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SVA SWEDISH VETERINARY AGENCY

Report on Salmon health monitoring in the Torne River 2020–2024

Swedish Agency for Marine and Water Management

Centre for Economic Development, Transport and the Environment

Content

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Front page photo: river boats at Lappea, Torne River. *Photographer*: Marjukka Rask

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Summary

Concerns about diseased and dead salmon began in 2014 in Torne River, when salmon with symptoms like skin hemorrhages and moribundity was reported. Since then, the Swedish Veterinary Agency (SVA) and the Finnish Food Authority (FFA) have conducted health monitoring of Torne River salmon. Here we report the health studies of salmon populations in the Torne River 2020 - 2024 by looking at the overall health status of caught salmon including morphometric data, necropsy, blood- and other parameters, monitoring possible pathogens present and specifically investigating the causes of ventral erythema and hemorrhage, since 2019 known as red skin disease (RSD).

During these years, 473 salmon were caught for general sampling where overall health was investigated, including physical, blood, and tissue parameters. In addition, 36 salmon were subjected to targeted sampling due to disease.

Focus areas included investigating potential causes of red skin disease (RSD), identifying pathogens and other valuable parameters to collect for salmon health monitoring.

The overall condition of the Torne River salmon was good. For nearly half of the salmon in general samplings lesions, mainly mechanical injuries/scars and skin hemorrhage, were recorded. However, few cases of skin hemorrhage could be said with certainty not to have been caused by capture. When only symptomatic salmon were considered, water mold and skin hemorrhage were most common. No pathogenic bacterial or viral infections were detected, except that piscine orthoreovirus (PRV) was detected in one salmon. However, no signs of the related disease heart and skeletal muscle inflammation (HSMI) were found, suggesting that the salmon was a latent carrier of the virus. The skin hemorrhage syndrome Red skin disease (RSD) may involve an intracellular organism similar to the Midichlorian-like organism that causes red mark syndrome in rainbow trout, but its role remains unclear due to difficulties in culturing and identification of this organism. *Saprolegnia parasitica*, a pathogenic oomycete that causes water mold, was present in Torne River salmon and results indicate that it is especially problematic during autumn and might affect survival to spawning. Continued monitoring of PRV, potential MLO-like organisms and causes for water mold is recommended.

Non-lethal health indicators (morphometric measurements, visible lesions, blood chemistry and blood cell composition and morphology) were evaluated. Blood glucose and lactate were especially investigated due to deviations from set reference levels, however much of the variation is interpreted as caused by stress from capture and massive exercise rather than disease. No single parameter was deemed sufficient for health interpretation. Instead, a fixed set panel of variables should be used and is proposed for monitoring salmon health. This panel includes morphometric measurements, visual evaluation of health status and scoring of health status, blood chemistry and blood cell morphology. Additional samples for pathogen isolation and histopathology should always be taken in salmon with lesions, or optionally in non-symptomatic salmon. In addition, records of water temperature and water flow (in rivers), proximity to rapids etc. could be useful to aid in interpretation of blood chemistry values.

Background

The Torne River constitutes the border between Finland and Sweden, and it is the largest river in the Baltic Sea area where Atlantic salmon (*Salmo salar*) and other salmonids can reproduce naturally. The production capacity of the river has been estimated to be 1 318 000 smolts of salmon and sea trout (HELCOM, 2011). In 2012, the number of salmon returning to spawn increased, and peaked at approx. 100 000 individuals in 2014 and 2016 (**Figure 1**), whereafter the number of returners has fluctuated between 40 000- 95 000 individuals per year until a sudden drop occurred in 2023 and 2024, where numbers were extremely low compared to the previous 11 years (**Figure 1**).

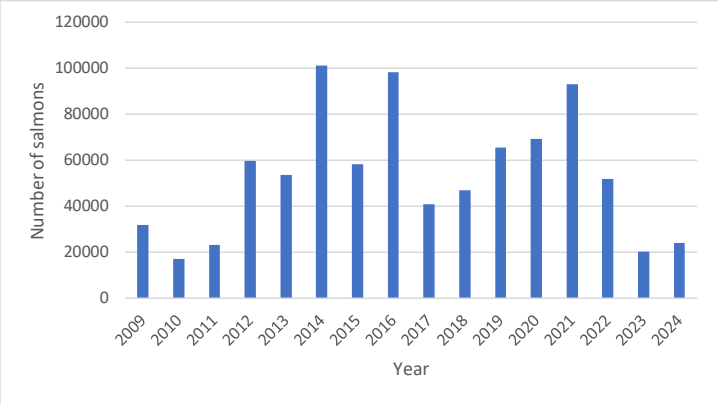

Figure 1. Number of salmon returning to the Torne River in 2009 – 2024, counted by sonar at Kattilankoski (Natural Resources Institute Finland. Kalahavainnot: kalahavainnot.luke.fi/fi/seurannat/tornionjoen-nousulohiseuranta/).

Concerns of an increasing amount of diseased and dead returning salmon were raised already in June-July 2014. The salmon were reported to have skin hemorrhage, wounds and water mold. This problem re-occurred the next year, and for 2016, the Swedish Veterinary Agency (SVA) and the Finnish Food Authority (FFA) received funding to investigate the matter. Since then, we have worked together to monitor the health of the salmon, and to some extent sea trout in Torne River. In 2016, sampling was performed to investigate the situation, with the aim to unravel pathology and any infectious cause (Axén & Koski 2017). Multiple factors affecting salmon health were identified. In diseased salmon, abdominal skin hemorrhage, UDN-like lesions, mechanical injuries and wounds of unknown origin were all described. Secondary infection with water mold, caused by *Saprolegnia parasitica*, other oomycetes or fungi were also found, and usually this complication is the reason for mortality. In a few salmon with wounds, different bacteria were detected, but a common pathogen causing hemorrhage or wounds could not be found. In salmon with skin erythema and hemorrhage, samples from a few salmon were sent for next generation sequencing, where short sequences of herpesviruses, iridoviruses and totiviruses were detected (Axén & Koski 2017; SVA, 2017). Similar lesions have been reported in aquaculture salmonids, for instance red mark syndrome (RMS) in rainbow trout. RMS is not known to cause high mortalities, but causes skin lesions, predisposing the trout to opportunistic infections, such as bacterial and water mold infections. The *Midichloria*-like organism (MLO) has been identified as a potential cause of RMS (Metselaar et al., 2020). In 2018, the association of different physiological parameters with the hemorrhaging skin syndrome was investigated (SVA, GU & SLU, 2019). Deviation in several physiological parameters were associated with being diseased (SVA, GU & SLU, 2019; Weichert et al, 2021), however no specific “driver” of disease was identified. Thiamine deficiency, which is associated with the M74 syndrome of salmonid fry and with adult salmon health around spawning

(Vuorinen et al. 2021), could not be identified. In 2019, at an international workshop in Oslo, the syndrome with ventral erythema and skin hemorrhage in salmon was named red skin disease (RSD). More research was needed to clarify causal pathways, and health surveillance has been continued to gather more data and set up a proper monitoring program.

Since 2020, SVA and FFA have annual surveillance of the Torne River salmon health. We monitor the population by looking at 1) the overall health status of caught salmon including morphometric data, necropsy, blood- and other parameters; 2) monitoring possible pathogens present and 3) specifically investigating the causes of RSD.

In addition, there is a reporting site for Sweden and Finland, where the public can report diseased or dead fish. All species of fish can be reported, but the site was originally set up in 2016 due to the salmon disease situation. The site is an important way for us to follow the Baltic salmon health. Adding pictures or short videos of the observed fish is optional but encouraged because it is important for proper interpretation of the situation. Adding contact information is also optional but encouraged because it allows us to get extra information if anything is unclear regarding the report. It is of course also useful if the reporter wants some feedback on what he/she has observed. The site can be accessed here: https://rapporterafisk.sva.se and from a Finnish IP-address, text will appear in Finnish. It has just come to SVA: s attention that the site has a bug that disables reporting from Finland. The issue is currently being worked on and reporting from the Finnish side should be possible again around mid-June. To enable a quick solution of the problem, any new text will appear in English at first and then be translated to Finnish as soon as possible.

Materials and methods

Sampling locations

Salmon samples were collected at different times and locations on both the Swedish and Finnish side of the river. Sampling locations are indicated in **Figure 2**. Samplings were conducted either as general samplings to estimate population health status or specifically aimed at salmon with disease symptoms.

Figure 2: Sampling locations used in 2020-2024. Blue dots show Finnish sampling sites; yellow dots show Swedish sampling sites.

Samplings

Sweden

SVA performed general samplings with the help from local fishermen, and in 2021 also with help from Vattenfall Vattenkraft AB. Every year, the aim was to sample 20 salmon upstream in Torne River and 20 salmon close to the estuary (in the archipelago) and in 2021, River Luleälven was sampled as a reference river only supporting restocking salmon. In 2020, repeated sampling was conducted, with one sampling in the early spawning run (mid-June) and one four weeks later. Upstream samplings were conducted at Risudden/Vitsaniemi, and the estuary samplings were conducted at Seskarö. Estuary sampling in River Luleälven was conducted at Lövskär and upstream sampling was conducted at the Boden hydropower dam. In the river, salmon were caught by drift net (Vitsaniemi) or in a fish elevator for the restocking facility (Boden) and in the archipelago by push up traps (Seskarö) or combi traps (Lövskär). In 2020-2022 the aim

was to keep the salmon alive from trapping to sampling, but due to technical difficulties this approach was abandoned in 2023 for estuary samplings. At Vitsaniemi the salmon was easier to keep alive until sampling.

The salmon was euthanized by a stunning blow to the head followed by exsanguination, using a heparinized syringe and needle to withdraw 10-20 ml of blood from the heart or from the caudal vein, and if necessary one or two gill arches were cut to facilitate exsanguination.

Necropsy was then conducted; photos were taken and external and internal abnormalities as well as intestinal parasite burden (*Eubothrium* sp.) were noted in the logbook. External abnormalities were scored as follows:

UDN-like (presence of non-mechanical skin necrosis on the head): 1) minimal and 2) significant to extensive

Skin erythema and hemorrhage: 1) erythema, 2) acute hemorrhage (possibly mechanical), 3) subacute hemorrhage 4) chronic/ healing hemorrhaging lesion

Mechanical injuries on the body: 1) scale loss from capture, 2) acute abrasions, 3) acute injury, 4) subacute injury, 5) healing injury, 6) scarring

Non mechanical injuries on the body: 1) superficial erosions, 2) scale loss (not from capture), 3) acute wounds, 4) subacute wounds, 5) chronic wounds, 6) lice

Fin damage: 1) erythema, 2) acute mechanical damage, 3) chronic mechanical damage, 4) fin rot, 5) healed injury.

Fungal infection: 1) initial/minimal, 2) significant/extensive

For inner abnormalities no scoring was done but the injuries were described in the logbook.

For parasite burden the scale 0) none, 1) minimal, 2) significant, 3) extensive, 4) massive was used

The biological parameters sex, length, weight, liver weight, gonad weight and the weight of the gastrointestinal package were registered. The condition factor (CF) was calculated according to Fulton: (somatic weight in grams x 100)/(length in cm)^3. The liver somatic index (LSI), gonadosomatic index (GSI) and gastrointestinal-somatic index (GISI) were calculated by (liver-/gonad-/gastrointestinal weight in grams/(somatic weight in grams x 100).

From all salmon, the following samples were collected: Blood for laboratory analyses (hematological and metabolic parameters and thiamine status) and for cytological evaluation of red and white blood cells (blood smear). Liver, kidney, spleen and heart for histopathology were placed in 10% formalin for fixation. In 2021, pyloric caeca were also fixed for histopathologic control of parasite status.

In 2021-2022, small pieces of kidney, heart, and spleen were taken and pooled per 10 salmon to Eagle's minimal essential medium (EMEM, LGC) with addition of 10 % FBS (fetal bovine serum) +1 % PS (penicillin - streptomycin) +1 % L-glutamine for virus isolation.

Targeted samplings were performed in symptomatic salmon accordingly: If UDN like changes or other skin lesions where present skin was sampled to formalin. In addition, heart and mid-kidney were sampled to RNAlater (Qiagen) for SAV analysis from five salmon. In 2021-2024, skin from salmon with ventral hemorrhage was also stored in RNAlater for MLO analysis. In 2022 dorsal (unaffected) skin was also taken from these individuals as reference material.

In individuals with suspected bacterial infection, samples for bacterial cultivation were taken from the affected organs (e.g. skin, spleen) and if general infection was suspected also from kidney.

In individuals with water mold, skin was sampled for oomycete/fungal cultivation.

Finland

In 2020, sampling was conducted by the FFA during Natural resources Institute Finland (Luke) capture of broodstock salmon and sea trout 11.6.-16.7. at the Torne River estuary, with the help of local fishermen and using fyke nets, and at Ylitornio 26.-27.6.2020, during regular drift net fishing by locals. From these samplings there are data on visible health status for all salmons, and sex, weight and length were recorded for all salmon at the Torne River estuary. Luke provided morphometric data of healthy salmon caught in fyke nets at Torne River estuary, while FFA collected data and samples mainly from symptomatic salmon. Targeted sampling was done in salmon with lesions. Samples were taken from spleen, kidney and heart to Minimal essential medium (MEM, Biowest) with 10 % FBS (fetal bovine serum) +2 % PS (penicillin - streptomycin) +1 % L-glutamine +1 % NEAA (non-essential amino acids), for virus isolation and/or to RNAlater for virological or bacteriological PCR analyses. Samples for bacteriology were taken from the kidney, spleen and heart. In salmon with water mold, samples were taken for oomycete/fungal isolation. Some salmon with skin lesions were sampled for bacterial cultivation. In addition, blood samples were taken from a total of 28 salmon for thiamine analysis.

