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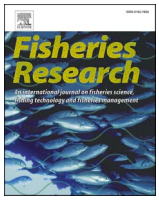

Full length article

Survey of the invasive alien pink salmon (*Oncorhynchus gorbuscha*) for infective agents in the Fennoscandian Rivers Tana and Neiden

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ABSTRACT

Pink salmon (*Oncorhynchus gorbuscha*, Walbaum 1792) is an invasive alien species in the Fennoscandian rivers, and its growing populations cause concern for their impact on the native salmonids and river ecosystems. One of the major concerns is fish diseases and the potential ability of pink salmon to transmit pathogens to the native salmonid species. In this study, pink salmon were sampled during their spawning migration in the Rivers Tana and Neiden in northern Norway and Finland in 2021. The fish were surveyed for viral hemorrhagic septicemia virus (VHSV), infectious pancreatic necrosis virus (IPNV), infectious salmon anemia virus (ISAV), salmonid alphavirus (SAV), piscine orthoreovirus (PRV), *Renibacterium salmoninarum*, and *Gyrodactylus salaris*. No viral diseases or infections with *R. salmoninarum* or *G. salaris* were detected. However, 23 % of the fish were found infected with a parasitic roundworm, *Hysterothylacium aduncum*. Additionally, external anomalies, such as hemorrhages or lesions in the skin, were observed in 35 % of the fish studied, and some of the fish exhibited degenerative myopathy. Based on this study, pink salmon appears not to pose a high infection risk to the native salmonid fish populations in the Rivers Tana and Neiden. Nevertheless, the results demonstrate that pink salmon have the potential to carry salmonid pathogens. More information on the susceptibility of pink salmon to different pathogens and on the overall effect this invasive species has on native fish species is needed. As the number of migrating pink salmon in the Fennoscandian rivers appears to increase, the potential risk of parasites and other pathogens spreading between fish species will also rise.

1. Introduction

Pink salmon, *Oncorhynchus gorbuscha* (Walbaum, 1792), is an anadromous salmon species that has a wide geographic range in the northern Pacific Ocean and adjoining waters, including spawning rivers in the North American as well as the Asian continent (Heard, 1991). Pink salmon have a strict two-year life cycle, in which the adults return to spawn in a river after approximately 18 months in the sea in segregated odd- and even-year broodlines (Scott and Crossman, 1973). Most adults return to the river in which they hatched, but some stray and use another river for spawning (Scott and Crossman, 1973). It has been hypothesized that compared to other Pacific salmon species, pink salmon is the most likely to stray from its home river, depending on the local conditions and life history (Quinn, 2005). The fact that pink salmon populations generally have lower levels of genetic variation than other Pacific salmon species supports this hypothesis (Beacham et al., 2012).

In the 1950s, pink salmon were introduced to the Barents and White Sea river systems in northwest Russia via transplantations of eggs from Russian Pacific rivers, and the stockings continued for decades (Bjerknes and Vaag, 1980). The transplantations of eggs ended in 1998, while releases of fry from local hatcheries ended in 2003 (Niemelä et al., 2016). Following a change in the pink salmon stock used for transplantations to odd-year broodline from a more northern origin in 1985, self-sustaining pink salmon stocks have been present especially in the White Sea rivers (Gordeeva et al., 2015; Gordeeva and Salmenkova, 2011). Moreover, since 2001, the odd-year catches of adult pink salmon in Russian rivers have significantly increased, from below 100 tonnes to a mean of 220.5 tonnes between 2001–2017, with the highest amount being caught in 2017 (Sandlund et al., 2019). Meanwhile, the catches of the even-year pink salmon have been variable but small since 2000 (Sandlund et al., 2019). In Norwegian waters, pink salmon was first detected in 1960 (Sandlund et al., 2019), and official catch statistics of

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this invasive alien species in the Rivers Tana (Deatnu in North Sámi and Skolt Sámi, Teno in Finnish) and Neiden (Njávdámjohka/Njauddâmjokk in Northern Sámi/Skolt Sámi, Näätämö in Finnish) have been recorded since the mid-1970s (Niemelä et al., 2016).

The River Tana is a large subarctic river that forms the border between northernmost Norway and Finland (catchment area 16,386 km^2, of which 69 % is located in Norway and 31 % in Finland) (Ekholm, 1993), and it supports the largest wild stock of Atlantic salmon (*Salmo salar*) in the world (Anonymous, 2012). The River Neiden (catchment area 2962 km^2: 20 % in Norway, 80 % in Finland) is a smaller transboundary river between northern Finland and Norway that discharges to the Barents Sea through Neidenfjord, and like the River Tana, it supports naturally reproducing stocks of Atlantic salmon.

The catches of pink salmon in Norway remained low until 2017 (Fig. 1), when an invasion of tens of thousands of pink salmon was experienced in rivers all along the coast, including the River Tana in the Finnmark region (Pauli et al., 2022). In 2019, the numbers of migrating pink salmon were again as high as in 2017 in the River Tana (Tana Monitoring and Research Group, 2019), and in 2021, a ten-fold increase in the occurrence of the odd-year population was reported (Tana Monitoring and Research Group, 2021).

