense, have been occupied by pink salmon [\(Nielsen 1961;](#page-7-13) [Broberg 2017\)](#page-7-25). Although it remains unknown if successful spawning has occurred, it must be noted that of the five specimens removed with harpoon, females contained hydrated eggs and males running sperm suggesting that spawning efforts were made (data not shown).

Regardless of how progressed the invasion is, our findings from across Greenland, and in the Kapisillit River, suggest that a potentially very large disturbance is imminent toward an already stressed population of "Kapisillit salmon". Moreover, Arctic char might be abundant in Greenland now, but climate change and local fisheries are also negatively affecting this species locally [\(Jacobsen et al. 2023\)](#page-7-23). Whether global warming would be an advantage for pink salmon in Greenland is beyond the scope of this study to evaluate, but a hypothesis could be that warming would enhance the pink salmon invasion in Arctic areas. The exact ecological consequences of the novel presence of pink salmon remain unknown, yet they are expected to potentially have a significant effect——both on water chemistry from decomposing pink salmon carcasses post-spawning, as well as altered ecological interactions [\(Mo et al. 2018;](#page-7-2) [Sandlund et al. 2019;](#page-7-3) Berntsen et al. 2022; [Lennox et al. 2023\). Pink salmon have been found to](#page-7-4) spawn in Norway from August/September [\(Lennox et al. 2023\)](#page-7-1) which also is supported by our findings from the Kapisillit River, where they occurred from September and onward. This is earlier than spawning of both Arctic char and At[lantic salmon in Greenland and Norway \(](#page-7-14)[Nielsen 1961](#page-7-13)[;](#page-7-14) Krohn 2013; [Hedeholm et al. 2018;](#page-7-15) [Jacobsen et al. 2023\)](#page-7-23) minimizing the potential damages for disturbances of spawned eggs of indigenous salmonids buried in gravel nests. However, pink salmon exhibit aggressive mating behavior, which potentially can be a stressor to other salmonids during their spawning migration [\(Mo et al. 2018;](#page-7-2) [Sandlund et al. 2019;](#page-7-3) [Lennox et al. 2023\)](#page-7-1). While parr of Atlantic salmon and Arctic char typically remain in the Kapisillit River for 4–6 years upon hatching [\(Nielsen 1961;](#page-7-13) [Broberg 2017\)](#page-7-25), pink salmon migrate quickly to the sea before they return to spawn the following year [\(Lennox et al. 2023\)](#page-7-1). In this period of a little more than 1 year at sea, a dietary overlap with both Arctic char and Atlantic salmon is possible and if pink salmon become highly numerous in Greenland waters a significant dietary competition could occur with potential depressing effects on native fauna [\(Bengtsson et al. 2023;](#page-7-9) [Lennox et al. 2023\)](#page-7-1). In the native range of the pink salmon in the Pacific Ocean, dietary overlap has been shown to have significant effect on prey availability for other salmonids during strong pink salmon years leading [to depressive effects on populations \(Ruggerone and Nielsen](#page-7-26) 2004).

If large-scale conservation actions are to be executed to protect the "Kapisillit salmon", it is important that these are accompanied by thorough monitoring programs of the Kapisillit River surveying both Atlantic salmon, Arctic char, and pink salmon. In northern Norway, large-scale mitigation efforts have been made. These comprise of physical barriers and cages across rivers and streams catching all larger fish migrating up the river [\(Anon 2021\)](#page-7-27). Only native fish are physically released again whereas invasive pink salmon are removed. Whether it is necessary to make similar efforts in the Kapisillit River, is too early to determine. Pink salmon are however expected to be present in the river in higher numbers than documented here within few years. Due to the 2-year life cycle, pink salmon will occur in Greenland rivers in odd years and we encourage that experiences from Norway are carefully evaluated and considered if usable for protecting the "Kapisillit salmon" in Greenland.

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### Data availability

All data are available upon request to the corresponding author or Kim Præbel [\(kim.praebel@uit.no\)](file:kim.praebel@uit.no). Raw data is available via the National Center for Biotechnology Information (NCBI) in the Sequence Read Archive (SRA) acces[sion number: PRJNA1118713 \(https://www.ncbi.nlm.nih.gov/](https://www.ncbi.nlm.nih.gov/sra/PRJNA1118713) sra/PRJNA1118713).

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### Competing interests

The authors declare no conflict of interest.

### **Supplementary material**

[Supplementary data are available with the article at](https://doi.org/10.1139/as-2023-0068) https: //doi.org/10.1139/as-2023-0068.

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## **Appendix A**

<span id="page-7-18"></span>Public request for pink salmon made in 2020 and 2021 and posted on Facebook. The request was written in Greenlandic and Danish and posted with the pictures below.

Facebook text:

*If you catch or have already caught a pink salmon, then the Greenland Institute of Natural Resourcesis in the following information:*

- *How many pink salmon have you caught?*
- *On what location/position did you capture them (or it)?*
- *We would like a photo*.
- *What was the length from snout to tip of tail fin?*

*Please send the information* via *messenger or by email: juni@natur.gl.*



*Pink salmon can be distinguished from Arctic charr and Atlantic salmon by black dots on the tail fin of the pink salmon. When spawning season is close (late summer/early autumn) the males develop a characteristic hump on the back, which makes them easy to recognize. The black dotted tail fin is a trait that can be applied for both males and females of pink salmon year around.*