Besides these samplings, the FFA performed sampling of symptomatic salmon which had hemorrhagic or other lesions during 2020-2024. Two salmon, who had no lesions on closer inspection, were also included. Samples were collected from the Torne River main branch and the Muonio River, the Bothnian Bay near the Torne River estuary and at the River Oulujoki estuary or in the river (**Figure 2)**. For this targeted sampling, help was requested from the public in 2020, 2021 and 2024. If they found diseased salmon or trout near the shore and could capture them by nets or by hand, the salmon was sent to FFA for disease investigations. In 2022 and 2023, symptomatic salmon were collected from fyke nets and lure fishing fishermen. In these two years, sampling was done in collaboration with a project (Härkönen et al., 2024), which was partly funded by the fishing license research fund (via the ELY-centre for Lapland). The symptomatic salmon were sampled as described above the FFA samplings at Torne River estuary and Ylitornio. The intestinal parasite burden was estimated in most of the symptomatic salmon. In addition, samples from skin, liver and any internal organs with lesions were taken to formalin for histopathological examinations.

Laboratory analyses

Blood

General blood parameters (Sweden)

A few drops of blood were used for measuring hematocrit (Htc) (HAEMATOKRIT 200, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany), hemoglobin concentration (Hb) (HemoCue® Hb 201+ System, HemoCue AB, Ängelholm, Sweden), glucose (HemoCue® Glucose 201 RT Analyzer, HemoCue AB, Ängelholm, Sweden) and lactate (Lactate Scout Vet, EKF Diagnostics GmbH, Barleben, Germany) within minutes from sampling.

Thiamine analysis

1 ml of whole blood was used for thiamin analysis and was directly frozen in an Eppendorf tube (SVA) or serum tube (FFA) on dry ice. Thiamine analysis (total thiamine, total free thiamine, thiamine monophosphate (TMP), and thiamine pyrophosphate (TPP)) was performed by FFA according to Koski et al., 1999.

Blood for research (SVA)

For the remaining blood, the plasma was separated from blood cells by centrifugation at 5 000 *x* g for 3 min. The plasma was immediately frozen on dry ice and for long term stored at -80ºC. The plasma was not further analyzed but stored for future research. In 2020 the separated erythrocytes were also frozen on dry ice for further analysis.

Detection of pathogens

Virus isolation on cell culture is performed routinely according to the Commission Delegated Regulation (EU) 2020/689 and the specific EU reference laboratory (EURL) diagnostic manual for hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV) at SVA and FFA. This methodology picks up a relatively wide spectrum of viruses beyond VHSV and IHNV, including infectious pancreatic necrosis virus (IPNV) and epizootic hematopoietic necrosis virus (EHNV). Briefly, prepared samples are inoculated on two cell lines (Bluegill fry (BF-2) and Fathead minnow (FHM) or BF-2 and Epitheloma Papulosum Cyprini (EPC)) and cultivated at 15°C for one week before passage to fresh cells for an additional week of culturing. The cells are inspected for the presence of a cytopathogenic effect (CPE), e.g. cell death induced by viruses, every three to four days. If CPE is noticed the type of virus is determined by an enzyme linked immunosorbent assay (ELISA) followed by quantitative Polymerase chain reaction (qPCR) or reverse transcriptase (RT)-qPCR for verification. Many viruses are also sequenced to determine genotype.

For viruses that are hard or impossible to cultivate, analyses were performed as follows. Analysis for Infectious salmon anemia virus (ISAV) was performed by RT-qPCR according to the EURL diagnostic manual at SVA (Swedish samples), FFA (some Finnish samples) or at the EURL at the Danish Technical University, Aquatic Department (DTU Aqua, some Finnish samples). Screening for salmonid alphavirus (SAV) was performed by RT-qPCR at SVA and FFA according to (Hodneland et al. 2006). Examination for the Salmon Gill Pox Virus (SGPV) by qPCR and for piscine myocarditis virus (PMCV) by RT-qPCR was performed at DTU Aqua. Screening for piscine orthoreovirus (PRV-1 and PRV-3) was performed by RT-qPCR at DTU Aqua in 2020-2021. In 2022 and 2023 screening for PRV-1, PRV-2 and PRV-3 was performed by RT-qPCR in the FFA (Zhao et al. 2021).

Bacterial cultivation was done using horse blood and tryptone yeast extract salts (TYES) agar plates incubated at 20°C for up to five days (SVA) and by using sheep blood agar, Shotts-Waltman agar at 20°C and TYES agar at 15°C (FFA) for up to seven days. If a mixed culture was obtained, a secondary culture was set, only picking bacterial colonies identified as significant from the primary agar plate. From pure primary or secondary cultures, bacterial species was determined by biochemical analysis and/or MALDI-TOF. Analysis for bacterial kidney disease (BKD) was done from kidney samples by cultivation on selective kidney disease medium (SKDM) agar at 16,5°C for 6 weeks or by PCR (FFA). Some of the FFA examinations for BKD were done by DTU Aqua by qPCR. Analysis for *Piscirickettsia salmonis*, the causative agent of Salmonid rickettsial septicemia (SRS) was done by PCR in DTU Aqua. MLO analysis was performed by qPCR at DTU Aqua.

Apparent or potential water mold (oomycetes/fungal infections) were cultivated on peptone glucose (PG-1) agar (SVA) or Sabouraud agar (FFA) at 15°C for 7-14 days. In 2020-2022, FFA analysed cultivated mycelium from two salmon to species level by sequencing (Engblom et al. 2023). In 2023, species was determined by qPCR followed by conventional PCR and sequencing of the ITS gene region to determine species of fungus or oomycete (Härkönen et al., 2024). SVA used a qPCR for the ITS region to determine presence of *Saprolegnia* sp. or *S. parasitica* according to Rocchi et al. (2017) and an in-house qPCR for *S. diclina* if Saprolegnia-like mycelium had grown on the agar plates.

Some of the intestinal parasites found were identified by ITS sequencing in FFA. Parasite DNA was extracted using Qiagen's Mini Kit and the sequence of the ITS1-5.8S-ITS2 region was amplified using the PCR method (Zhu et al., 1998). The sequence obtained was compared with Genbank data using BLAST (Nucleotide BLAST: Search nucleotide databases using a nucleotide query).

Histology and cytology

After fixation the tissue pieces were cut into thin slices and placed into cassettes for embedding, slicing and staining. The heart was, if possible, cut through the middle plane from apex to base to enable evaluation of

both ventricle, valves, atrium, and bulbus arteriosus. At SVA, skin tissues were decalcified before embedding due to the presence of scales (body) and underlaying cartilage/bone (snout). The tissues were embedded in paraffin, sliced, mounted onto cover slides, and stained using Hematoxylin Eosin according to standard procedures at our histopathology laboratories. The sections were observed using 40 – 1 000 *x* magnification.

The blood smears were fixed in methanol and stained using Giemsa or Diff-quick and analyzed at 400-1 000 *x* magnification.

In depth investigations about associations between health and measured variables

A health index (HEALTH1) was generated based on visible external lesions in salmon from the general samplings performed by SVA. Salmon were scored according to a five-degree scale:

1: healthy
2: mildly affected health
3: moderately affected health without signs of secondary fungal/oomycete infection
4: moderately to severely affected health with early fungal/oomycete infection
5: extensive fungal/oomycete infection, severe/terminal illness

To reduce the number of classes, and see if there was any difference between salmon with mechanical injuries or those with other lesions (hemorrhage, other wounds, severe water mold infection) the index HEALTH2 was also created and scored accordingly:

1: healthy
2: mechanical injuries
3: disease

Salmon that only had acute mechanical lesions or erythema/acute hemorrhage were classified as healthy under both HEALTH1 and HEALTH2, because the lesions were not suspected to have affected health (yet). The two variables were then used to investigate the association between visible health/disease and measured variables. In HEALTH2, score 2 (mechanical injuries) were used for mild water mold infections because these were not deemed yet to have caused more problems than the mechanical injury itself. Examples for scoring can be found in **Appendix 1**.

Statistical analysis

Data was transferred to Stata 15 (StataCorp, Texas) or IPM SPSS Statistic software for statistical calculations and to GraphPad for visualization of data distribution. The Wilcoxon rank-sum test was mainly used for comparison, as this allows both for normally and non-normally distributed population data.

Simple/univariate regression analysis and multivariate regression analysis were also performed for in-depth analysis of some variables. Prior to multivariate regression, each potential variable was explored in univariate regression analysis. A $p<0.2$ was set as the required significance for a variable to be included in multivariate regression. Interaction between included variables was investigated. Post modelling analysis was done by variance estimations of included variables, likelihood ratio tests, information criterions etc.

Results

All the salmon sampled from Torne River or Bothnian Bay near to the Torne River estuary or Oulujoki River estuary or River Oulujoki River were salmon, except one sea trout was sampled from Oulujoki River estuary. In total, 473 salmon were caught during the SVA samplings and FFA samplings at the Torne River estuary and at Ylitornio (2020), and 35 salmon and one sea trout were collected for targeted sampling (FFA). In addition, a fungal culture was collected from a diseased sea trout that was accidentally caught during the SVA sampling at Seskarö in 2023, but no notes were taken for morphometry etc.

Samplings with data on both healthy and diseased salmon

For samplings where both healthy and diseased salmon was assessed (SVA samplings, FFA samplings at Torne River estuary and Ylitornio in 2020); sampling times, location, capture method, number of salmon and a summary of morphometric parameters can be seen in **Table 1**. In total, 473 salmon were investigated, 252 by SVA and 221 by FFA. For the samples from Ylitornio length and CF are lacking, as well as weight for all but three salmon. Females dominated in all samplings except in the SVA July sampling 2020. The salmon in this sampling was also significantly smaller than in all other SVA samplings ($p<0.001$), and the salmon from the 2024 sampling was significantly smaller than the salmon caught in 2022 and 2023 ($p<0.05$). The salmon in the June 2021 and 2022 samplings had lower CF compared to the other samplings ($p=0.051$ – $p<0.001$), and the fattest salmon was caught in 2023 ($p<0.05$ – $p<0.001$ compared to all other samplings). Two females sampled by SVA (one in 2020 and one in 2023), and one male sampled by FFA (2020) were kelt (overwintered spawners).

Table 1. Information on samplings in 2020 – 2024, where both healthy and symptomatic salmon have been assessed, including summarized morphometric data.

Sampling	SVA/FFA	Location	Capture method	N	Sex (male/female)	Length, cm median (interval)	Weight, kg median (interval)	Condition factor median (interval)
2020 June	SVA	Seskarö	PU trap	19	13/15	88 (73-120)	7.56 (2.74-23.16)	1.09 (0.65-1.34)
2020 June	SVA	Vitsaniemi	Driftnet	9				
2020 June	FFA	Ylitornio	Driftnet	47	14/32[2]	NA	14.9 (12.20-15.30)[3]	NA
2020 June-July	FFA	Torne R. estuary	Fyke nets	174	78/96	83 (55-113)	5.95 (1.70-16.70)	1.01 (0.73-1.24)
2020 July	SVA	Seskarö	PU trap	20	35/5	62 (48-123)	2.61 (1.10-17.92)	1.04 (0.89-1.42)
2020 July	SVA	Vitsaniemi	Driftnet	20				
2021 June	SVA	Seskarö	PU trap	20	9/31	92 (71-122)	6.84 (2.80-19.11)	0.98 (0.79-1.36)
2021 June	SVA	Vitsaniemi	Driftnet	20				
2021 July[1]	SVA	Lövskär	Combi trap	20	10/30	93 (73-107)	8.41 (4.05-13.45)	1.02 (0.87-1.31)
2021 July[1]	SVA	Boden	Elevator	20				
2022 June	SVA	Seskarö	PU trap	20	11/29	93 (74-117)	8.03 (3.70-16.96)	0.94 (0.72-1.16)
2022 June	SVA	Vitsaniemi	Driftnet	20				
2023 June	SVA	Seskarö	PU trap	11	10/21	99 (70-117)	10.10 (4.06-20.60)	1.13 (0.69-1.50)
2023 June	SVA	Vitsaniemi	Driftnet	20				
2024 June	SVA	Seskarö	PU trap	13	2/31	86 (68-116)	6.94 (3.40-18.16)	1.07 (0.93-1.17)
2024 June	SVA	Vitsaniemi	Driftnet	20				
Total				**473**	**182/290**[2]	**87 (48-123)**[4]	**6.70 (1.10-23.16)**[5]	**1.03 (0.65-1.50)**[4]

N=Number of salmon; NA=Not Applicable; PU=Push up; [1] River Luleälven; [2] sex not recorded for one salmon; [3] Weight recorded for three salmon; [4] data from 429 salmon; [5] Data from 426 salmon

External lesions

Data on observed external lesions during general samplings are summarized in **Table 2**. For erythema and hemorrhage, acute lesions have been included in the table because it cannot be determined whether they stem from capture or were early signs of red skin disease (RSD). For mechanical wounds, those that were deemed to be caused by capture have been left out of the table. Fin damage was included under mechanical lesions, as (potential) bacterial fin rot was rarely observed. Scars were included in the wound statistics, both for mechanical and other wounds. "Other wounds" include non-mechanical wounds, UDN-like lesions and potential red vent syndrome (RVS/*Anisakis simplex* infection at the cloaca).

Table 2. External lesions observed during samplings conducted 2020-2024, where both healthy and symptomatic salmon have been assessed. Each salmon in the "Total N (%) with lesions" column can be represented in one or more of the following columns.