The growing numbers of the invasive pink salmon have caused concern regarding possible fish disease transmission from pink salmon to wild Atlantic salmon and other wild and farmed salmonids in the river environment. According to the Report of the Norwegian Scientific Committee for Food and Environment (VKM et al., 2020), abundant spawning pink salmon in a river may have a substantial impact on native salmonids, and the likelihood of spreading disease to native wild fish, as well as to farmed fish, is higher when the numbers of pink salmon increase. Even though the spawning of pink salmon occurs earlier in the season than the native salmonids, in 2019, there were observations that indicated possible overlap with native salmonids in northern Norway. Pink salmon enter the Norwegian rivers during summer and early autumn and the spawning time varies between the beginning of August and early days of September, whereas for anadromous brown trout (*Salmo trutta*) the spawning time is between 10th September and 10th October, and for Atlantic salmon between 15th September and 15th October (Berntsen et al., 2018). The preferred spawning sites for pink salmon are like the ones of Atlantic salmon and brown trout: coarse gravel with a flow trough of well-aerated water (Heard, 1991). In the northern Norwegian rivers, there has been observations that both pink salmon and brown trout have been spawning at the same time in September (Erkinaro et al., 2022). Additionally, in Norway, there is a current strategy to battle pink salmon invasion in the most severely affected rivers using river barrier traps as well as nets and angling (Anonymous, 2024). Dense aggregations of fish can occur by the river barriers and in the bag-nets, forcing pink salmon and other salmonid species in contact with each other. An overlap in the timing of spawning and increased contact between different fish species would increase the likelihood of the spread of pathogens.

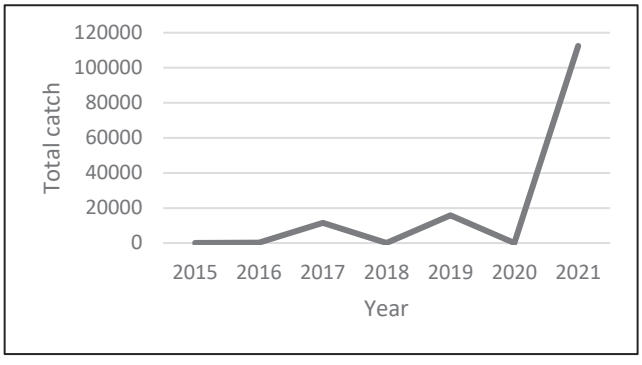

Fig. 1. Total annual river catches of pink salmon (number of fish) in Norway in 2015–2021 (Pauli et al., 2022).

Limited information on the susceptibility of pink salmon to viral, bacterial, or parasitic diseases is available at this date. As for viruses that are known to be pathogenic for variety of salmonid species, viral hemorrhagic septicaemia virus (VHSV) has been isolated from wild pink salmon in Alaska (Meyers et al., 2019). Piscine orthoreovirus, the causative agent of heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon, has been detected in wild pink salmon in 2012–2014 in North America and later in 2021 in northern Norway (Purcell et al., 2017; Norwegian Veterinary Institute, 2021). Additionally, viral erythrocytic necrosis virus (VENV), has been reported from pink salmon based on light and electron microscopic studies (Evelyn and Traxler, 1978). No reports on salmonid alphavirus (SAV), infectious haematopoietic necrosis virus (IHNV), infectious salmon anaemia virus (ISAV) or infectious pancreatic necrosis virus (IPNV) in pink salmon are currently available. However, these viruses can infect several salmonid species, and therefore they should not be excluded when potential pathogens of pink salmon are studied (VKM et al., 2020).

Bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum* is a significant disease affecting wild and farmed salmonids worldwide (Wiens, 2006), and pink salmon is known to be susceptible to *R. salmoninarum* infection (Meyers et al., 1993). Transmission of the disease between different salmonid species has been documented (Mitchum and Sherman, 1981). A distinct subspecies of *Aeromonas salmonicida* (subsp. *masoucida*) has been reported to cause furunculosis-like disease symptoms in pink salmon in Pacific regions (Gulla et al., 2019; Kimura, 1969).

Parasites that pink salmon can host may have an impact on the welfare of native salmonids. Some of the parasites are also zoonotic and may have an impact on the public health. Anisakid nematodes (genera *Anisakis*, *Psudoterranova* and *Contracaecum*) are roundworms of marine mammals (and birds) and can cause anisakidosis in humans if intermediate host fish are consumed raw. Anisakidosis is usually caused by larval forms of nematodes and manifests as gastrointestinal symptoms. Even dead larvae are often regarded as cause of allergic reactions. Also *Hysterothylacium* spp. (family *Raphidascarididae*), which have fish as their final hosts, have rarely been implicated in human infections (Shamsi and Barton, 2023). *Gyrodactylus salaris* is a monogenean ectoparasite of various salmonid fish species, regarded as highly pathogenic to the Atlantic salmon, especially to salmon parr in the Norwegian rivers (Paladini et al., 2021). The species does not live in the salinity of sea water. Thus far, the potential of the pink salmon to host *G. salaris* infection is unknown (Mo, 2020).

Due to the increasing numbers of the invasive pink salmon in the northern rivers, there is a growing need for more information on possible fish diseases, both native and invasive, that pink salmon is susceptible to or a carrier for, and whether these diseases could pose a risk to the native salmon populations and have serious implications for the fishery resources. The aim of this study was to investigate the infection status of the pink salmon migrating to the Rivers Tana and Neiden in the summer season of 2021. The focus was on the causative agents of fish diseases listed in Finnish national and EU legislation, including VHSV, IPNV, IHNV, ISAV, SAV, *R. salmoninarum*, and *G. salaris*. Due to the recent findings in northern Norway (Norwegian Veterinary Institute, 2021), PRV was also included in the survey.

