How pathogens affect the marine habitat use and migration of sea trout (*Salmo trutta*) in two Norwegian fjord systems

Running Head: Ecology of sea trout with pathogens

Authors

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Abstract

Wild fish are confronting changing pathogen dynamics arising from anthropogenic disturbance and climate change. Pathogens can influence animal behaviour and life histories, yet there is little such data from fish in the high north where pathogen dynamics may differ. We aimed to compare the pathogen communities of 160 wild anadromous brown trout in two fjords in northern Norway and determine whether pathogens influenced area use or return to spawn. Application of high-throughput qPCR detected 11 of the 46 pathogens screened for; most frequently encountered were *Ichthyobodo* spp., *Flavobacterium psychrophilum*, and *Candidatus* Branchiomonas cysticola. The rate of returning to freshwater during the spawning season was significantly lower for the Skjestadfjord fish. *Piscichlamydia salmonis* and *F. psychrophilum* were indicator species for the Skjerstadfjord and pathogen communities in the two fjords differed according to perMANOVA. Individual length, Fulton's condition factor, and the time between first and last detection of the fish were not related to the presence of pathogens ordinated using non-metric multidimensional scaling (NMDS). However, there was evidence that pathogen load was correlated with expression of smoltification genes, which are upregulated by salmonids in freshwater. Correspondingly, percentage of time in freshwater after release was longer for fish with greater pathogen burdens.

Introduction

Pathogens may have complex life histories and are transmitted to hosts either horizontally through the environment or in their food, or vertically from mother to offspring (Marcogliese, 2002). Infection by pathogens cause endemic diseases and can therefore be observed consistently within host populations, having population dynamics linked to their host populations (Dobson, 2004). Depending on pathogen prevalence, host condition/immunity, and environmental conditions (e.g. temperature), infections can have variable impacts on host condition and performance. Drastic changes to the landscape including an increase in host abundance, shifts in climate, or other factors can enhance the abundance or virulence of pathogens and result in epidemics. Epidemics in wild animals have significant negative consequences to their host populations and many can affect human institutions including agri/aquaculture (e.g. bovine tuberculosis, Woodroffe et al., 2006; salmon lice, Vollset et al., 2017) and recreation (e.g. chronic wasting disease, Needham et al., 2007; ciguatera, Cooke et al., 2018). Migratory species may serve as reservoirs of pathogens and their movements across environments can expose them to a higher diversity of potential pathogens or allow them to escape spatially discrete pathogen reservoirs (Altizer et al., 2011).

Interest in characterizing the pathogen dynamics of wild fishes and the potential role that they have in regulating their host populations is expanding. Although host-pathogen ecology is less studied relative to processes such as predator-prey relationships, pathogens also have a critical role in regulating their hosts and can influence host behaviour. Pathogens themselves can have etiological effects that can also interact with other stressors to enhance vulnerability to disturbance and catalyze the development of disease (Altizer et al., 2013). Recent interest in

investigating the influence of pathogens on fish ecology has yielded insights into interactions of certain pathogens with other stressors, particularly fisheries, and how pathogens can enhance vulnerability of individuals to anthropogenic disturbance (Miller et al., 2014). Many fish pathogens are known to occur among salmonids in Norway including *Flavobacterium psychrophilum*, Piscine orthoreovirus-1 and -3 (PRV), salmonid gill pox virus (SGPV), infectious salmon anemia (ISA), and more (Zubchenko and Karaseva, 2002; Garseth et al., 2013a, 2103b, 2018). However, little is known about the prevalence, distribution, and impacts on performance of key pathogens on wild anadromous salmonids in the North Atlantic.

Anadromous brown trout (*Salmo trutta*; aka sea trout) are a relevant model system for surveying salmonid pathogens because (1) they occupy and spawn in small creeks often highly impacted by climate change and (2), are targeted by recreational fisheries and spend most of the summer months in coastal areas overlapping with many areas exploited by humans (e.g. salmon farming; Eldøy et al., 2015; Thorstad et al., 2015; Bordeleau et al., 2018).