Sampling	SVA/ FFA	Location	N	Total N (%) with lesions	Skin hemorrhage[1] N (%)	Mechanical injury[2] N (%)	Other wounds[3] N (%)	Water mold N (%)
2020 June	SVA	Seskarö	19	10 (53)	2 (10)	8 (42)	1 (5)	1 (5)
2020 June	SVA	Vitsaniemi	9	4 (44)	2 (22)	2 (22)	1 (11)	1 (5)
2020 June	FFA	Ylitornio	47	17 (36)	6 (13)	9 (19)	7 (15)	0
2020 June-July	FFA	Torne R. estuary	174	56 (32)	39 (22)	38 (22)	8 (5)	3 (2)
2020 July	SVA	Seskarö	20	9 (45)	2 (10)	7 (35)	3 (15)	0
2020 July	SVA	Vitsaniemi	20	3 (15)	0	3 (15)	0	0
2021 June	SVA	Seskarö	20	9 (45)	3 (15)	7 (35)	0	2 (10)
2021 June	SVA	Vitsaniemi	20	8 (40)	5 (25)	5 (25)	0	2 (10)
2021 July[4]	SVA	Lövskär	20	11 (55)	6 (30)	7 (35)	2 (10)	0
2021 July[4]	SVA	Boden	20	10 (50)	7 (35)	2 (10)	2 (10)	0
2022 June	SVA	Seskarö	20	5 (25)	1 (5)	4 (20)	0	0
2022 June	SVA	Vitsaniemi	20	12 (60)	9 (45)	6 (30)	0	1 (5)
2023 June	SVA	Seskarö	11	2 (18)	1 (9)	0	1 (9)	0
2023 June	SVA	Vitsaniemi	20	14 (70)	11 (55)	6 (30)	2 (10)	2 (10)
2024 June	SVA	Seskarö	13	11 (85)	11 (85)	7 (54)	3 (23)	0
2024 June	SVA	Vitsaniemi	20	11 (55)	7 (35)	5 (25)	3 (15)	0
Total			**473**	**194 (41)**	**112 (24)**	**116 (25)**	**33 (7)**	**10 (2)**

[1] Includes erythema and acute hemorrhage that could be caused by capture; [2] Includes scars and fin erythema/ damage, excludes injuries deemed to be caused by capture; [3] Includes UDN-like necrosis, RVS and scars; [4] River Luleälven

Skin hemorrhage and mechanical injuries were the most common findings in samplings presented in **Table 2**. However, of the 67 cases of erythema and hemorrhage registered by SVA, only seven were subacute (e.g. **Figure 3E**), meaning they were more than a few days old and certainly not caused by capture. There is no information available about the stage (acute/subacute/chronic) of hemorrhage in the salmon sampled by FFA. Of the 69 salmon sampled by SVA with mechanical wounds, 15 only had scars (**Figure 3A**) and 17 had wounds that were nearly healed (**Figure 3B**), whereas scarring is only noted for one salmon from the FFA estuary sampling. Many of the mechanical wounds registered were caused by seals, but there were also wounds caused by hooking (**Figure 3C**). In addition, FFA registered collar-like lesions deemed to be caused by nets around the head in 11 salmon from the estuary and 2 from Ylitornio. Of these, eight lesions were old and five were fresh. SVA registered two old collar-like lesions in 2021, one at Seskarö (**photo 4, Appendix 1**) and one at Lövskär.

Figure 3. A) Healed bite wounds. **B)** Almost healed bite wound where the cloaca has been torn, and reactive hyperplasia of the intestinal mucosa has occurred during healing. **C)** Chronic inflammation after escape from hooking, where the line got entangled in the pelvic fin. **D)** Carlin tag embedded in the dorsal muscle. **E)** Subacute skin hemorrhage (RSD) in kelt female. **F)** Old wound of unknown (potentially lamprey) origin, with early *Saprolegnia* infection (brown discoloration). **G)** Deep UDN-like necrosis and *Saprolegnia* infection on the head of a sea trout.

Two salmon sampled by SVA in 2020 had been tagged using Carlin tags. One only had deep scars left, but in the other, the tag was still in place, embedded in the dorsal muscle (**Figure 3D**). The tag was from Finnish restocking, and the salmon was of Torne River origin. This is also likely the origin of the other salmon, since Sweden abandoned Carlin tagging about 10 years ago.

UDN-like lesions/Skin necrosis on the head were identified in three salmon, one each from the Seskarö samplings in 2020 and one from the Boden sampling in 2021, and in addition in a sea trout that was accidentally caught in the Seskarö trap in 2023 (**Figure 3G**). Potential red vent syndrome (*Anisakis simplex* infection at the vent/anus) was identified in seven salmon (SVA). The other wounds were of unknown origin.

The kelt females both had skin hemorrhage and early water mold infections, whereas the kelt male had no lesions.

Lesions registered as water mold (N=9) by SVA and by FFA (N=3) were extremely mild (**Figure 3F**), except for one sea trout that SVA did not include in the general sampling but collected a sample for oomycete cultivation from (**Figure 3G**).

HEALTH was scored in 251 of the 252 salmon sampled by SVA. For one salmon sampled in 2024, photo documentation was lacking, and thus health status could not be verified although no notes on lesions except for acute ventral hemorrhage were taken in the logbook. No salmon was scored as HEALTH1 5, i.e. severe/terminal illness with massive saprolegniosis. The sex representation in HEALTH categories were as follows:

HEALTH1 Score	N	Males (% of all males)	Females (% of all females)
1	166	51 (57.3)	115 (71.0)
2	62	31 (34.8)	31(19.1)
3	14	6 (6.7)	8 (4.9)
4	9	1 (1.1)	8 (4.9)
5	0	0	0
HEALTH2 Score			
1	166	51 (57.3)	115 (71.0)
2	46	19 (21.3)	27 (16.7)
3	39	19 (21.3)	20 (12.3)
Total	251	35.7 (of all 252 salmon)	64.3 (of all 252 salmon)

A larger proportion of females (115 of 162, 71%) were scored as healthy (HEALTH1/2 score 1) compared to males (51 of 89, 57.3%). For HEALTH1 score 2-4 and HEALTH2 score 2-3 it was only in HEALTH1 score 4 that females had a larger proportion compared to males.

Internal lesions

The most common observation in the abdominal cavity was parasites. Tapeworms (*Eubothrium* sp.) in the intestinal pyloric caeca region were present in every salmon, from a few worms to massive amounts. In two salmon, Anisakid worms, most probably *Contracaecum osculatum* (seal worm), were found in the liver. Cysts, probably caused by parasitic larvae (although no larvae were seen) were found in four spleens and on one intestine.

Melanisation of the adipose tissue or peritoneum was seen in 11 salmon. This is indicative of an immune reaction, usually to parasites or viral infections such as infectious pancreatic necrosis and pancreas disease.

Internal hemorrhages were observed in the liver of four salmon and in the ovary of one salmon. The ovary lesion is most likely due to an injury during capture. The spleen was enlarged in 10 salmon and extremely fragile in five salmon.

The kelt females lacked peritoneal fat deposits, indicating that they were emaciated. There is no information about the fat deposits of the kelt male, but they can be supposed to be lacking or extremely low.

In addition, in the River Luleälven salmon, 9 out of 40 salmon had heart abnormalities in form of a displaced bulbus arteriosus, which also led to an abnormal positioning of the heart within the heart cavity (**Figure 4**). In a follow-up sampling in October for another project, six salmon were found to have the same mailformation. Most of the salmon were estimated to be 2 sea-winter fish, and fin clips were sent for DNA analysis at the Swedish University of Agricultural Science to check for kinship. None of the salmon were siblings.

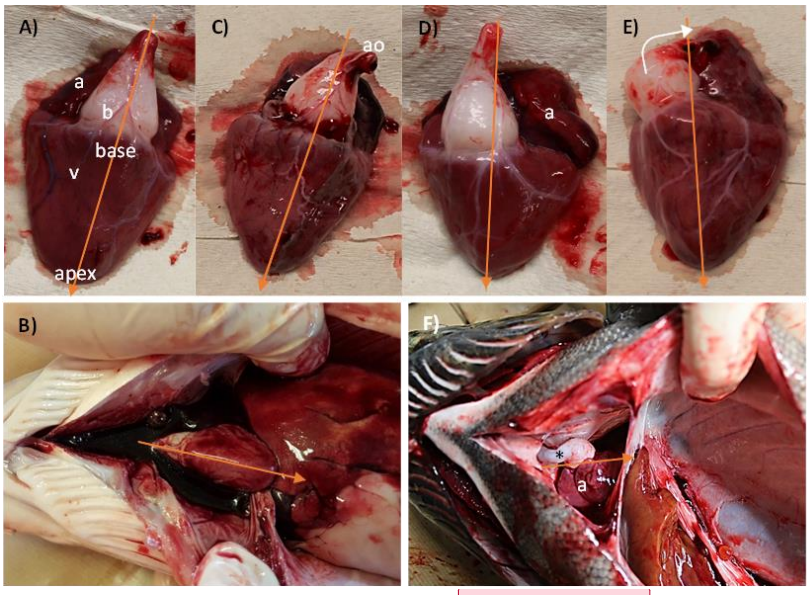

Figure 4. Heart abnormalities identified in River Luleälven salmon in 2021. The yellow arrows indicate the natural longitudinal base to apex-axis of the heart. All views are from the ventral/abdominal side. **A)** & **B)** Normal heart, where the ventricle (v), bulbus arteriosus (b) and atrium (a) are aligned. **C)** Minor malformation, with normal axis except that the transition between the bulbus arteriosus and the aorta (ao) is bent. **D)** Right displacement of the bulbus arteriosus at the heart base, with a slight bend at the aortic transition, and exposing the slightly left displaced atrium. **E)** Right displacement of the bulbus arteriosus with severe bend at the aortic transition (white arrow) and exposing the slightly left displaced atrium. **F)** Rotated position of the heart in the heart cavity, exposing the bent aorta (*) and the atrium (a), whereas the apex has been shifted dorsally.

Kommenterad [CA1]: Fix colour of arrows

Sampling of symptomatic salmon

In total, 35 salmon and 1 sea trout with visible lesions were sampled by FFA (**Table 3**) in addition to sampling of symptomatic and healthy fish. In 2020 and 2021 most of the symptomatic salmon was received from the public late in the autumn, while in 2022 and 2023 salmon with symptoms were collected from fyke net and lure fishing catches between June and August. The condition factor of symptomatic salmon was significantly lower in 2020 and 2021 when compared to condition factors in years 2022 and 2023 ($p<0.05$). Also, a difference in sex distribution was found between sampling years, where more females were studied in years 2020 – 2021 and more males were studied in 2022.

Table 3. Targeted samplings conducted in symptomatic salmon and sea trout by FFA in 2020 – 2024.

Sampling	Locations	Capture method	N	Sex (male/female)	Length, cm median (interval)	Weight, kg median (interval)	Condition factor median (interval)
2020 July, October, November	Tornio, River Oulujoki Estuary	net	8	1/6[1]	90 (11 – 110)	6 (1.7-10.6)[3]	0.79 (0.65 – 0.86)[3]
2021 June, October, November	Torne River and River Oulujoki estuaries, Tornio, Kolari, Pello	NA	12[2]	3/9	92 (54-110)	6.8 (1.5-14.0)	0.80 (0.66-1.11)
2022 June, August	Torne River estuary, Tornio, Kolari, Pello	Fyke nets or lure fishing	8	6/2	112 (86-117)	14 (6.7 – 16.5)	1.01 (0.89-1.26)
2023 June-August	Torne River estuary, Pello, Kolari, Muonio	Fyke nets or lure fishing	7	3/4	91 (82-103)	7.14 (5.08-14.7)	1.03 (0.83-1.23)
2024 August	Pello	NA	1	1/0	93	NA	NA
Total			**36**	**14/21**	**92 (11-117)**	**6.97 (1.5-16.5)**	**0.91 (0.65-1.26)**

N=Number of salmon; NA=Not Applicable; [1] sex not determined for one smolt; [2] includes one sea trout; [3] weight and CF not determined for one smolt.

Lesions were recorded for almost all the symptomatic fish (**Table 4**). Most commonly the lesions in fish were skin hemorrhage and/or water mold. Hemorrhage was detected on ventral skin and/or around the fin bases. Wounds and mechanical injuries were only recorded from three and two symptomatic salmon, respectively. The mechanical injuries were most likely caused by seals. Other wounds were usually necrotic lesions on skin or fins. One salmon had collar-like lesions on the skin because of scale loss from that area, which could have been caused by net injury.

Water mold was seen in 21 symptomatic fish in 2020, 2021 and 2023 (**Table 4**). In 2020, one smolt sampled in July had water mold on the gills and the symptomatic salmon sampled in October and November were heavily infected in skin and fins. In 2021, salmon sampled in June had mild water mold lesions, while salmon and one Sea trout sampled in November were heavily infected. Salmon sampled in June and August in 2023 had mild water mold lesions.

Of the symptomatic fish, 24 were inspected for intestinal tapeworms, and 19 (79 %) had tapeworms (*Eubothrium* sp.) in the pyloric caeca region of the intestine. The estimated tapeworm burden was most often high or very high. One salmon, which was caught from River Oulujoki, had an Ascarid nematode in the liver and was confirmed as *Contracaecum osculatum*.

Table 4. Lesions recorded in symptomatic salmon and sea trout studied by FFA in 2020 – 2024. Each salmon in the "Total N (%) with lesions" column can be represented in one or more of the following columns.