2. Materials and methods

2.1. Sampling

Altogether, 69 pink salmon were sampled in June-July 2021 from the Rivers Tana and Neiden. The sampling sites were located in the lower main stems in both system and are indicated on the map in Fig. 2. Sampling site A in the River Neiden was located at the Skoltefossen fishway and samples were obtained from the pink salmon removal fisheries conducted there. Sampling sites B, C and D were located in the

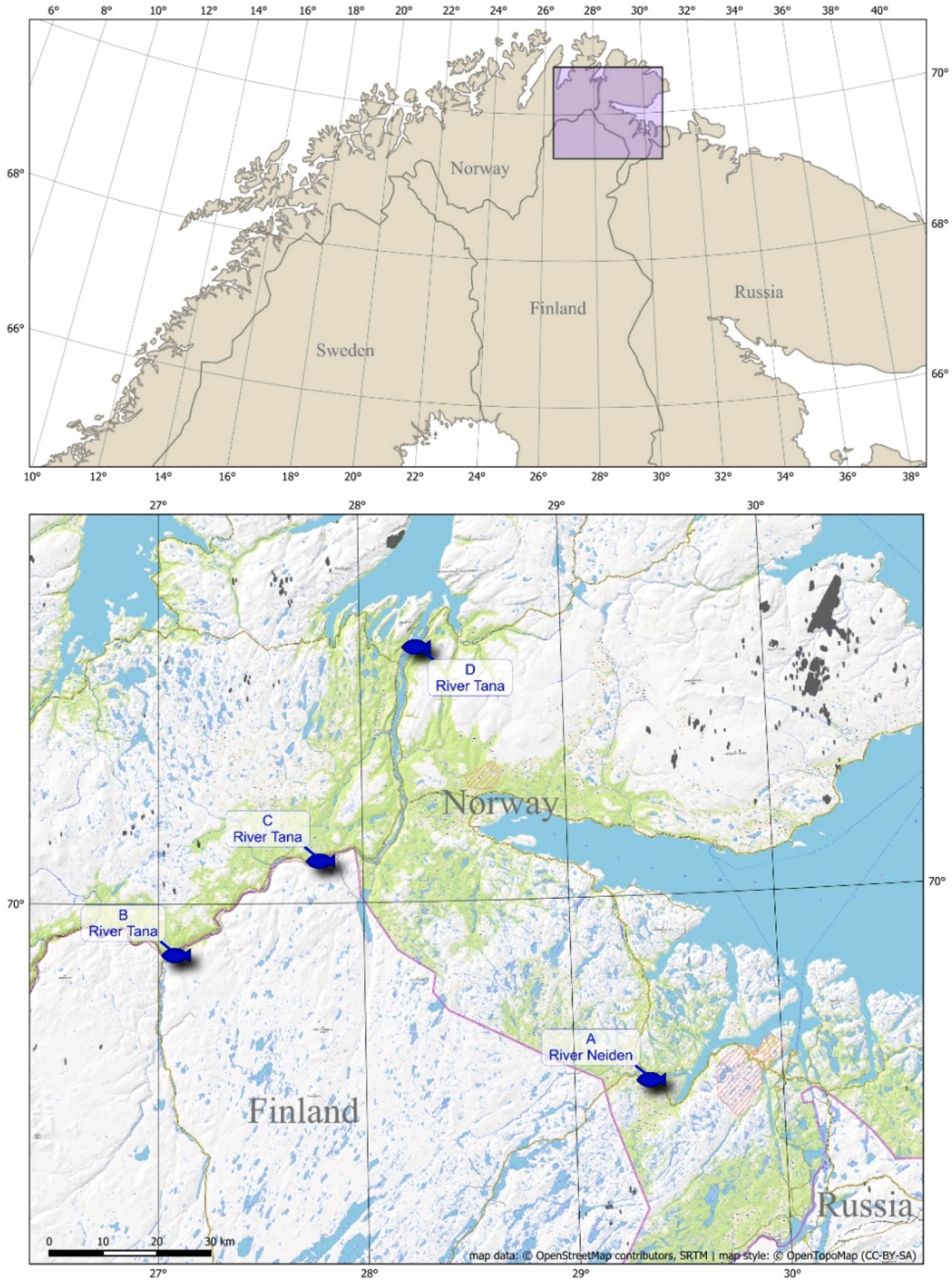

Fig. 2. Upper map: location of the sampling area marked with a square. Lower map: location of the sampling sites in the River Neiden (A) and River Tana (B, C, and D).

River Tana and samples were obtained through another study where pink salmon were caught for telemetry tagging. Multiple sampling sites were needed to acquire sufficient number of samples. The fish were caught by netting and killed by percussive stunning. The sampling date, body weight, total length from the snout to the tip of the longer lobe of the caudal fin, sex, and any visible injuries on the skin or in the body cavity were recorded from each fish at the time of sampling. Selected organs were collected directly in RNAlater solution, and the sample size did not exceed 5 mm in any dimension to avoid sample degradation. For further molecular analysis each fish was sampled as follows: the posterior kidney for *R. salmoninarum* analysis and a pooled sample of the heart, spleen, and anterior kidney for analyses of VHSV, IHNV, IPNV, ISAV, SAV, and PRV. Samples from four fish (internal organs and affected skin and muscle) from the River Neiden were collected in neutral-buffered 10 % formalin for histological analysis. Pectoral fins were collected in ethanol for screening of *G. salaris*. Additionally, any other parasites detected during sampling were collected in ethanol for further identification. The internal organs and the peritoneal wall were visually examined for extraintestinal macroparasites, but no further examination of muscle tissue was performed.

2.2. Analysis for Renibacterium salmoninarum

DNA was extracted from the kidney samples stored in RNAlater using the QIAamp DNA Mini Kit (Qiagen, Germany) and a DNA extraction robot (Qiagen) according to the manufacturer's instructions. At the beginning of extraction, kidney samples were placed in sterile 2-mL tubes with beads (NucleoSpin Bead Tubes, Marchery-Nagel), after which sterile 1xPBS was added to the samples, and they were homogenized with a MagNA Lyser instrument (Roche). PCR analysis for the detection of *R. salmoninarum* was conducted using primers and a PCR assay from Chase et al. (2006), with minor modifications. The TaqMan MGB probe (Thermo Fisher Scientific) was used instead of TAMRA quencher, and iTaq Universal Probes Supermix (Bio-Rad) was used for the PCR reaction. To detect possible inhibition in samples, an internal amplification control (IAC) consisting of approximately 500 copies of pUC18 plasmid was included in each PCR reaction, and pUC18 amplification primers and probes were used (Fricker et al., 2007). In addition to pUC18 plasmid, DNA extracted from *R. Salmoninarum* ATCC 33209 culture was included in each PCR run as a positive control sample. For each extraction batch, one blank DNA extraction control template was included, to detect possible contamination during the DNA extraction. Furthermore, every 13th sample in the PCR run contained only PCR-grade water as a no-template control (NTC) sample, to detect possible contamination during the PCR run.