Pathogens can exert substantial influence on performance and fate of their hosts (e.g. Bradley and Altizer, 2005) and we aimed to investigate how viruses, bacteria, and parasites present on wild sea trout as they exit freshwater in multiple locations in northern Norway affect marine behaviour and fate. We also applied a new salmon Fit-Chip technology to assess relationships between pathogens and movement metrics with indices of stress, disease, and osmoregulatory state of the sea trout host (Miller et al., 2017; Houde et al., 2019a,b). We tagged fish in river systems belonging to two fjords in northern Norway to compare pathogen abundance and diversity and to relate movement patterns within arrays of acoustic receivers to disease. Our objective was to describe pathogens and physiological states relevant to these ecological communities and identify how these factors contributed to the fate of the wild fish. As climate

change and human stressors are projected to have substantial impacts on these northern ecosystems, our research will contribute to establishing a baseline state in areas relatively unimpacted by human activity that are dominated by sea trout.

Methods

Study Site

This study took place in the two fjord systems Tosenfjord and Skjerstadfjord in Northern Norway (Figure 1). The study site in Tosenfjord consists of two interconnected fjords with approximately 150 km^2 surface area, more than 270 km of shoreline and is connected to the open sea by a 15 km long strait. In Tosenfjord, the tracked fish were tagged in the two watercourses Urvold and Åbjøra. The Urvold watercourse has a common water discharge of 5 m^3s^{-1} and is characterised by a 200 meter steep river stretch draining from a lake, as well as an approximately 1 km river stretch upstream of the lake available for anadromous populations. Åbjøra watercourse have about 23 km of river stretch available for anadromous salmonids. The Skjerstadfjord system consists of four interconnected fjords (Skjerstadfjord, Misværfjord, Valnesfjord and Saltdalsfjord). The study system in the Skjerstadfjord is more than 40 km long, with a surface area of about 230 km^2, and is connected to the open coast by an approximately 15 km long strait, including the strong tidal current of the Saltstraumen Maelstrom. In the Skjerstadfjord, the fish were tagged in the three watercourses Saltdalselva (65 km anadromous stretch), Botnvassdraget (10 km anadromous stretch), and Laksåga in Sulitjelma (27 km anadromous stretch).

Multiple open net-pen aquaculture operations are located within both of the two study sites (Fig 1). In Tosenfjord, there were five registered marine aquaculture sites for salmonids in 2017 with a combined total allowed biomass of 15 300 tons. In Skjerstadfjord, there were seven registered marine aquaculture sites for salmonids in 2018 with a total allowed biomass of 17 800 tons. The farmed salmon are regularly screened for diseases by the salmon farmers, and detection of two diseases, pancreas disease and infectious salmon anemia, requires mandatory reporting to the national authorities. In Tosenfjord, farmers reported suspicion of a pancreas disease outbreak in October 2016 with confirmed pancreas disease on farmed fish in the fjord from 17 November 2016 and throughout the study period in this fjord system. Neither pancreas disease nor infectious salmon anemia were reported on farmed fish in the Skjerstadfjord fjord system during the study period.

Sampling and Tracking of Wild Sea Trout

All brown trout were sampled during March 28 – May 31, 2016, September 22-25 2016, or April 28 – May 29, 2017. The trout had 2-7 seasonal feeding migrations before the sampling. A total of 340 trout (135-730 mm TL) in Tosenfjord (2015-2017) and 267 trout (169-890 mm TL) in Skjerstadfjord (2016-2018) were caught using fishing rods and gillnets that were continuously monitored, and kept in holding nets for up to four hours prior to tagging. The fish were sedated using 2-phenoxy ethanol, and acoustic transmitters (Thelma Biotel AS, Norway, various models depending on fish size) were surgically implanted in the body cavity using a validated protocol for wound opening and closure with sutures (Bordeleau et al., 2018). Morphometric measurements and blood-, scale-, adipose fin, and gill-tissue were sampled before

recovery from the sedation in a tank for up to 15 minutes followed by release at a calm site at the tagging location. Scales were visually analyzed to determine individual age and migration history. The experimental procedures were approved by the Norwegian National Animal Research Authority (permission number 2012/22965 & 2015/8518).

In both fjord systems, arrays of acoustic receivers (Vemco Inc., Halifax, Canada; models VR2, VR2W and VR2-AR) were deployed in fresh- and saltwater to monitor the movements of the tagged fish. Acoustic receivers (i.e. hydrophones) are listening stations tuned to the same frequency as tag transmissions so that they can identify individual tags from detections when in range. Detection ranges in the two fjords ranged from 200-400 m. In Skjerstadfjorden, detection efficiency at the outermost array of receivers were 100 %, will the efficiency of the receivers arrays in Tosenfjord ranged from 81-100% (Bordeleau et al., 2019; Davidsen et al., 2019). Based on size of the fish and sampling and tagging in the springtime, fish were expected to have been animals that had previously been to sea, overwintered in freshwater for several months, and now beginning to move back into the marine environment for the summer.