Sampling	Locations	N	Total N (%) with lesions	Skin hemorrhage[1] N (%)	Mechanical injury[2] N (%)	Other wounds[3] N (%)	Water mold N (%)
2020 July, October, November	Oulo River Estuary, Tornio	8	8 (100)	3 (37,5)	1 (12.5)	1 (12.5)	8 (100)
2021 June, October, November	Torne River and Oulo River estuaries, Tornio, Kolari, Pello	12[4]	12 (100)	6 (50)	0	2 (16.7)	9 (75)
2022 June, August	Torne River estuary, Tornio, Kolari, Pello	8	7 (88)	7 (88)	1 (12.5)	0	0
2023 June-August	Torne River estuary, Pello, Kolari, Muonio	7	6 (85.7)	2 (28.6)	0	1 (14.3)	4 (57.1)
2024 August	Pello	1	1	1	0	0	0
Total		**36**	**34 (94)**	**19 (53)**	**2 (6)**	**4 (11)**	**21 (58)**

[1] Includes erythema and acute hemorrhage that could be caused by capture; [2] Includes scars and fin erythema/ damage, excludes injuries deemed to be caused by capture; [3] Includes UDN-like necrosis, red vent syndrome and scars; [4] Includes one sea trout

Laboratory analyses

Virology

Viral examinations for IHNV, IPNV, VHSV, SAV, ISAV and PMCV were all negative. 25 salmon were examined for PRV (**Table 5**). Of these, one salmon caught in the Bothnian Bay near the Torne River estuary in 2023 was PRV positive.

Bacteriology

Bacterial culture was made from 37 salmon and specific bacterial growth was found in only seven salmon, three salmon that were caught from Torne River and four reference salmon that were caught in River Oulujoki or in the Bothnian Bay. All seven had *Iodobacter limnosediminis* infection.

Twenty-four salmon and the sea trout were examined for BKD. All results were negative.

56 salmon were examined for Midichloria-like organisms (MLO) by qPCR. All samples were negative for MLO, but 24 samples had unspecific amplification of DNA. Further investigations by DTU Aqua showed that the so-called melting curves in the amplification process peaked at the same temperature but at another temperature than the peak of the MLO melting curve (**Figure 5**), indicating presence of identical DNA in the salmon samples, but different DNA than in MLO samples. The qPCR and melting curve results together indicate that there is an organism, potentially related to MLO, present in the samples. We call this an MLO-like organism from here on in this report.

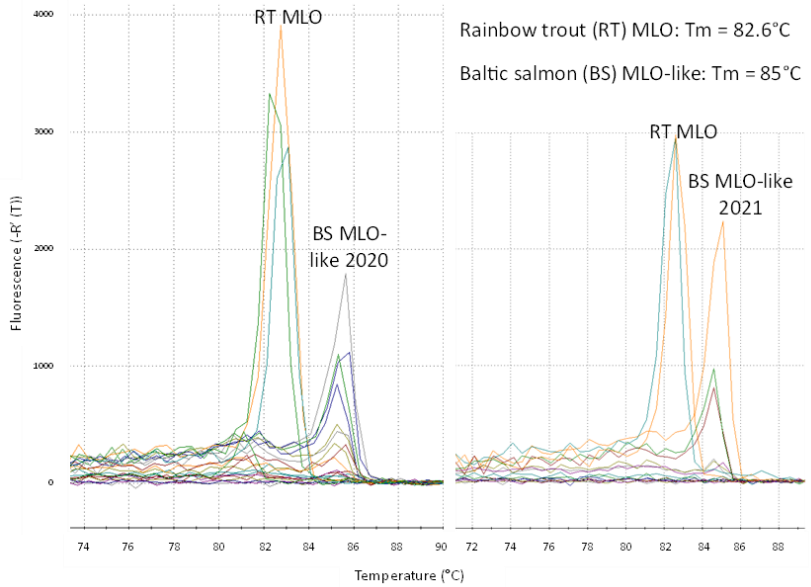

Figure 5. Melting curves for MLO samples and our samples. DNA from MLO has a melting temperature of 82.6°C whereas DNA from the MLO-like organism has a melting temperature of 85°C. RT=rainbow trout; BS=Baltic salmon, Tm=melting temperature.

Water mold

Five of the six suspected or mild water mold cases from SVA's samplings were investigated further. In addition, one sample was collected from a sea trout with massive skin necrosis and water mold, that was also caught in the Seskarö trap in 2023. In 2021, only qPCR for *Saprolegnia* sp. was performed (N=3) and no infection was detected. In 2022 and 2023, cultivation followed by qPCR was performed, identifying one case of *S. diclina* (2022), two cases of *S. parasitica* (2023, including the sea trout) and one case with undetermined *Saprolegnia* sp. From 21 symptomatic salmon with water mold (**Table 4**) in the FFA samplings, seven were analyzed (**Table 5**). In 2020 water mold isolated from two salmon were determined as *Saprolegnia parasitica* (Engblom et al. 2023). In 2023 all four investigated water mold isolates tested negative for *S. parasitica* by qPCR and were instead specified as *Didymella* sp., *Mucor* sp., *Pithomyces* sp., and *Cladosporium* sp. by ITS sequence analysis (Härkönen et al. 2024).

Table 5. Number of samples and laboratory diagnostic results for pathogen detection in 2020-2024. Results are shown as number of positives/total number of samples.

Year	Sampling	Symptomatic salmon (N)/total	Virology						Bacteriology				Water mold[4]
			Cultivation[1]	SAV	ISAV	PRV	SGPV	PMCV	Cultivation	BKD	MLO-like[2]	SRS	
2020	SVA	26 / 68	-	-	-	-	-	-	-	-	-	-	-
2020	FFA Y	8 / 62	0/20	0/20	0/3	-	0/3	-	0/6	-	1/3	-	-
2020	FFA E	19 / 47	0/6	0/6	0/3	-	0/3	-	0/6	-	2/3	-	-
2020	FFA T	8/8	0/4	0/4	0/3	0/3	0/2	0/3	3/8	0/2	4/6	0/3	2/2
2021	SVA To	26 / 40	0/4	0/5	-	-	-	-	-	-	2/6	-	0/3
2021	SVA L	31 / 40	0/4	-	-	-	-	-	-	-	5/7	-	-
2021	FFA T	12/12[3]	0/12	0/12	0/12	0/11	-	-	4/8	0/11	3/11	-	-
2022	SVA	17 / 40	0/4	-	-	-	-	-	0/1	-	0/11	-	1/1
2022	FFA T	7/8	0/5	0/5	0/2	0/5	-	-	0/1	0/5	0/8	-	-
2023	SVA	16 / 31	-	-	-	-	-	-	-	-	7/7	-	3/3
2023	FFA T	6/7	0/5	0/5	0/5	1/5	-	-	0/6	0/6	0/4	-	0/4
2024	SVA	22 / 33	-	-	-	-	-	-	-	-	?/7 - RP	-	-
2024	FFA T	1/1	0/1	0/1	0/1	0/1	-	-	0/1	0/1	-	-	0/1
Total		**182**	**0/65**	**0/58**	**0/29**	**1/25**	**0/8**	**0/3**	**7/37**	**0/25**	**24/56**	**0/3**	**6/14**

Y=Ylitornio sampling; E=estuary sampling; T=Targeted sampling; To=Torne River; L=River Luleälven; RP=results pending; [1] IHNV, IPNV, VHSV, EHNV; [2] MLO-like organism identified by PCR targeted at detecting MLO; [3] Includes four salmon and one sea trout from River Oulujoki estuary and upstream in the river; [4] Analyzed by cultivation, qPCR and/or sequencing.

Blood analysis

A summary of Hemoglobin (Hb), hematocrit (Htc), glucose, lactate and thiamine results are presented in **Table 6**. Hemoglobin and hematocrit were analyzed for all years, glucose was analyzed in 2020-2021 and 2023-2024, lactate was analyzed 2020 and 2024, and finally thiamine was analyzed all years.

Table 6. Summary results of blood analyses in Torne River and River Luleälven salmon 2020 – 2024.

Sampling	SVA/FFA	N	Hb (g/L) median (min, max)	Htc (%) median (min, max)	Glucose (mmol/L) median (min, max)	Lactate (mmol/L) median (min, max)
2020 June	SVA	28	126 (81, 158)	53 (37, 62)	3.7 (0.6, 10.0)[1]	14.8 (10.6, 18.9)
2020 July	SVA	40	120 (52, 159)	50 (36, 66)	4.4 (0.4, 9.8)	14.3 (1.5, 19.4)
2021 Torne	SVA	40	113 (64, 143)	51.5 (28, 62)	3.2 (1.1, 7.4)	-
2021 Lule	SVA	40	113 (84, 125)[2]	48.0 (38, 71)	6.0 (2.7, 12.9)	-
2022	SVA	40	131 (80, 162)	53.5 (41, 66)	-	-
2023	SVA	31	120 (89, 147)	49 (40, 65)	3.7 (0.9, 6.8)	-
2024	SVA	33	109 (64, 149)	55 (41, 87)	2.0 (0.6, 7.2)	12.8 (6.3, 18.1)
Total			**118 (52, 162)**	**51 (28, 87)**	**3,8 (0,4 - 12,9)**	**13.9 (1.5, 19.4)**

Sampling	SVA/FFA	N	Ttot nmol/g median (min, max)	TPP nmol/g median (min, max)	TMP nmol/g median (min, max)	Tfree nmol/g median (min, max)
2020 June	SVA	24	0.90 (0.57, 1.60)	0.68 (0.43, 1.04)	0.16 (0.09, 0.38)	0.07 (0.03, 0.18)
2020 June-July	FFA[3]	28	0.73 (0.52, 1,43)	0.37 (0.28, 0.62)	0.31 (0.17, 0.58)	0.08 (0.04, 0.28)
2020 July	SVA	39	0.80 (0.45, 1.11)	0.61 (0.32. 0.85)	0.14 (0.08, 0.24)	0.06 (0.03, 0.12)
2021 Torne	SVA	40	1.03 (0.26, 1.53)	0.76 (0.22, 1.15)	0.18 (0.04, 0.63)	0.07 (0, 0.12)
2021 Lule	SVA	30	0.96 (0.32, 1.62)	0.61 (0.27, 1.05)	0.19 (0.05, 0,40)	0.10 (0, 0.29)
2022	SVA	40	1.06 (0.51, 1.51)	0.75 (0.39, 1.07)	0.20 (0.08, 0.38)	0.07 (0.03, 0.15)
2023	SVA	31	1.12 (0.63, 1.54)	0.81 (0.49, 1.09)	0.19 (0.10, 0.28)	0.10 (0.04, 0.19)
2024	SVA	33	0.96 (0.65, 1.38)	0.71 (0.50, 1.08)	0.18 (0.11, 0.29)	0.08 (0.05, 0.20)

[1] Values from 27 salmon; [2] Values from 37 salmon; [3] Samples from both Torne River estuary (N=13) and Ylitornio (N=15); Hb=hemoglobin; Htc=hematocrit; Ttot=total thiamine; TPP=thiamine pyrophosphate; TMP=thiamine monophosphate; Tfree=free thiamine

For both hemoglobin and hematocrit, there was an annual variation in measured levels. Hemoglobin could be measured in 248 of the 252 salmon sampled by SVA and varied from 52 g/L to 162 g/L, with an overall median value of 118 g/L (**Table 6, Figure 6A**). This is in accordance with previously published values for salmon (approx. 60-160 g/L at 8°C water temperature, Porter et al., 2022) but much lower than in another publication (95% of population interval 408-687 g/L in adult salmon, Rozas-Serri et al., 2022). Hematocrit could be measured in all salmon sampled by SVA. The hematocrit varied from 28 to 87%, with a median value of 51% (**Figure 6B**). This is a larger span than what has been published for adult farmed salmon, and our median is close to or above the reported limits (40–60 % (Currie et al., 2022); approx. 22–38 % (Porter et al., 2022); 29–53 % (Rozas-Serri et al., 2022); 44–49 % (Sandnes et al., 1988)).

There are no published cut-off values for hemoglobin regarding anemia in fish. For hematocrit, Currie et al. (2022) states that veterinarians use 25% as a cut off for salmon but found that hematocrit values in clinical anemic cases in Scotland ranged from 25-35%. As the clinical signs of disease in these cases could also be related to other factors such as infection, 30% is used as cut-off by us. One salmon sampled at Vitsaniemi in

2021 was anemic based on an hematocrit value of 28% (**Figure 6B**). There is also a cut-off for severe anemia, at hematocrit ≤ 10% (Anonymous, 2016).

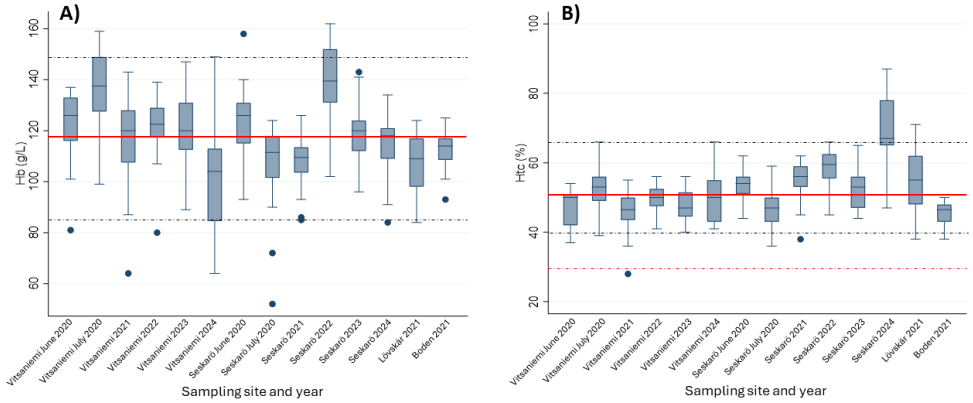

Figure 6. Box and whiskers plots for: **A)** Hemoglobin (Hb) in grams per liter (g/L) and **B)** Hematocrit (Htc) in % packed cell volume for all individuals. Solid red lines indicate median and dashed black lines show the range of values for 95% of the sampled population. Dashed red line in **B)** indicates cut-off for anemia. Note that the Y-axis does not start at 0, but at approx. 50 g/L (Hb) and 18% (Htc).