2.3. Viral analyses

RNA was extracted from the pooled organ samples stored in RNAlater using the QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's instructions. All samples were tested by real time RT-PCR with protocols previously described for viral hemorrhagic septicemia virus (VHSV) (Jonstrup et al., 2013), infectious pancreatic necrosis virus (IPNV) (Ørpetveit et al., 2010), infectious salmon anemia virus (ISAV) (Snow et al., 2006), and piscine orthoreovirus (PRV) (Zhao et al., 2021). All samples were also tested by real time RT-PCR for salmonid alphavirus (SAV) using a protocol previously described by Hodneland and Endresen (2006) with a modified probe, PDV 5' FAM-CAG GGT TCG AAG TGG TGG CCA GC-BHQ 3', and for infectious hematopoietic necrosis virus (IHNV) using two real time RT-PCRs, the first published by Purcell et al. (2013) and the second with the protocol and primers described by Purcell et al. (2013) and the probe described by Hoferer et al. (2019). For each RNA extraction batch, two blank RNA extraction control templates containing only PCR-grade water, and one positive RNA extraction control were included. Furthermore, in each RT-PCR run, one virus specific positive control sample was included, as well as one NTC sample containing only PCR-grade water.

2.4. Parasitological analyses

From the parasitic nematode worms stored in ethanol, DNA was extracted with the Qiagen DNA mini kit (Qiagen) according to manufacturer's instructions, using a 25-mg piece from the middle part of each specimen. The DNA was tested with the primers NC5F (forward; 5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and NC2R (reverse; 5′-TTAGTTTCTTTTCCTCCGCT-3′) published by Gasser et al. (1993), Gasser and Hoste (1995), which amplify a nematode ribosomal RNA (rRNA) fragment that includes a partial sequence of the 18S rRNA gene, internal transcribed spacer 1, the 5.8S rRNA gene, internal transcribed spacer 2, and a partial sequence of the 28S rRNA gene from nematodes of the family *Anisakidae*. Each reaction mixture contained 2 μL of genomic DNA, isolated as described above, 1 U DyNAzyme II DNA Polymerase (Finnzymes), dNTPs (200 μM each), and 800 pmol of each primer in a total reaction volume of 25 μL. The PCR program used was: 94 °C (5 min), 60 °C (30 s), 72 °C (90 s), followed by 30 cycles of 94 °C (30 s), 60 °C (30 s), 72 °C (1 min), and a final extension of 72 °C (5 min). PCR products were run on 2 % agarose gel to confirm the size and quality of the amplicons. Prior to sequencing, the PCR products were cut from the gel and purified using the E.Z.N.A.® Gel Extraction Kit (Omega Bio-Tek). The sequencing was performed in both orientations from each PCR product, using the primers NC5F, NC13F (forward; 5′-ATCGATGAAGAACGCAGC-3′), NC13R (reverse; 5′-GCTGCGTTCT TCATCGAT-3′), XZ1R (reverse; 5′-GGAATGAACCCGATGGCGCAAT-3′), and NC2R (Zhu et al., 1998). The PCR products were sequenced with Sanger sequencing at the Biocenter Oulu Sequencing Center. *Anisakis pegreffii* and *Contracaecum osculatum* DNA were used as positive amplifying controls in PCR. Control DNAs were kind donations from the European Union Reference Laboratory for Parasites (Rome, Italy).

To identify the nematode species, phylogenetic analyses for the rRNA sequences were performed with MEGA 10.0.11 software (Tamura et al., 2013) using the maximum likelihood method based on the general time-reversible model. The reliability of the analyses was assessed using a bootstrap method with 1000 replicates. Previously published nematode rRNA sequences of *Hysterothylacium aduncum, H. amoyense, H. fabri, H. sinense, H. reliquens, H. bidentatum, H. longilabrum, Raphidascaris acus, Raphidascaroides brasiliensis, Anisakis typica, Anisakis physeteris, Anisakis simplex, Contracaecum muraenesoxi, Contracaecum osculatum,* and *Pseudoterranova decipiens* were obtained from the NCBI GenBank and included in the analyses.

The pectoral fins collected in ethanol were screened for *G. salaris* using a stereo microscope.

2.5. Histological analysis

Tissues fixed in neutral-buffered 10 % formalin were routinely processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE). Slides were assessed under a standard light microscope.

2.6. Statistical analyses

To reveal possible differences in the physical condition of the fish between the Rivers Tana and Neiden, the numbers of fish with external skin anomalies and internal parasites were compared using Pearson's chi-squared test. The weight and length of the studied fish were compared between the two rivers using the independent samples t-test. All analyses were performed using IBM SPSS Statistics software. The significance level was set at 5 %.

3. Results

Altogether, 50 fish (28 females, 20 males; sex was not determined

from 2 fish) from the River Neiden and 19 fish (5 females, 14 males) from the River Tana were examined (Table 1). The mean weight of the fish from the River Neiden was 1.25 kg (SD 0.33) and the mean length was 49.3 cm (SD 3.9). The respective measurements for the fish from the River Tana were 1.37 kg (SD 0.34) and 50.8 cm (SD 4.1). Neither the weight nor the length of the studied fish differed significantly between the two rivers (weight $p = 0.17$, length $p = 0.15$). External anomalies, such as hemorrhages or skin lesions, were observed on 35 % of all the examined fish (Fig. 3), with no significant difference in their occurrence between the two rivers ($p = 0.08$).