Genomic Analysis

For this study, 160 of a total of 607 trout were sub-selected for genomic analysis. Ninety three of the trout originated from the Tosenfjord, of which 60 were from Urvoll and 33 from Åbjøra. The remaining 67 were from the Skjerstadfjord system, 29 from Sulitjelma Laksåga, 23 from Botnvassdraget, and 15 from Saltdalselva. Sea trout from the Skjerstadfjord were larger ($t = 2.90$, $df = 102.07$, $P < 0.01$) on average (507 ± 139 mm) than those from the Tosenfjord (451 ±

86 mm) but there was no difference in Fulton's condition factor. Expression data for two fish failed and was excluded, so the maximum sample for data including genomic analysis was 158.

Gill tissues samples from the fish were preserved at-196° C and the expression of host- and microbe- related biomarkers were analyzed by quantitative real-time polymerase chain reaction (qPCR) as described in Teffer et al. (2019). On the first dynamic array run, nucleic acids obtained from gill samples were assessed for pathogens capable of causing disease (hereafter referred to as pathogens, but note that the detection of a pathogen does not imply the detection of a disease; Table 1: 12 viruses, 12 bacteria, and 14 microparasites) using 39 qPCR assays to pathogens and three host reference genes (S100 calcium binding protein, Coiled-coil domain-containing protein 84, 39S ribosomal protein L40, mitochondrial precursor described in Miller et al., 2017). All pathogen assays and host reference genes were run in duplicate. On a second dynamic array run, nucleic acids from gill samples were assessed for transcriptional activity of 63 host biomarkers comprised of panels of genes that when co-expressed are predictive of specific physiological processes of interest in our study (e.g. thermal (Akbarzadeh et al., 2018), hypoxia, osmotic (taken from smoltification studies (Houde et al., 2019a,b), viral disease (Miller et al., 2017), immune stimulation, general stress, and mortality related (Miller et al., 2011); See Table 2) run as singletons, along with duplicate assays to the same three host reference (housekeeping) genes. Dynamic arrays containing these curated host biomarker panels to assess host health and condition are termed "Salmon Fit-Chips" (Houde et al., 2019a).

Total RNA was extracted by homogenization of tissue in TRI reagent (Ambion Inc., Austin , TX) followed by aqueous separation using 1-bromo-3-chloropropane. Resulting supernatants were used to extract purified total RNA using the Magmax-96 for Microarrays RNA kit (Ambion Inc.) on a Biomek NXP (Beckman-coulter, Mississauga, ON, Canada)

automated liquid handler according to the manufacturers “spin method”. Extracted RNA (0.25ug) was reverse transcribed to cDNA using the SuperScript VILO master mix kit (Invitrogen, Carlsbad, CA) following the manufacturer’s method. The BioMark platform employs nanofluidics, as per manufacturer's recommendations, and specific target amplification (STA) of assays is required (Dhoubhadel et al., 2014). The cDNA (1.3 µl) from each sample was preamplified with a mixture of 0.2 µM of primer pairs for each of the assays applied in a given dynamic array run using TaqMan Preamp MasterMix (Applied Biosystems, Foster City, California) in a 5 µl reaction.The preamplification was run for 14 amplification cycles, as per the BioMark protocol. ExoSAP enzyme treatment (Affymetrix, Santa Clara, CA) was used to remove unincorporated primers from the assays, which were then diluted 1:5 in DNA Suspension Buffer (Teknova, Hollister, CA). For pathogen quantification, artificial positive constructs (APC) were created from each microbe assay region's sequence, with an additional sequence added that allowed for the detection of vector contamination (see Miller et al., 2016). A serial dilution of these APC clones was run on the dynamic array for calculation of assay efficiency. For the Salmon Fit-Chips, a serial dilution of gill cDNA was included in STA processing to be used in gene expression analysis. BioMark Fluidigm Dynamic Arrays were run according to the manufacturer's instructions. Cycle threshold (CT) values were determined using the BioMark Real-Time PCR analysis software (Fluidigm Corp., CA), and duplicates handled with limits of detections applied (Miller et al., 2016) through an access database. For pathogens, only samples with detections for both duplicate assays were considered positive. For host biomarkers, sample gene expression was normalized with the ΔΔCt method (Livak and Schmittgen, 2001) using the non-diluted pool sample as the calibrator. Gene expression was then log transformed: $\log_2(2^{-\Delta\Delta Ct})$.