Glucose was analyzed in a total of 211 salmon and ranged from 0.4 - 12.9 mmol/L (**Table 6**), which is a larger variation than the published normal range for 95% of adult salmon (4.0-7.3 mmol/L, Rozas-Serri et al., 2022). The overall median value was 3.8 mmol/L (**Figure 7A**), which thus is slightly below the lower normal range value. To allow some margins for exercise and stress elevation of glucose values, as well as allowing for that the measuring device was not developed or calibrated for fish blood and considering that the published reference values cover 95% and not 100% of the population included in that reference, 2-10 nmol/l was set as the normal range for healthy salmon in our dataset. This limit was set for the whole Swedish dataset for 2020-2022 (also including Rivers Vindelälven, Klarälven and Ätran, data not published yet). In the Torne River and River Luleälven dataset, three salmon from Lövskär, River Luleälven exceed the 10 mmol/L limit, whereas 22 salmon from Vitsaniemi and 9 salmon from Seskarö fell below the 2 nmol/L limit. The glucose values differed significantly between Seskarö and Vitsaniemi by Wilcoxon rank-sum test (median 4.5, range 0.7-10 mmol/L, N=82 vs. median 3.0, range 0.4-6 mmol/L, N=89, $p<0.001$). Glucose was higher in Luleälven (Lövskär median 5.05, range 2.7-12.9 mmol/L, N=20; Boden median 6.35, range 5-9 mmol/L, N=20) compared to Vitsaniemi ($p<0.001$ for both) and Seskarö (Lövskär not significant, Boden $p<0.001$). In addition, males in general had higher glucose levels than females (males median 4.5, range 0.4-12.9 mmol/L, N=79; females median 3.5, range 0.6-10.7 mmol/L, N=132; $p<0.05$, Wilcoxon rank-sum test). The association was even more significant for only Torne River salmon (males median 4.2, range 0.4-9.8 mmol/L, N=69; females median 3.1, range 0.6-10 mmol/L, N=102; $p<0.001$, Wilcoxon rank-sum test). Lactate was successfully measured in 101 salmon from Torne River in 2020 and 2024. Lactate values ranged from 1.5 – 19.4 mmol/L, with an overall median of 13.9 mmol/L (**Table 6, Figure 7B**). This range is much larger than the published normal range for 95% of adult salmon (2.0-5.7 mmol/L, Rozas-Serri et al., 2022). To allow some margins for exercise and stress elevation of lactate values, as well as allowing for that the measuring device was not developed or calibrated for fish blood and considering that the published reference values cover 95% and not 100% of the population included in that reference, an upper limit of 10 nmol/l was set as the normal range for unstressed and healthy salmon in our dataset. Salmon with values >10 nmol/L were considered to suffer from lactic acidosis due to exercise or stress, although blood pH could

not be measured. This limit was set for the whole Swedish dataset for 2020-2022 (also including Rivers Vindelälven, Klarälven and Ätran, data not published yet). There were no significant differences between Seskarö (overall median 13.8 mmol/L) and Vitsaniemi (overall median 14.2 mmol/L), Wilcoxon rank-sum test, but in July 2020 there was a marked spread of values at Seskarö, compared to the other samplings (**Figure 7B**). There were also no significant differences between males and females (males median 14.6 mmol/L, range 1.5-19.4 mmol/L, N=50; females median 13.5 mmol/L, range 2-18.9 mmol/L, N=51; p>0.05, Wilcoxon rank-sum test).

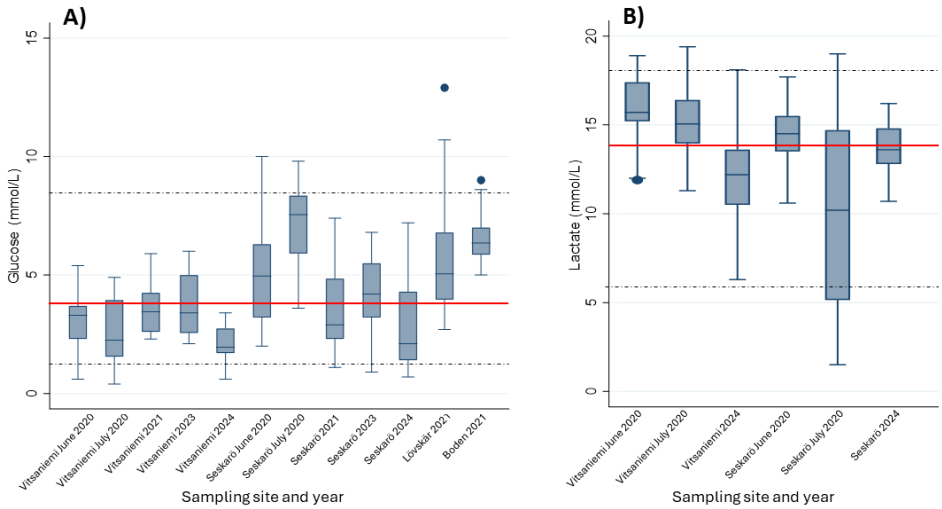

Figure 7. A) Blood glucose (mmol/L) and **B)** Blood lactate (mmol/L) plotted against capture site and year. Solid red lines indicate median and dashed black lines show the range of values for 95% of the sampled population.

A total of 237 samples from SVA and 28 samples from FFA were analyzed for thiamine content (**Table 6**). Values ranged from 0.26 – 1.62 nmol/g (Ttot), 0.22 – 1.15 nmol/g (TPP), 0.04 – 0.63 nmol/g (TMP) and 0 – 0.29 nmol/g (Tfree). Median and range values per sampling can be found in **Table 6**.

Looking at the summary values and comparing data from 2020 (**Table 6**), there seemed to be differences in the median and range of the thiamine vitamers between the SVA and FFA sampling, although total thiamine values are similar. To test this, SVA June + July thiamine samples from 2020 were merged into one group and the Wilcoxon rank-sum test was performed. Ttot did not differ significantly, however Tfree and TMP were significantly lower in SVA samples ($p<0.05$ and $p<0.001$ respectively), and TPP was significantly higher in SVA samples ($p<0.001$). When the SVA and FFA values were plotted together, the divergence in TPP, TMP and Tfree values was obvious (**Figure 8A-F**). From these graphs, it is also apparent that there is a strong correlation between the values of the different vitamers, especially between TPP and Ttot (**Figure 8A**).

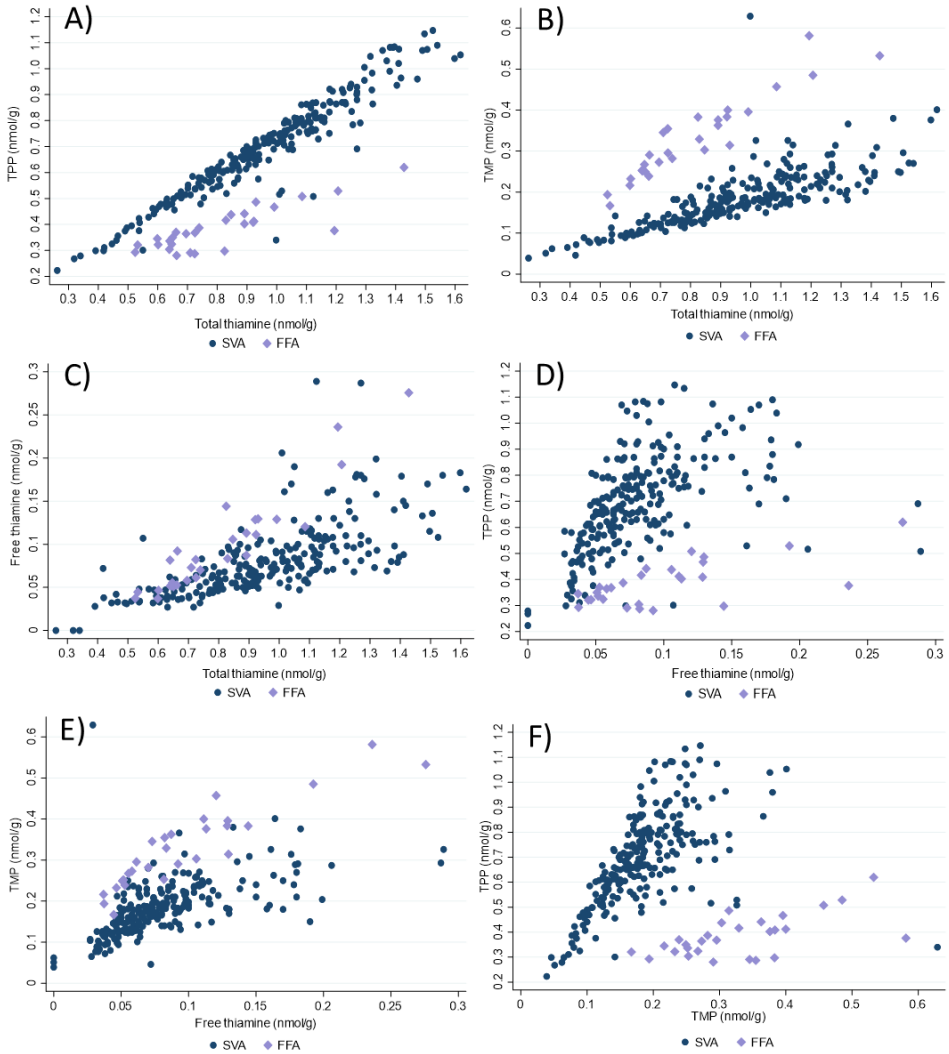

Figure 8. Comparison of thiamine vitamers and total thiamine in blood from 256 salmon. **A)** TPP vs. total thiamine, **B)** TMP vs. total thiamine, **C)** free thiamine versus total thiamine, **D)** TPP vs. free thiamine, **E)** TMP vs. free thiamine and **F)** TPP vs. TMP.

The two kelt females, who had been starving for approximately a year, had normal hemoglobin (122 and 113 g/L) and Glu (5.4 and 5.1 nmol/L) values, hematocrit was a bit low (42 and 48 %) and thiamine values were median or in the lower range in one (Ttot 0.94 nmol/L, TPP 0.7 nmol/L, TMP 0.16 nmol/L, Tfree 0.08 nmol/L) but in the higher range in the other (Ttot 1.54 nmol/L, TPP 1.09 nmol/L, TMP 0.27 nmol/L, Tfree 0.18 nmol/L). Lactate was measured to 15 mmol/L in the female from 2020.

Histology and cytology

In SVA samplings, internal organs were collected from all 252 salmon, but one or two organs were accidentally missed in a few salmon. Thus, liver, heart and kidney were analyzed for 251 salmon. Blood smears were done for all salmon, but two were so thick and intensely stained that they could not be interpreted. Spleen was collected systematically in 2021, 2022 and 2024 (in total from 147 of 153 salmon sampled in these years), and from one salmon with a parasite cyst in 2023. Pyloric caeca were sampled from all 80 salmon in 2021. Skin lesions were sampled from a total of 36 salmon (7 in 2020, 16 in 2021, 8 in 2022, and 5 in 2024). In addition, ovaries with lesions were sampled from three females.

Liver vacuolization (fat/glycoprotein storage vacuoles) was present in 232 salmon, i.e. 19 salmon (including the kelt females) lacked signs of nutrient deposits in the liver. Mild-moderate vacuolization was the most common finding (N=167), with maximum vacuolization present in 13 salmon. Six livers were diagnosed with fatty degeneration, and 56 had evidence of beginning fatty degeneration or uneven vacuolization. In 2021, pigment deposition (indicative of an immune response) but without visible inflammation, was seen in 14 livers and in 2023 in three livers. Inflammation was noted in 107 livers, and it was usually focal. Massive granulomatous inflammation associated with parasites was noted in 14 of these 107 livers, and an additional 21 livers had relatively massive inflammation. Of these four were granulomas without an obvious parasite, one had massive focal inflammation and associated hepatocyte necrosis, and one had an inflamed biliary duct. Otherwise, inflammations were mainly seen as perivasculitis (inflammation around blood vessels). In addition to the 14 livers with parasite granulomas, parasites with mild or without inflammatory response were seen in four livers.

Inflammatory reaction was identified in 17 (6.8%) kidneys. In seven of these kidneys, mild to moderate focal inflammation was observed. Granulomas (one to several, and in one salmon containing a parasite) were identified in five salmon. Thickened glomeruli capsules (part of the nephron/urine filtration unit) were observed in four salmon. In one salmon, degeneration of tubules (part of the nephron/urine filtration unit) and corresponding inflammation was present in approx. ¼ of the investigated tissue, but the glomeruli were still intact and there were no signs of nephron regeneration in response to the damage. In addition, some sporadic degenerative changes, with enlarged glomeruli capsules and/or swollen or shrunk glomeruli were observed.

Regarding hearts, the aim was to check both atrium, ventricle and bulbus arteriosus (for visual reference, see **Figure 4**). However, in many salmon, either atrium or bulbus or both were lacking in the final organ section. The ventricle could always be assessed. Lesions were identified in 92 (37%) hearts. Seventy-three hearts had inflammatory infiltrates, most commonly in the heart muscle (myocarditis, N=54), 19 had inflammatory infiltrates in the outermost/epithelial layer of the heart (epicarditis) and 6 salmon had both myocarditis and epicarditis. In most salmon, only single small foci of inflammation were detected, but some had multifocal or disseminated inflammation. In one of the hearts with multifocal (granulomatous) myocarditis, nematodes were observed within the lesions. In addition, endocarditis (inflammation of the heart valves) was noted in one salmon. Fibrous tissue/scarring was noted in many hearts, indication previous episodes of myocarditis. In the two female kelts there was mild degeneration of the myocardium. There were no apparent defects in the myocardium of the River Luleälven salmon with displaced bulbus arteriosus.