Parasites were detected in 23 % of the fish, mostly in the abdominal cavity. All the parasitic worms detected during sampling were identified as *Hysterothylacium aduncum* using PCR and subsequent sequencing and phylogenetic analyses. The *H. aduncum* rRNA sequence obtained in this study was identical to other previously published *H. aduncum* rRNA sequences (data not shown), and it was deposited in the NCBI GenBank with the accession number OR039062. A significantly higher number of fish had *H. aduncum* in the River Tana (9/19) than in the River Neiden (7/50) ($p = 0.03$). No *R. salmoninarum*, viruses, or *G. salaris* were detected in any of the studied fish.

No histopathological anomalies were detected in the internal organs of the fish. Light vacuolization of the hepatocytes was observed in one of the fish, while in others, the hepatocytes were non-vacuolated. Reddened areas of the skin were partly due to erosion of the epidermal layer and the accumulation of erythrocytes in the underlying tissues. However, in other lesions, the epidermis appeared intact, and the main finding was degenerative myopathy with increased cellularity, including macrophages invading the affected myofibrils, erythrocytes, and increased endomysial connective tissue (Fig. 4).

4. Discussion

In this study, no viral diseases, *R. salmoninarum*, or *G. salaris* were detected in pink salmon sampled from the Rivers Tana or Neiden in northern Fennoscandia. Based on a Norwegian risk assessment (VKM et al., 2020), PRV, IPNV, and viral erythrocytic necrosis virus (VEN) have a high probability of infecting pink salmon. However, reports on pathogens detected in pink salmon are relatively scarce in the available literature. According to the Norwegian Veterinary Institute (2021), PRV-1 was detected in eight pink salmon sampled from Færsetvassdraget, Skibotnelva, and Skallelv in northern Norway. PRV RNA was also detected in pink salmon caught between 2012 and 2014 on the Washington Coast, North America (Purcell et al., 2017). Another viral pathogen, VHSV genotype Iva, has been isolated from wild pink salmon in Alaska on two occasions (Meyers et al., 2019). Additionally, *R. salmoninarum* infection has been reported in ocean-caught pink salmon in British Columbia (Kent et al., 1998).

In the current study, *H. aduncum* was detected in 23 % of the 69 pink salmon examined. No other parasites were detected by visual inspection of the body cavity. *H. aduncum* infects a wide variety of aquatic species in marine, and to a lesser extent, in freshwater environments. It is a highly abundant nematode parasite in the North Atlantic Ocean (Adroher-Auroux and Benítez-Rodríguez, 2021). It has invertebrates as intermediate hosts and fish as paratenic and definitive hosts (González, 1998). Salmonids are typical definitive hosts, with adult worms infecting the digestive tract. In this study, digestive tracts were not surveyed, and the recorded nematodes were found in the body cavity. Previously, pink salmon caught in their feeding area in the Norwegian Sea have been found to be infected with 13 marine parasite species, including the nematodes *H. aduncum* and *Anisakis simplex*, as well as trematode, cestode and crustacean species (Rullestad, 2021). In the Kandalaksha Bay of the White Sea, 100 % of the 25 pink salmon studied were infected with *H. aduncum*, and 64 % were infected with *A. simplex* (Sokolov et al., 2024). Sea lice (both *Lepeophtheirus salmonis* and *Caligus clemensi*) and renal myxosporean parasites have been observed in juvenile pink salmon in their early seawater stage in British Columbia, Canada (Saksida et al., 2012). In this study, *H. aduncum* was detected more often in pink salmon caught from the River Tana than from the River Neiden. This result may partly be a consequence of the different sampling strategy in the two rivers: in the River Neiden, all fish were caught near the river mouth, whereas in the River Tana, all fish except for one were caught further upstream a week or more later than the fish in River Neiden. It cannot be excluded that after a longer stay in freshwater, the fat deposit in the abdominal cavity of the fish was further depleted, making the visual detection of the parasites easier. However, the Tana fish were on average somewhat heavier and longer than those of the River Neiden, even though the difference was not statistically significant.

Interestingly, no *Anisakis* larvae were discovered in this study. However, in Alaska, it has been reported that most *Anisakis* larvae in salmon occur in muscle tissue, which was not examined in the current study. In 12 pink salmon examined in Alaska, the mean abundance of *Anisakis* larvae was 21.4 in flesh and 2.1 in viscera (Karl et al., 2011). In a study of 80 pink salmon from three rivers in Hordaland (West Norway), 25 % of the fish were infected with *A. simplex*, and the mean abundance was only 0.5. All larvae were found in the viscera, but the musculature was not specifically examined (Fjær, 2019). In another study, all 90 Atlantic salmon caught from the Namsen Fjord in central Norway had *A. simplex* larvae in the viscera and 88 also had them in the musculature. The mean number of larvae was 31.2 in viscera and 13.2 in fillets (Mo et al., 2021). In 21 Atlantic salmon from the Tana Fjord examined in 1995, the mesenteries of 38 % were infected with *A. simplex*, while all 31 sea trout (*Salmo trutta*) were found negative. The prevalence of *H. aduncum* was 95 % and 39 % in the digestive tract of Atlantic salmon and sea trout, respectively (Bristow et al., 1996). Our study does not prove the absence of *Anisakis* larvae in pink salmon in the Barents Sea but indicates that pink salmon may not be an important host species strongly affecting the ecology of *A. simplex*. While *H. aduncum* is not really regarded as zoonotic, it is important in the differential diagnosis of the zoonotic *Anisakis* spp.

In the present study, no salmon lice were found. The salmon lice species *L. salmonis* and *C. elongatus* were found in 90 % and 5 %, respectively, of the 21 Atlantic salmon examined by Bristow et al. (1996) from Tana Fjord, but their combined prevalence in 31 sea trout was 3.2 %. This was hypothesized as being caused by the trout staying in or close to Tana Fjord and not migrating to pelagic waters, like the Atlantic salmon (Bristow et al., 1996). Salmon lice are known not to live in freshwater, and freshwater treatments are used for de-lousing in aquaculture (Guttu et al., 2024). Adult lice are less susceptible to low salinity than immature ones but have been seen expelled from fish in freshwater within 15 days (Guttu et al., 2024). Some of the epidermal injuries observed in this study might have been caused by sea louse detached after the fish entered the rivers.