Data Analysis

Pathogen Data

Pathogen loads were measured as the number of cycles for initial detection in the qPCR runs (C_T values) executed with a maximum of 45 cycles. For analyses and visualizations, qPCR results are transformed by subtracting the C_T value from 45 with not detected pathogens (negative results) given as 0, such that high values (pathogen loads) are then closer to 45. Pathogen loads were then transformed to relative load to scale the values for multivariate ordination; this was preferred to standardization because ordination cannot handle negative values produced by z-scores (Teffer et al., 2017). Relative infection burden was calculated as the sum of the relative load of each pathogen. Shannon diversity, a measure of abundance and evenness of an ecological community (Hurlburt, 1971) was computed with the *diversity* function in the R package vegan (Oksanen et al., 2019) and compared between the Tosenfjord and Skjerstadfjord by a t-test with the *t.test* function in R. Indicator species, which are species associated with sites based on pattern matching, were investigated using multilevel pattern analysis (*multipatt* function in indicspecies package; De Caceres and Lagendre 2009). Pathogen readings were unsuccessful for two of the 160 individuals.

Summarising Observed Movements

Acoustic telemetry detections from each of the two fjords were used to identify movement patterns and spatial area use by the tagged sea trout. We used movement to estimate 1) survival; 2) network use; and 3) time spent in freshwater. All analyses were carried out using R.

1. Survival to spawn

Despite not having details of the death of any fish, we had detection histories for each individual that we used to estimate fate of all 160 individuals. Each fish is expected to return to rivers between August and October, where they then overwinter. Given that we had good coverage of rivers in the system, we registered fish that were detected in freshwater between August and October following tagging; those that were not recorded at freshwater receivers were coded as missing spawning (note that this does not necessarily mean they died). Survival time was modeled by time to event analysis (event being the last detection) by the *cph* function in the rms package (Harrell, 2019). Time to event was the number of days between the event and the date of release and was modeled against fish length, condition factor, fjord of origin, tagging year, and pathogen diversity for that individual (see below). A second model was run with only fjord of origin. Assumption of proportionality of hazards was checked by the *cox.zph* function in the rms package. Three individuals were excluded from the survival analysis owing to lack of data.

2. Network analysis metrics

To classify individual movement patterns, we extracted detection data from the acoustic telemetry arrays in the Tosenfjord and Skjerstadfjord. Network analyses were conducted for each individual to summarise their use of the available receiver array. From individual networks, we calculated mean betweenness, mean degree, and diameter. Betweenness is a measure of the shortest paths through a receiver and is measured for each receiver, degree is the number of other receivers visited directly after visiting that given receiver, and the diameter is the shortest distance through all nodes (receivers) in a network (Csardi and Nepusz 2006). Network metrics were compared between fjords using a t test with the *t.test* function in R.

3. Time spent in freshwater

The proportion of time spent in freshwater was calculated by subtracting the time between two detections and adding them for all receiver locations grouped by habitat type (river, estuary, fjord). This yielded an estimated time interval spent in each habitat type, but we focused on freshwater.

Non-metric multidimensional scaling

Non-metric multidimensional scaling (NMDS) is an ordination method often used to analyze predictor variables explaining ecological community data based on species counts at sites (Oksanen et al., 2019). We implemented NMDS with the *metaMDS* function in the vegan package using 999 permutations, 100 iterations and three dimensions, rather than the default of two dimensions due to lack of convergence. We ordinated information on pathogens for 126 of

the 160 sea trout that were sampled, excluding all that had all zero pathogen values because NMDS would not run with rows having all zero values. Twelve of these 126 were captured by gill net and 114 were captured by angling. We were interested in relationships between these community data and the site as well as gene expression data and individual metrics. Gene expression data were ordinated onto the NMDS using the *envfit* function, which ordinates additional variables into the analysis. For visualisation, only significant genes ($P < 0.05$) are displayed with unscaled arrow segments. A second *envfit* function was passed to the NMDS to add individual information: length, condition factor, total detection interval (days from first to last detection), proportion of time in freshwater, and three network analysis summary statistics (degree, betweenness, diamater; see above for calculation details in *Summarising Observed Movement*). Two fish were missing condition factor; instead of deleting them, we imputed them as having the mean of the fish from that fjord. To test results of the NMDS we used permutated analysis of variance (perMANOVA) implemented with the *adonis* function in vegan, with fjord, length, condition factor, total detection interval (days from first to last detection), proportion of time in freshwater, network betweenness, degree, and diameter as predictors. The perMANOVA was run with 999 permutations. Plots were drawn with ggplot2 (Wickham et al., 2016).