Spleen lesions were rare. Hemosiderosis (accumulation of hemosiderin/iron due to erythrocyte breakdown) was seen in 35 salmon. Inflammation was noted in five salmon, of which two had granulomas, one had acute inflammation, one had thrombophlebitis (inflammation and thrombus forming in a blood vessel and one had perivasculitis. In one spleen, a cyst was sectioned, but the cyst was empty meaning that the cause for the cyst formation could not be determined.

Intestinal sections from 2021 all showed a varying degree of an inflammatory response common at parasite infections, with mild to massive infiltration of eosinophilic leukocytes in the lamina propria, which is the connective tissue layer supporting the mucous intestinal epithelium that comes in direct contact with the parasites.

Skin samples were collected from 24 salmon with skin hemorrhage, four with RVS and two with melanisation in the dermal skin layer of the abdominal wall, three with potential UDN, two with healing mechanical injuries that had penetrated the abdominal wall and one with an old wound, potentially caused by a lamprey.

The skin hemorrhage samples showed epidermal necrosis, epidermal desquamation or hemorrhage in or around the scale pockets. Degeneration of underlying muscle was common. Lesion status ranged from acute (without any inflammatory response) to severe inflammatory reaction in the dermal and/or hypodermal layer or in degenerating muscle (subacute to chronic). Some samples had fibroblast activity in the dermis. Both superficial necrosis (outer layer) and deeper necrosis was observed in the epidermis. Muscle edema or degeneration or necrosis was observed in 12 salmon. Epithelial erosion, inflammation of the hypodermis, and muscle degeneration /necrosis are consistent with what has been described for RMS (Metselaar et al., 2020). However, some criteria for RMS, like acanthosis (reactive thickening of the epithelium) were not seen. Seven of the salmon were subjected to MLO analysis, and three were positive for the MLO-like organism. Lesions of these three salmon were subacute, whereas the lesions of the four that were negative for the MLO-like organism were acute.

The four salmon with RVS all had massive inflammatory reactions in the dermal and/or hypodermal skin layers. In three of them, nematodes were present, confirming the diagnosis. In the two salmon with melanisation in abdominal dermal skin layers, some remnants of parasites were found in association with the melanisation. Of the three salmon with UDN-like necrosis on the head, one had pyknosis and early bullae formation in the deep epidermis, indicating early signs of UDN. The other two salmon had necrosis from the surface of epidermis, which is not consistent with UDN. The two salmon with healing abdominal penetrating injuries had significant inflammatory reaction and scarring so that the intestine had grown into the abdominal wall. The old wound had chronic inflammation in the dermal layer of the skin and slight infiltration of white blood cells in the fat of the hypodermis.

Cytology of blood smears was done to estimate the composition of the white (leukocyte) and red (erythrocyte) blood cell populations. Regarding leukocytes, there was an annual variation in the composition, both within and between sampling sites. Plotting of % neutrophils, monocytes, lymphocytes and the neutrophil/lymphocyte quota against the other blood parameters or the health indexes did not produce any significant trends. Regarding erythrocytes, focus was on the approximate percentage of immature erythrocytes (erythroblasts and proerythrocytes). Occurrence of proerythrocytes (1-10%) was seen in 41 smears. The presence of a single erythroblast was noted in one smear.

FFA collected skin and liver samples for histology from 17 symptomatic salmon in 2021-2024. Different, but usually mild lesions were observed. Water mold and desquamation of the epidermis was observed in some salmon. A few salmon showed more severe lesions in the skin and in the underlying muscle. Severe lesions were usually observed in salmon with water mold infection. Histological skin lesions typical for RMS were not observed in the skin samples analyzed by FFA. In most of the liver samples analyzed by FFA, vacuolization was mild to moderate.

Association of measured variables to health status

An overall analysis of the population sampled by SVA in 2020 - 2024 was made by plotting the health indexes against different parameters. First, HEALTH1 was plotted against length, weight and sex (**Figure 9A**).

Most of the salmon examined (189 (89.6%) of 211 Torne River salmon, or 228 (90.8%) of 251 if River Luleälven is included). were in good condition with health index 1-2. No salmon had health index 5 (severe water mold infection). The largest and smallest (length and weight) salmon are males but there is no apparent difference in health depending on sex or size of the salmon. To investigate whether condition is affected in salmon assigned as "diseased" compared to healthy salmon or salmon with mechanical injuries, condition factor and liver-somatic index were examined against HEALTH2 (**Figure 9B**). Since healthy salmon (light blue) gather in the same area of the graph as diseased salmon (orange) and those with mechanical injuries (purple), there is no obvious effect of disease on condition in the sampled population.

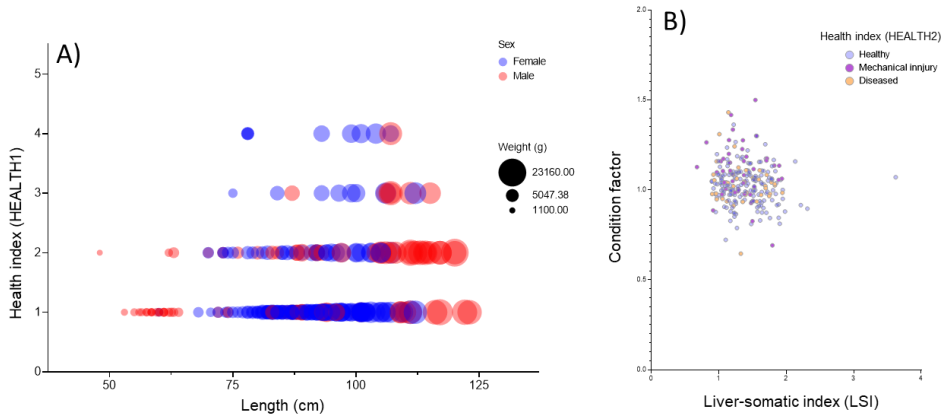

Figure 9. A) Visual multivariate analysis of HEALTH1 in relation to length, weight and sex. **B)** Visual multivariate analysis of condition factor and liver-somatic index in relation to HEALTH2.

When hemoglobin and hematocrit levels were put in relation to HEALTH1, no specific correlations were found (**Figure 10 A & B**), and both the highest and the lowest (anemic) Htc levels were found in apparently healthy salmon.

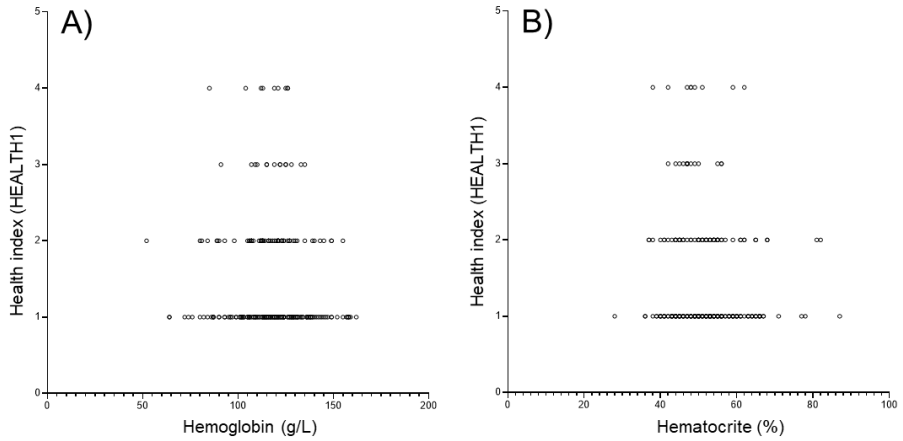

Figure 10. HEALTH1 in relation to **A)** the amount of Hemoglobin (g/L) and **B)** Hematocrit (%).

As for hemoglobin and hematocrit, no specific correlation between HEALTH1 categories and blood glucose or lactate levels were found when mapping data in graphs, but the highest glucose levels and the lowest lactate levels were found in healthy or mildly affected salmon (HEALTH1 categories 1 and 2). As written under laboratory results, glucose and lactate levels differed significantly between salmon caught at Seskarö and Vitsaniemi, and glucose values differed between males and females. Thus, to separate the potential effects of method of capture and the influence of gender or health on the glucose/lactate levels, multivariable graphs including these parameters were made for Torne River data. **Figure 11A** visualizes the difference in glucose levels between salmon caught by drift nets and salmon caught in traps. Lactate levels seem to have the opposite correlation compared to glucose (**Figure 11B**), although this was not significant by Wilcoxon rank-sum test. was found for lactate. The gender difference for glucose can possibly be spotted when looking at HEALTH1 categories 3 & 4 in **Figure 11A**. In addition, glucose seems to decrease with increasing health index and lactate seems to increase with increasing health index.

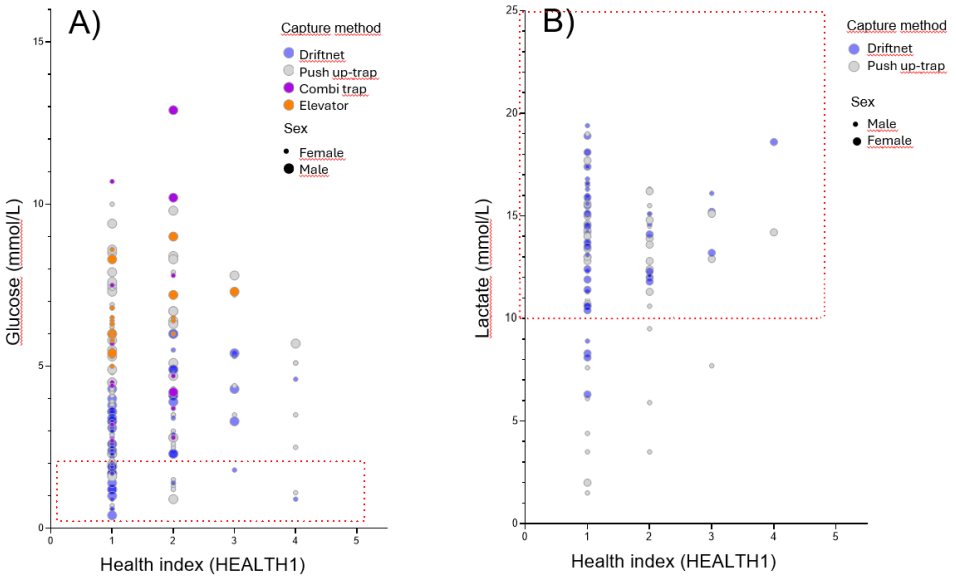

Figure 11. Visual multivariate analysis of **A)** glucose and **B)** lactate levels in relation to HEALTH1, capture method (circle color) and sex (size of circle). Please note that the circle size for sex has been switched between **A)** and **B)**. Red dashed boxes indicate hypoglycemia (low glucose levels) and elevated lactate levels in the respective graphs.

The results for glucose and lactate respectively with respect to capture method indicated an inverse relationship between the variables, i.e. that when glucose is high, lactate is low and vice versa. This could be considered normal because lactate is formed if glucose is metabolized anaerobically. Glucose and lactate values were therefore plotted against each other to confirm the relationship (**Figure 12**). Statistically, a simple (univariate) regression of lactate against glucose showed a significant negative relationship, showing that as lactate increases by one unit (1 mmol/L), glucose decreases by 0.67 mmol/L (N=100 samples, $p<0.001$, 99 degrees of freedom, $R^2=0.16$).

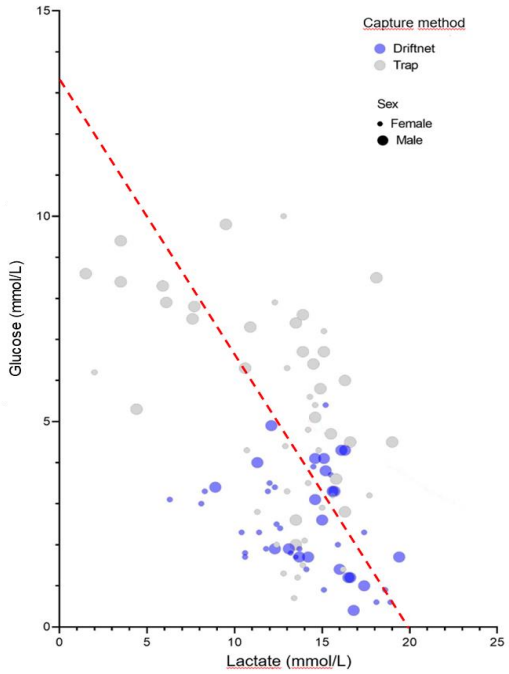

Figure 12. Visualization of the relationship between glucose and lactate levels of the 100 Torne River salmon where both parameters were measured. Capture method is indicated by gray/blue color and sex by size of the dot. The dashed line indicates the univariate regression result, which indicated that as lactate increases with 1 mmol/L, glucose decreases by 0.67 mmol/L.