The river catchments of the Rivers Tana and Neiden are officially free of *G. salaris*. Even though *G. salaris* was not detected in this study, it does not rule out the possibility of pink salmon acting as a carrier for this potentially highly damaging parasite for wild salmonid populations. According to the World Organization for Animal Health (2022), susceptible hosts for *G. salaris* are the Atlantic salmon, Arctic char (*Salvelinus alpinus*), brook trout (*Salvelinus fontinalis*), brown trout, grayling (*Thymallus thymallus*), and rainbow trout (*Oncorhynchus mykiss*). However, *G. salaris* parasites may attach onto any fish species not considered as a susceptible species for short periods of time. The parasite has recently been reported to have spread to the River Tuloma and Lake Imandra in northwestern Russia due to rainbow trout farming (Hansen et al., 2022). There is a spawning population of pink salmon in the River Tuloma (Niemelä et al., 2016) that could potentially spread *G. salaris* via individuals migrating through the Barents Sea and therefore pose a threat to the susceptible wild salmonids in northern rivers. However, it has been reported that *G. salaris* parasites are immobilized at sea water salinity (33 ‰) within a few minutes, but can survive in lower salinities, depending on temperature (Soleng and Bakke, 1997). Therefore, the risk

Table 1
Individual information and results for the studied pink salmon. Sampling site, A: River Neiden, B, C, and D: River Tana. Sex, F: female, M: male. External anomalies: hemorrhages, a: on the lower jaw, b: in the anal area, c: in the abdominal area, d: at the base of the fins; lesions, e: in the skin around the mouth, f: at the base of the tail; g: excessive accumulation of mucus on the fins; * fish sampled for histology. Internal anomalies: parasites, h: in the abdominal cavity, i: in the internal organs, j: in the muscle; k: hemorrhages in the intestine; l: granular liver tissue. Internal parasites: Ha: *Hysterothylacium aduncum*. ND: not determined; N/D: not detected. All fish were negative for viruses (VHSV, IHNV, IPNV, ISAV, SAV, and PRV), *R. salmoninarum*, and G. salaris.

Fish no	Sampling site	Sampling day/month/year	Weight (kg)	Length (cm)	Sex	External anomalies	Internal anomalies	Internal parasites
1	**A**	7/7/2021	1.09	47.0	F	N/D	N/D	N/D
2	**A**	7/7/2021	1.49	49.0	F	N/D	N/D	N/D
3	**A**	7/7/2021	1.65	42.0	M	a,*	N/D	N/D
4	**A**	7/7/2021	0.84	44.0	F	N/D	N/D	N/D
5	**A**	7/7/2021	1.53	51.0	F	N/D	N/D	N/D
6	**A**	7/7/2021	1.22	48.0	F	a,*	N/D	N/D
7	**A**	7/7/2021	0.78	45.0	M	a	N/D	N/D
8	**A**	7/7/2021	1.33	49.0	F	a	N/D	N/D
9	**A**	7/7/2021	1.02	48.0	M	N/D	N/D	N/D
10	**A**	7/7/2021	1.24	49.0	F	N/D	N/D	N/D
11	**A**	7/7/2021	1.12	47.0	F	b	N/D	N/D
12	**A**	7/7/2021	1.25	49.5	F	N/D	h	Ha
13	**A**	7/7/2021	1.50	41.8	M	N/D	h	Ha
14	**A**	7/7/2021	1.03	46.0	F	N/D	h	Ha
15	**A**	7/7/2021	1.85	57.5	M	b	h, k	Ha
16	**A**	7/7/2021	1.23	48.0	F	N/D	N/D	N/D
17	**A**	7/7/2021	1.20	49.0	M	N/D	N/D	N/D
18	**A**	7/7/2021	0.80	45.5	F	a, c	N/D	N/D
19	**A**	7/7/2021	1.15	47.0	M	N/D	N/D	N/D
20	**A**	7/7/2021	1.58	54.0	M	N/D	N/D	N/D
21	**A**	7/7/2021	1.65	50.0	F	N/D	N/D	N/D
22	**A**	7/7/2021	0.91	47.0	M	N/D	N/D	N/D
23	**A**	7/7/2021	2.75	62.0	M	a, b, d, e,*	N/D	N/D
24	**A**	7/7/2021	0.89	49.0	M	e	N/D	N/D
25	**A**	7/7/2021	0.89	45.0	F	b	N/D	N/D
26	**A**	7/7/2021	1.31	52.0	ND	N/D	N/D	N/D
27	**A**	7/7/2021	1.60	49.0	F	b	h	Ha
28	**A**	7/7/2021	1.36	52.0	M	N/D	N/D	N/D
29	**A**	7/7/2021	0.95	47.0	F	N/D	N/D	N/D
30	**A**	7/7/2021	1.07	48.5	F	N/D	h	Ha
31	**A**	7/7/2021	1.34	56.0	M	a	N/D	N/D
32	**A**	7/7/2021	0.82	44.0	F	N/D	N/D	N/D
33	**A**	7/7/2021	1.30	52.0	ND	c	N/D	N/D
34	**A**	7/7/2021	1.28	50.0	F	c	N/D	N/D
35	**A**	7/7/2021	1.15	50.5	F	c	N/D	N/D
36	**A**	7/7/2021	1.18	50.5	M	N/D	N/D	N/D
37	**A**	7/7/2021	1.17	48.0	F	N/D	N/D	N/D
38	**A**	7/7/2021	1.52	55.0	M	N/D	N/D	N/D
39	**A**	7/7/2021	1.11	48.0	F	a, e	N/D	N/D
40	**A**	7/7/2021	0.95	45.5	F	a, f	N/D	N/D
41	**A**	7/7/2021	1.08	49.0	M	N/D	N/D	N/D
42	**A**	7/7/2021	1.25	52.0	M	N/D	N/D	N/D
43	**A**	7/7/2021	1.21	52.5	F	N/D	N/D	N/D
44	**A**	7/7/2021	1.44	53.0	F	N/D	h	Ha
45	**A**	7/7/2021	1.41	51.0	M	e	N/D	N/D
46	**A**	7/7/2021	1.13	47.5	M	c	N/D	N/D
47	**A**	7/7/2021	1.48	52.0	F	a,*	l	N/D
48	**A**	7/7/2021	0.81	44.0	F	a	N/D	N/D
49	**A**	7/7/2021	1.43	55.0	F	N/D	N/D	N/D
50	**A**	7/7/2021	1.00	47.0	M	N/D	N/D	N/D
51	**B**	13/7/2021	2.04	59.3	M	g	N/D	N/D
52	**C**	13/7/2021	1.35	51.0	M	N/D	h	Ha
53	**C**	13/7/2021	1.46	51.5	M	N/D	N/D	N/D
54	**C**	13/7/2021	1.33	49.0	M	N/D	h	Ha
55	**C**	13/7/2021	1.48	53.0	M	b, c	h	Ha
56	**C**	13/7/2021	1.36	49.0	M	b	N/D	N/D
57	**C**	13/7/2021	1.17	48.2	F	N/D	N/D	N/D
58	**C**	13/7/2021	1.44	52.5	M	b, c	N/D	N/D
59	**C**	13/7/2021	0.88	45.9	F	N/D	N/D	N/D
60	**C**	13/7/2021	1.49	54.5	M	N/D	N/D	N/D
61	**B**	14/7/2021	0.95	45.5	F	N/D	h	Ha
62	**B**	14/7/2021	2.00	56.5	M	N/D	N/D	N/D
63	**B**	14/7/2021	1.40	51.4	M	N/D	h	Ha
64	**B**	14/7/2021	0.90	44.5	M	N/D	h	Ha
65	**B**	15/7/2021	1.10	47.3	F	N/D	i	Ha
66	**B**	15/7/2021	0.95	45.0	F	N/D	j	Ha
67	**D**	15/7/2021	1.80	53.0	M	N/D	N/D	N/D
68	**B**	22/7/2021	1.40	54.5	M	N/D	N/D	N/D
69	**B**	22/7/2021	1.55	53.5	M	N/D	h	Ha

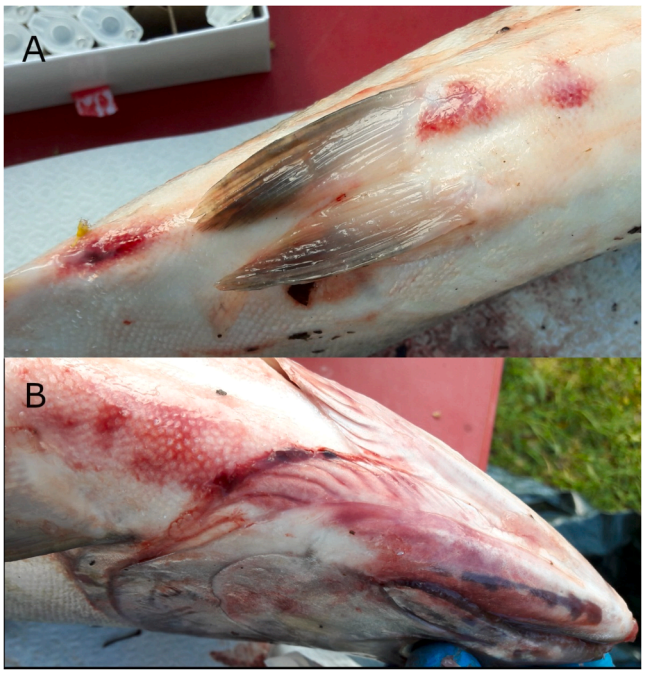

Fig. 3. External anomalies of the pink salmon sampled in this study. Hemorrhages and lesions in the abdominal skin and around the anus (A), and in the lower jaw, operculum, and abdominal skin (B).

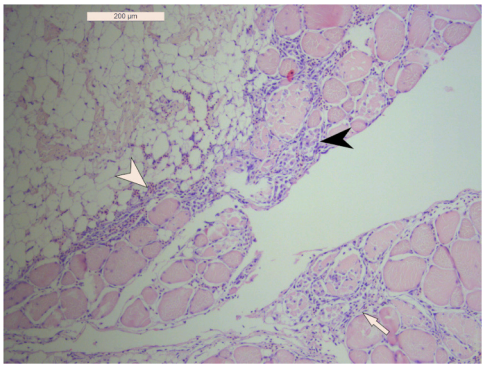

Fig. 4. Degenerative myopathy in connection with the skin lesion of a pink salmon, with increased cellularity, including macrophages invading the affected myofibrils (black arrowhead), erythrocytes (white arrowhead), and increased endomysial connective tissue (white arrow). HE, bar = 200 µm.

of pink salmon transmitting *G. salaris* into river environments is low but not non-existent. Thus far, the suitability of pink salmon for hosting *G. salaris* is unknown (Mo, 2020).