However, there were several individuals who had glucose values (>7 mmol/L) while also having elevated lactate values (>12 mmol/L) (**Figure 12**), and the model only explained approx. 16% of the variance (R^2) in the data, indicating that other factors also affect lactate values. Thus, multivariate linear regression modelling was done, after first testing variables in univariate regression models to confirm an association. Post estimation showed that the variance of lactate values estimates was uneven (heteroscedastic) for different values of glucose, and even variance (homoscedasticity) must be present for a linear regression model to be valid. To overcome the problem, a variable can be transformed. Transformation to squared values (lactate x lactate) removed the heteroscedasticity. Thus, modelling was done using "$Lactate^{2}$". In addition to "Glucose", "Year", "Site" (capture method), "Condition factor", "HEALTH1", "HEALTH2", "Hematocrit", "Hemoglobin", the three thiamine vitamers and total thiamine were explored in univariate regression to rule these variables in or out of multivariate modelling, using $p<0.2$ as significance level for inclusion. For HEALTH1, categories 3 & 4 were joined for the analysis. Only "glucose", "Hb", "Hematocrit" and "year" were significant enough for further modelling. "Hematocrit" was not significant in combination with "glucose" and "Hb", and the variable was dropped. When "year" was added to the model, approximately 36.5% of the variation in lactate levels was explained. However, "year" also introduced an unequal variance again. Other transformations of lactate were tested (square-root, natural logarithm (ln),

common logarithm (log10) of lactate and lactate^3. Lactate^3 was the only converted variable with equal variances for different glucose values, and a model containing “glucose”, “Hb” and “year” could be produced without breaking the criterion of homoscedasticity. “Hb” had a positive relationship with lactate (higher Hb values are associated with higher lactate values) and “year” had a negative relationship with lactate (lower lactate values in 2024). No significant interactions between variables were encountered. This model explained 34.9% of the variance and was highly significant ($p<0.001$, 100 samples and 99 degrees of freedom). Thus, a higher degree of the variation in lactate levels could be explained than by just comparing lactate levels to glucose. However, the model residuals were unevenly distributed, indicating that there still were problems with the model. Further attempts to fit the model were not made.

Regarding thiamine status, there was no visible association between either general health status/index and TPP level (**Figure 13A**) or any difference in TPP levels depending on condition factor or whether mechanical injuries or other lesions (disease) were seen (**Figure 13B**).

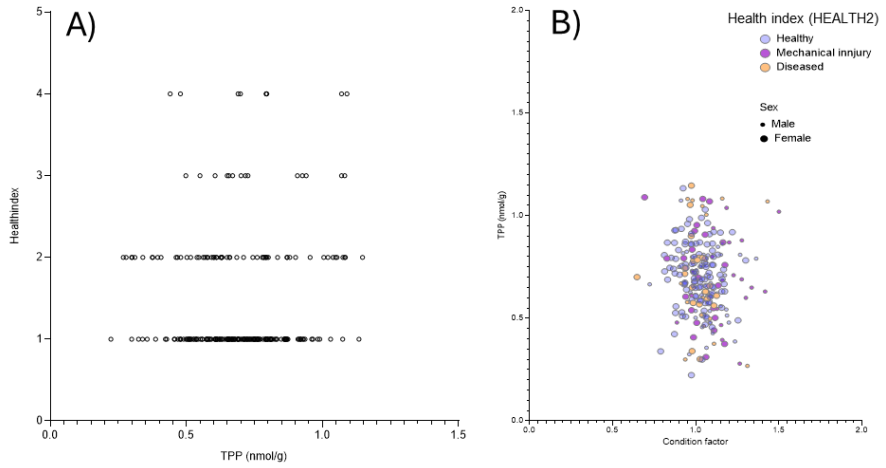

Figure 13. **A)** HEALTH1 versus blood TPP level and **B)** Condition factor vs. blood TPP level, HEALTH2 and sex.

Discussion

For five consecutive years (2020-2024), we have collected data to record the health status of returning Torne River salmon. The combination of a general sampling, where many salmon are sampled independent of visible lesions or not, and targeted sampling when salmon with lesions are identified, enables us to get a better overview of the health of Torne River salmon than by just either of these sampling strategies. In addition, the reporting site https://rapporterafisk.sva.se can provide a complement that both allows for the targeted sampling and for a better overview of the total number of diseased salmon per year. However, using the reporting site for interpretation of the disease situation requires appropriate reporting, i.e. that people have knowledge of the reporting site and are willing and able to report.

Lesions were recorded in almost half of the salmon from general samplings and of course in the symptomatic salmon. The most common lesions in the general samplings were skin hemorrhages and mechanical injuries, which is in line with previous reports on Torne River salmon (Axén & Koski 2017; SVA, GU & SLU, 2019). In the targeted samplings, the most common lesions were hemorrhages and water mold, while mechanical injuries were scarce.

Skin hemorrhage occurs in several infectious diseases, but no specific disease agent was identified, except in one female salmon with RSD, that was found to be positive for Piscine orthoreovirus (PRV). PRV was first described in 2010 in farmed salmon with heart and skeletal muscle inflammation (HSMI) (Palacios et al. 2010). Since that PRV has been identified in both wild and farmed salmonids around the world (Sørensen et al. 2020). However, no symptoms of HSMI were seen in our study. Indeed, PRV is often isolated from asymptomatic salmonids, both farmed and wild ones (Wessel et al., 2015; Sørensen et al. 2020). However, PRV infection will develop into HSMI under the right circumstances and its presence and possible effects on wild Baltic salmon should continuously be monitored. Fortunately, no serious viral or bacterial disease outbreaks were detected at Torne River salmon in this study, as several disease agents were ruled out by negative analyses (**Table 5**). The reason for not systematically performing virus analysis in the general samplings were that no viruses had been indicated to be present during the season 2016, when samplings were started, and that all wild broodstock salmon and sea trout females at the restocking farms are sampled each year, with to date only IPN virus identified in 2 of 25 510 females from 2007-2024, indicating populations of salmon and sea trout with extremely low prevalence of virus infections. The reason for conducting virological samplings in 2021 and 2022 was an outbreak of IHN in farmed rainbow trout at Åland, meaning that the salmon would potentially pass through the contaminated zone on their way north from the Southern Baltic Sea. However, the virus had not been picked up by the salmon in this study, neither has it been found in any broodstock females sampled since the outbreak.

The RSD syndrome is somewhat similar in appearance to RMS of Rainbow trout, but the location of lesions differs (abdomen vs. around the lateral line). A rickettsial-like bacterium called *Midichloria*-like organism (MLO) has been associated with RMS (Metselaar et al. 2020). Thus, in this study, we systematically sampled skin from salmon with ventral hemorrhage/RSD and analyzed them for the presence of MLO. MLO was not detected; however, several samples were un-specifically amplified, indicating presence of other genetical material, and melting curves indicated that there was a specific DNA sequence present. Thus, it is possible that an organism similar to MLO is present in the salmon with RSD. Whether this organism is then causing hemorrhage or not is unclear. Rickettsia and rickettsia-like organisms are hard to culture and thus isolation of live organisms is extremely difficult. This poses challenges, since experimental challenges to prove its infectious potential become difficult, and the lack of live organisms that can be propagated also means that mapping the whole genome for proper identification is much harder. Fulfilling Koch's postulates for an infectious disease is thus hard. More studies are needed to better understand the role of this "MLO-like organism" in the health of wild salmon populations. In addition to molecular genetic analysis, a number of skin samples from hemorrhaging lesions were studied histologically. Some samples showed similarities with RMS, but no samples fulfill all the histological criterions, provider further evidence that RMS/MLO is not the cause but adding extra evidence that an "MLO-like organism" could be present.

The bacterium *I. limnosediminis* was isolated from several salmon, especially in salmon with saprolegniosis. *Iodobacter limnosediminis* is considered relatively apathogenic for fish but may cause lesions in trout (Korkea-aho et al. 2021) and has been commonly isolated from salmonids with saprolegniosis (Korkea-aho et al. 2021; Carbajal-González et al. 2011). However, the bacterium's possible role in co-infection with Saprolegnia sp. is still unknown.

Regarding water mold, heavily infected salmon have been observed in the Torne River in recent years (Axén & Koski 2017, Härkönen et al. 2024). Water mold usually affects individuals that have skin lesions, are stressed or are immune suppressed (van den Berg et al. 2013), and massive infection leads to water logging and death due to osmotic imbalance causing e.g. hemolysis and thereby hypoxia in the tissues. The overall effect of saprolegniosis on the salmon populations is still not well known, because even if fewer salmon survive to spawning, the total number of offspring might not be affected (due to less competition between those fry that hatch). In this study we got more knowledge on water mold species and their occurrence

patterns in Torne River salmonids. *Saprolegnia parasitica* is considered the most pathogenic species of water mold, causing high mortalities in both farmed and wild salmonids (van den Berg et al. 2013). Few salmon caught in June and July had signs of water mold infection, and those that were infected only had mild, local infection, except for one sea trout with massive UDN-like lesions. Both *S. parasitica*, *S. diclina* and *Saprolegnia* of unidentified species, as well as some fungi existing in water environment but not known to be harmful for salmon were identified from these lesions. On the other hand, all salmon caught in late autumn were usually heavily infected with water mold that was found to be *S. parasitica*. According to these results, it can be concluded that *S. parasitica* is present in Torne River salmon populations and is causing heavy infections in salmonids, especially during the autumn. At this time, salmon is more susceptible to diseases due to the physiological processes related to spawning, where their metabolic resources are invested in the offspring and the immune system becomes suppressed. Because mechanical injuries were only found 6% of the salmon from targeted sampling, and water mold was detected in 58% of the same sub-population, this is an indication that mechanical injuries are not necessarily a big risk factor for water mold infection compared to for instance stress or skin hemorrhage. There was also a difference in the prevalence of water mold in symptomatic salmon between the years. Saprolegnia was present in most symptomatic salmon in years 2020 and 2021 and this could be related to a higher number of returning salmon (higher infection pressure) in these years compared to the three following years. However, the most probable explanation is that 2020 and 2021 were the only years when salmon was sampled in late autumn, when water mold infections are most common.

Most of the salmon in Torne River were infected with the tapeworm *Eubothrium* sp. *Eubothrium* sp. are commonly found in Baltic salmon, a result from feeding on pelagic fish which are intermediate hosts of the parasite. In salmonids, who are the definite hosts, the tapeworm is found in the intestine, where it feeds on nutrients by absorbing them directly from the intestinal content. Massive infections might cause nutrient deficiencies, but there was no apparent association between parasite burden and condition factor in the salmon studied by us. The tapeworms have hooks to anchor themselves to the intestinal mucosa (inside of the intestine). From histopathology it was apparent that this results in a local immune reaction, sometimes massive. In addition to tapeworm, single nematode larvae identified as *Contracaecum osculatum* were found in a few livers. This nematode has seal as its definite host and is commonly found in Baltic cod livers, which can be heavily infected (>100 worms). One study identified *C. osculatum* in 46% of the investigated Baltic salmon, which is much higher than what we found in the Torne River salmon, but always at low numbers (1-4 worms) and with immune reactions killing the parasites (Setyawan et al., 2019). Although the parasite is uncommon in salmon, it is still worth highlighting that *C. osculatum* can cause disease (allergic reaction or pain due to live parasites trying to dig through the gastric ventricle) in humans if raw fish is consumed (EFSA, 2011). In contrast, fish with intestinal (adult) tapeworms are safe to eat, because the tapeworms are already in their definite host. However, if tapeworms are found in the abdominal cavity (without the intestines having been injured during opening of the fish), in muscle or in cysts on the intestine or other internal organs, care should be taken because these will be larvae in their intermediate host. Special care should be taken when tapeworms are found in the muscle tissue, as these may be the human tapeworm *Dibothriocephalus latus*. To avoid zoonotic transmission, all fish to be consumed raw or marinated should always be frozen at least 48 hours before preparation to kill any potential parasite larvae.

During these five years, an aim of the general sampling has been to identify, if possible, proper parameters to enable monitoring of salmon health without killing the animal. To do this, many animals must be sampled to create a baseline for all parameters. The animals used for the baseline should preferably be in perfect health, but that is not possible since all wild salmon are in some way exposed to immunological challenges such as parasites. It is unlikely that only one or two single parameters are enough to estimate health status, but looking at several parameters simultaneously, the possibility of interpreting the health

status increases significantly. Therefore, we have evaluated a panel of different parameters that can be collected without killing the fish, including externally visible lesions (apparent health status).

In general, the salmon was in good condition and considered to be in good health although mechanical lesions or skin hemorrhage were commonly encountered. However, most of the hemorrhages were acute, meaning that they might have been caused by handling during capture. To catch salmon effectively for large samplings, it is impossible to use rod fishing, and thus we must live with this potential confounder and keep noting the stage of the hemorrhages for comparison with other parameters. In addition, many of the mechanical lesions recorded were scars or healing lesions, some of those with obvious penetration or tearing away part of the abdominal wall, meaning the internal organs have been exposed to the surroundings. It is thus apparent that salmon have a fantastic healing ability if their nutritional and immune status are good.

Scoring visible lesions in different ways based on how affected the salmon looked or dividing it into mechanical or non-mechanical lesions was a way of categorizing lesions for easier evaluation and comparison to other collected parameters. Although no specific associations of either health index to the different measured parameters were found, it is still a good way to visually evaluate the current health status of the fish. It must be kept in mind that fish with visible HEALTH1 index score 1 or 2 (i.e. visible healthy or mildly affected) might be in early disease stages, thus masking the effect of some parameters that have already been elevated or lowered. However, more importantly, because most fish were in these two categories, few evaluation points were generated for HEALTH1 score 3 and particularly score 4, and none for score 5, which makes it harder to conclude any relation of parameters to advanced disease. Continued comparison of a range of parameters against each other might provide evidence that some parameters are highly correlated to disease, and to get more data it is important to be able to measure these parameters also when targeting diseased fish, where more advanced disease stages are likely to be encountered.

The two female kelts that were in bad condition by visual examination (low body condition, early saprolegniosis) were determined as emaciated due to lack of visceral fat deposits, in combination with histological evidence of no nutrient storage in the livers and signs of muscle degeneration in the heart. The latter is due to recruitment of muscle amino acids for metabolism when fat and glucose storages have been emptied. Still, blood values were relatively normal. Emaciation at this late kelt stage is normal, and the condition is reversed to normal once the fish starts feeding again. The fate of these females had they not been caught is however unclear since they were both affected by early stage saprolegniosis.