Lesions, hemorrhages, and other external anomalies were detected in 35 % of the pink salmon studied here. Lesions and hemorrhages were detected in fish from both rivers. Additionally, it appeared that external anomalies were detected in fish caught near the river mouth in River Neiden as well as from further upstream in River Tana. Many of the lesions detected were most likely of mechanical origin, caused by predators, nets, fishing tackle, or other physical contact, for example with stones on the river bottom. Additionally, degenerative myopathy was detected in lesions where the epidermis had remained intact. The number of histological samples studied here was limited, but the myopathy observed could be indicative of migratory stress. Wilson et al. (2014) reported that oxidative DNA damage in the heart and plasma of pink salmon increased during migration. The increased oxidative stress of these tissues is correlated with the senescence and deterioration associated with a semelparous reproductive strategy of the Pacific salmons. In white and red muscle tissue, however, the oxidative stress levels were higher at the beginning of the freshwater migration, indicating that the aerobic demands are less for individuals that have reached the spawning grounds. This is consistent with our findings from pink salmon sampled close to the mouth of the River Neiden. Due to the small number of histological samples of affected skin tissues, and the focusing of viral and bacterial testing on internal organs, it cannot be excluded that the external changes observed in pink salmon in this study could be of infective origin. Atlantic salmon returning to the River Tornio and other rivers in Sweden and Finland from the Baltic Sea have been reported to suffer from hemorrhages, erosion, and ulcerative/necrotic skin conditions (Axén and Koski, 2017; Weichert et al., 2021). No conclusive evidence of an infective agent was obtained from virological or bacteriological studies on the affected fish tissues (Axén and Koski, 2017). However, external anomalies in the fish were associated with diseases responses such as an inflammatory response and osmotic hemodilution (Weichert et al., 2021). Red mark syndrome (RMS) is a skin condition characterized by multiple skin lesions over the body of the fish. The etiology of the condition has not been confirmed, but two pathogens have been associated with the disease: a *Midichloria*-like organism (MLO) and *Flavobacterium psychrophilum* (Metselaar et al., 2022). Even though RMS has only been identified in cultured rainbow trout, similar lesions have been reported from other salmonids such as brown trout and cutthroat trout (*Oncorhynchus clarkii*) (Metselaar et al., 2022). In this study, the pink salmon were not tested for MLO or *F. psychrophilum*. Histopathological changes in the fish skin are often non-specific, and their origin can therefore be difficult to define.

In the current study, limited number of pink salmon were sampled in a restricted geographical area during one summer season. Additionally, the diagnostic testing was limited to selected pathogens with the focus on viral diseases. Despite these limitations, new information on the disease status and physical condition of the pink salmon migrating to the Rivers Tana and Neiden was obtained. At the time of writing, it is known that the pink salmon populations in the Fennoscandian rivers continued to multiply rapidly. According to the report from the Tana Monitoring and Research Group (2024), the pink salmon run estimate in the Tana mainstream was approximately 170 000 individuals in 2023, when the corresponding estimate in 2021 was approximately 50 000 pink salmon. At the same time, the numbers of adult wild Atlantic salmon decreased in the Tana River system in 2023 compared to the two earlier years and the one sea-winter Atlantic salmon abundance remained poor for the fifth successive season.

Even though no alarming findings of pathogens were made in this study, it is evident that more information on the diseases of pink salmon and the effects of this invasive species on native species on a broader scale is needed. More than one fifth of the pink salmon studied here were infected with *H. aduncum*. Based on previous studies of wild-caught Atlantic salmon and pink salmon in the Norwegian Sea and the Tana fjord, *H. aduncum* is a prevalent parasite in both species (Bristow et al., 1996; Rullestad, 2021), and both Atlantic salmon and pink salmon appear to be competent hosts for the parasite. One of the mechanisms by which parasitized pink salmon may influence native salmonid and other fish populations could be parasite spillback. In parasite spillback, nonindigenous species act as hosts for native infective agents, from which infection may spill back to native fauna (Kelly et al., 2009). In Chile, *H. aduncum* was spread from native fish to cultured salmonids and together with microbial disease outbreaks caused losses to the salmonid industry (González, 1998). There are also cases where native parasites occur in equal or higher numbers in introduced salmonids than in their native fish hosts (Kennedy et al., 1991). Whereas in New Zealand, introduced brown trout have been reported to lower the parasite burden of a native acanthocephalan species in native roundhead galaxias (*Galaxias anomalus*) and serve as an infection sink (Paterson et al., 2011). Whether invasive pink salmon affects the native parasite-host

dynamics of the Fennoscandian rivers by spillback, by acting as infection sinks, or by some other mechanism needs further study.

Recently, pink salmon were found infected with a parasite from the genus *Ichthyophonus* in the River Lakselva in Northern Norway (Erkinharju et al., 2024). This was the first detection of *Ichthyophonus* sp. in pink salmon in the North Atlantic Ocean, whereas in the North Pacific, the parasite is known to be prevalent in pink salmon. Monitoring the disease status and the numbers of rapidly increasing invasive pink salmon is essential not only for conserving native salmonid populations but also for protecting the health of cultured fish.

In conclusion, no viral diseases, *R. salmoninarum*, or *G. salaris* were detected in migrating pink salmon captured in the Rivers Tana and Neiden in northern Fennoscandia. Regarding these diseases, pink salmon appears not to pose a high infection risk to the native salmonid fish populations in these rivers. However, 23 % of the studied fish were shown to be infected with the nematode *H. aduncum*, indicating the potential of pink salmon to act as a carrier of salmonid pathogens. The number of migrating pink salmon in the Fennoscandian rivers is still increasing, thus increasing the potential risk of parasites and other pathogens spreading between fish species. Furthermore, information on the susceptibility of pink salmon to different pathogens and on the overall effect this invasive species has on native fish species is needed.

CRediT authorship contribution statement

Tuija Kantala: Writing – review & editing, Methodology. **Tiina Korkea-aho:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Riikka Holopainen:** Writing – original draft, Methodology, Funding acquisition, Conceptualization. **Satu Viljamaa-Dirks:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Panu Orell:** Writing – review & editing, Funding acquisition, Conceptualization. **Petra Heikkinen:** Writing – review & editing, Methodology. **Antti Oksanen:** Writing – review & editing, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Panu Orell and Riikka Holopainen report financial support was provided by Ministry of Agriculture and Forestry of Finland. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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