Evaluating the blood parameters is challenging, firstly because no measurement instruments for Hb, glucose and lactate available on the Swedish market have been developed for fish blood. Thus, there are no specified normal “fish ranges” set for the instruments we use, and there is always a variation between different instruments. In addition to this, the reference ranges found have been set for farmed fish which have a slightly different biology (bred to grow quickly), are massively fed (thus not in a catabolic state as returning salmons), not in an exercising stage (as especially the upstream salmon caught immediately after ascending the long Vuentokoski rapids, and potentially suffering less stress during catchment (just being netted at the farm). For these reasons, and to allow some sampling stress and measurement error, the range for normal Hb, glucose and lactate levels were allowed to be a bit wider than published ones. The reference values for Hb in Rozas-Serri et al. (2022) are way above what is normally used as reference values in animals and humans (e.g. 117-153 g/L is normal in human females (Karolinska Universitetssjukhuset)). All median Hb values (pre-smolt, smolt, post-smolt and adults of Atlantic and coho salmon) in Rozas-Serri et al. (2022) are extremely high (384-641 g/L) indicating a measurement error or wrong scale conversion. Potentially the values are in g/dL (i.e. 38.4-64.1 g/L) which would mean they are at the lower end of our range. Hematocrit is evaluated visually against a scale after spinning samples in specific Hematocrit

microtubes, and thus there is no instrumental reference value to keep to. Measurement errors can of course occur, and as with the other blood values, some slack was allowed to adjust for especially stress elevation of Hematocrit. When it comes to blood smears, thickness of the smear and intensity of staining affects the readability.

Hyperglycemia, or high glucose levels can be due to recent feed intake (especially in diabetic individuals), stress or due to chronic disease (elevated metabolism). Hypoglycemia, or low blood glucose can occur at starving or massive exercise. Here, three salmon were scored with hyperglycemia, and 31 salmon were scored with hypoglycemia. All but two (that were hypoglycemic) were in apparently good health (HEALTH1 score 1 or 2) and thus the values are believed to be caused by stress or massive exercise. Lactate levels in blood are low during aerobic metabolism but increase during anaerobic metabolism because the end product when breaking down glucose anaerobically is lactate. Normally, the body will clear lactate from the system, but if the production of lactate is higher than the clearance rate, lactic acidosis will occur. Acidosis is common due to septic shock (severe infection) or other processes that cause lowered blood (oxygen) perfusion to tissues (Agedal et al., 2023), forcing anaerobic metabolism to occur and without possibilities for clearance of lactate. Lactic acidosis can also be caused by thiamine deficiency, because thiamine is necessary for aerobic metabolism (Agedal et al., 2023). In humans, lactic acidocis is considered to be present at lactate levels above 4 mmol/L (Acute Lactic Acidosis: Overview, Treatment Overview, Prehospital Care) lactate levels of >8 mmol/L have been associated with significantly lower survival in critically ill patients (Bernhard et al., 2020). Thus, human lactic acidosis is considered present within the stated normal interval of 2.0-5.7 mmol/L for Atlantic salmon (Rozas-Serri et al., 2022). This could either mean that salmon have a higher blood lactate tolerance than humans, or that sampling can never be done in salmon without causing increased lactate levels or both. Anyway, 86% of the blood lactate levels measured in this study were above the set lactic acidosis level 10 mmol/L. In salmon most of the muscle mass works anaerobically, and only a small rim of muscle along the lateral line that works aerobically. The aerobic muscles are the ones that work most of the time, during slow controlled swimming, but when a short burst of force is needed, the white muscle must work. Thus, to combat rapids anaerobic metabolism will be necessary. Anaerobic metabolism will also occur during fighting against fishermen (in our case to escape nets and when traps are emptied), and lack of oxygen from suffocation in nets or in the air after emptying of traps will also increase lactate levels. Since the drift nets are in the water for approximately 30 min, fighting can be prolonged and this will likely affect the lactate levels. Thus, the relationship between glucose/lactate and site/capture method is believed to be a relevant association. However, there might also be a relation to different “normal” exercise levels at the two sampling sites, since capture at Vitsaniemi occurs immediately after the salmon conquers the Vuentokoski rapids. Sampling year was significantly associated with lactate level, which might seem odd. However, “year” is probably acting as a proxy for another, unmeasured parameter like differences in water flow (resistance when swimming) or water temperature, two parameters that will affect the metabolism. In addition, lactate levels at Seskarö varied a lot in the July 2020 samplings compared to other samplings, making associations harder. The association with an increase in Hb as lactate increases could be caused by a haemoconcentration with increased strain. This could be caused by increased fluid loss or recruitment of red blood cells from depots (spleen, kidney) in the body. No association with any of the thiamine vitamers was found, and thus we do not expect thiamine deficiency to be involved in the development of lactic acidosis measured here, but that the levels were mainly elevated due to massive exercise and stress. Even if salmon are better than humans at lactate clearance (which is unknown to us), it is uncertain if the levels measured here would allow survival of sampling was done on live fish that was released. Measuring post catch lactate levels in net-caught salmon with the possibility to follow up on survival by tagging would be necessary to evaluate the chance of survival before recommendation of live blood sampling and release. Also, measurement of lactate in rod-caught salmon would be interesting for comparison, since catch and release is a common way to fish for salmon.

Leukocytes, especially neutrophils, are recruited to the blood stream during stress, but are also recruited during inflammation due to mechanical injuries or disease. Here, we only looked at the leukocyte overall composition, and not in relation to red blood cells, and we didn't take the thrombocyte fraction into account. This was done because we were primarily interested in the relationship between different leukocytes as a proxy for stress and disease stage and type. We couldn't find any significant associations between different leukocyte fractions and disease, which might indicate that no salmon was in a severe or advanced disease condition. A few salmon were indeed affected by saprolegniosis but in an early stage of infection. In late stage saprolegniosis, blood parameters will be affected both due to inflammation and blood dilution (Weichert et al., 2021). Red blood cells were evaluated for deformities and amount of immature red blood cells. Late stage immature red blood cells are normally present to some extent in the blood, but high levels of earlier stages of immature red blood cells and even blasts (stem cells) indicate that erythropoiesis (production of red blood cells) is increased. This could occur due to severe blood loss (injuries or diseases that disrupt red blood cells) or hormonal imbalance. An increased erythropoiesis of unknown origin, but belived to be related to elevated thyreoid hormone levels, were seen in our investigations in 2018 (SVA, GU & SLU, 2019). No indications of increased erythropoiesis were seen during 2020-2024 indicating that the cause for the disturbance in 2018 is no longer present. Overall, monitoring different blood parameters, both regarding chemistry and cell composition and morphology is useful to interpret health stage, taking into account that other parameters such as capture method and recent strain might affect measurements.

Blood thiamine was measured because thiamine deficiency in salmon leads to serious illness and death, especially when no or little thiamine is transferred from the female to the offspring (Vuorinen et al. 2021; Koski et al., 2001). In nature, thiamine (or vitamin B1) is mainly produced by phytoplankton and is enriched upwards in the food chain. Thiamine is poorly stored in the body and therefore needs to be continuously supplied through food. This means that during starvation, like the spawning run, thiamine levels will successively drop. Thiamine exists in four different forms or vitamers (Tfree, TMP, TPP and thiamine triphosphate (TTP)) in nature. In salmon, TTP is basically not present and Tfree, together constitute the total thiamine level (Ttot) of blood, liver etc. Free thiamine is absorbed from the intestine and phosphorylated into TMP and TPP. TPP is the most prevalent vitamer, and the biologically active form, that e.g. acts as a coenzyme during the citric acid cycle (part of the glucose metabolism). In blood, about 90% of all thiamine is found in the red blood cells. There are no true reference values for blood thiamine in salmon, and thus it is hard to know what would be considered a deficiency level. This itself is an indication to sample for blood thiamine to create a baseline in salmon.

Interestingly, Ttot was similar in SVA and FFA samples, but the vitamer fractions differed significantly. A definitive explanation for this difference has not been discovered. All thiamine analyses were performed in the same laboratory, and thus the "error" should not have appeared at this stage. Perhaps there were differences in sampling and storing samples. For instance, FFA used serum tubes to store their samples while SVA used tubes without additives for coagulation. But why TMP would increase and TPP decrease due to using serum tubes cannot be explained. It would be more reasonable to believe that different handling would affect the total thiamine concentration. Thiamine is said to be unstable, and samples should be extracted immediately after death. 2020, when SVA tried to keep all salmon alive until sampling just for the purpose of thiamine sampling, all salmon died during transport to shore due to hard weather (stress). The last of the 20 salmon had been dead for 9-10 hours before sampling could be conducted. Thiamine levels were thus plotted against salmon id to check for postmortem effects, but no decreasing trend with time could be seen. Thus, blood thiamine levels seem to be stable at least if the blood is not drawn and left in a syringe before freezing.

Finally, histopathological analyses did not identify any serious diseases. However, the fact that some salmon didn't have any nutrient storage in the liver is a bit concerning, especially since they had a long period of starvation ahead of them, had they not been caught. In contrast, several livers have an excess of nutrient storage and were classified with beginning or developed fatty degeneration. Fatty livers are a common finding in cods, and they seem to cope well. It is unclear whether this is also valid for salmon, or if the livers get damaged. The common presence of myocardial inflammation and scarring indicates continuous exposure to irritants or pathogens, but also a good ability to heal these lesions. The disoriented anatomy of some River Luleälven hearts is somewhat similar to the tetralogy of Fallot, a human congenital heart condition where one of four defects is that the aorta is shifted to the right like in these salmon. Because salmon have a two-chamber heart (one atrium, one ventricle) and not four (two atria, two ventricles) as humans, the second defect, a hole between the right and left ventricle cannot be present. The third defect is pulmonary valve stenosis (thickening and narrowing of the valve from the right ventricle to the pulmonary artery). Of course, fish have gills and not lungs, and the artery leaving the only ventricle is the aorta (leaves the left ventricle in four-chambered hearts), but the aorta somewhat replaces the pulmonary artery as blood vessels to the gills branch from the aorta and stenosis of the aortic valve might have been expected. Valvular stenosis was not seen, and neither was the fourth defect in the tetralogy of Fallot, namely thickening of the (right) ventricle. The last defect occurs secondary to the increased workload that the heart suffers due to the other three defects, because it must work harder to supply the body with oxygen. In the River Luleälven salmon, no obvious signs of cardiac failure or damage due to the displaced aorta were seen microscopically. The cause of the malformation is unknown. If this is a hereditary malformation, it must thus have developed several generations before because no siblings were detected. More likely is perhaps that some disturbance like a (small) peak in temperature has occurred during egg incubation, since this can cause organs to develop too quickly, leading to among other things heart defects (Brijs et al., 2020).

Based on the results, as discussed above, we propose a general sampling scheme for salmon health monitoring including:

In all salmon

1) morphometric parameters (length, weight, sex)
2) visual evaluation including photos for later scoring according to the proposed HEALTH1 index
3) blood samples for measurement of Hb, Hematocrit, glucose, lactate, thiamine vitamers and blood cell composition and morphology

In salmon with lesions, based on nature of these or optional in non-symptomatic salmon

4) samples for virology, bacteriology, mycology (culture and/or PCR)
5) samples for histology – skin lesions (non-mechanical), internal organs with lesions or routinely liver, heart and kidney

In addition, records of water temperature and water flow (in rivers), proximity to rapids etc could be useful to aid in interpretation of blood chemistry values.

Conclusions

Our results indicate that there are no serious viral or bacteriological disease outbreaks in Torne River salmons. The most common infection was water mold, especially during autumn samplings, and more investigation is needed to find out the possible implications of the different oomycete and fungi species for salmon health, reproduction success and survival. In fish with red skin disease, the presence of DNA that

could be an organism related to the Midichloria-like organism causing a similar disease in rainbow trout warrants further investigations.

Whether performing general or targeted samplings, a common protocol for assessment of salmon health status as well as a common panel of samples would be good to enable interpretation and comparison of data. Photos that can be used for health scoring, morphometric variables and blood parameters need to be collected continuously to increase the reference library enable comparison of samples collected over the whole migration season as well as between years. Care must be taken when interpreting blood chemistry values, especially glucose and lactate, considering the potential effect of capture method, stress, water temperature etc. Thiamine levels also need to be continuously monitored as trends for M74 might be detected prior to spawning and indicate breeding success. These data can be collected without killing the salmon, but blood sampling requires either manual or anesthetic immobilization, which is stressful to the salmon. This would be in addition to the stress caused by capture, and with the lactate levels registered here, release post-sampling cannot be recommended. Thus, euthanasia is the best option for sampling. This also allows sampling for histopathology and pathogen detection, which might serve as early warnings that something is introduced into the population and might become a problem.

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Appendix 1 – Scoring of health indexes (HEALTH1, HEALTH2)

The following scoring system was used:

HEALTH1: 1) healthy, 2) mildly affected health, 3) moderately affected health without signs of secondary fungal/oomycete infection, 4) moderately to severely affected health with early fungal/oomycete infection and 5) extensive fungal/oomycete infection, i.e. severe/terminal illness

HEALTH2: 1) healthy, 2) mechanical injuries, 3) disease

No visible lesions (except sometimes scale loss caused by capture), incl. completely healed injuries

HEALTH1 score 1

HEALTH2 score 1

Acute to subacute minor to moderately sized lesions

HEALTH1 score 2

HEALTH2 score 2 if mechanical

HEALTH2 score 3 if not mechanical

More extensive lesions, subacute to chronic in nature, no signs of water mold

HEALTH1 score 3

HEALTH2 score 2 if mechanical

HEALTH2 score 3 if not mechanical

Moderately to severely affected fish, signs of mild/early fungal/oomycete infection

HEALTH1 score 4

HEALTH2 score 2 if mechanical injury is the primary cause

HEALTH2 score 3 if non-mechanical lesion is the primary cause

Extensive fungal/oomycete infection – severe/terminal illness

HEALTH1 score 5

HEALTH2 score 3