Results

Pathogen Data

All fish were sampled in freshwater rivers but had previously been to the ocean based on scale analyses. Eleven pathogens were detected, with at least one pathogen detected in 126 of the

158 fish tested (80%). The most common pathogen recorded in the sample was *Ichthyobodo* sp., present in 63% of the 158 sampled fish, followed by *Flavobacterium psychrophilum* (34%), *Candidatus* Branchiomonas cysticola (31%), and *Ichthyophthirius multifiliis* (18%; Table 3; Figure 2). Analysis of diversity was conducted on 158 individuals including individuals with no pathogens observed. Shannon diversity scores of pathogens ranged from 0-1.60 with a mean of 0.52. The individual having diversity=1.60 registered presence of five pathogens. Pathogen communities were more diverse ($t = 6.35$, $P < 0.01$) in the Skjerstadfjord (mean = 0.81 ± 0.50) than the Tosenfjord (mean = 0.32 ± 0.44). According to multilevel pattern analysis, *Flavobacterium psychrophilum* (stat = 0.62, $P = 0.01$) and *Piscichlamydia salmonis* were indicator species for the Skjerstadfjord. There were no significant indicator species for the Tosenfjord.

Summarising observed movements

Sea trout were tracked for a minimum of three and a maximum of 806 days (mean = 155 ± 132 d). Trout from the Tosenfjord were detected for a mean of 156 ± 102 d, similar to the Skjerstadfjord where they were tracked for a mean interval of 154 ± 166 d. By average, Tosenfjord trout spent 31% of the detections at freshwater receivers whereas Skjerstadfjord spent 18%. Overall only eight of 160 trout were never detected outside of freshwater, suggesting a 95% rate of marine migration in this sample. Eighty nine trout were detected between August and October (when they would be expected to return to spawn) following tagging (56%), only 45 of which were detected at freshwater receivers anytime in these months. A greater proportion of trout from the Tosenfjord (43%) were tracked to or beyond the beginning of the spawning

315 migration period in freshwater than in the Skjerstadfjord. The first survival analysis with all
316 terms failed the assumption of proportionality of hazards ($\chi^2 = 22.29$, $P < 0.01$) but the simple
317 single-term model revealed a significant difference between fjords with respect to return to
318 freshwater for spawning ($\chi^2 = 20.78$, $P < 0.01$; Figure 3).
319
320 *Non-metric multidimensional scaling*
321
322 Non-metric multidimensional scaling on the 126 individuals having non-zero pathogen
323 prevalence revealed significant overlap of disease profiles for individuals from the Tosenfjord
324 and the Skjerstadfjord and therefore no significant differences (Figure 4). Envfit revealed
325 significant associations with four of 11 smoltification genes on NMDS 1 and 2
326 (SMLT_CCL19_V1, SMLT_IL2B_V1, SMLT_WAS_V1, SMLT_CCL4_V1), two of eight viral
327 disease genes (VDD_GAL3_MGL2, VDD_MX_ONTS), three of six MRS genes (MRS_C7,
328 MRS_RPL7, MRS_NKA_B1), one of 14 heat shock genes (HX_PGK), and one of two
329 inflammation genes (INF_MMP25). No immune stimulation, general stress, osmotic stress,
330 stress-mortality, or thermal stress related genes were significantly associated with pathogens in
331 axis 1 or 2 of the NMDS (Figure 4). The smoltification family of genes being expressed are
332 consistent with NMDS2 positive fish being in freshwater for some time and ill-prepared for
333 saltwater entry at the time of tagging, consistent with the significantly longer post-release
334 "freshwater" residency displayed by these fish, as depicted by *envfit* layering in Figure 4. Also
335 consistent was the greater tendency of NMDS2 positive fish towards infection, especially with
336 freshwater transmitted agents (Table 1). Alternately, the negative end of NMDS3 revealed a
337 clear signature consistent with viral infection (up-regulation of multiple genes within the viral

disease development [VDD] panel), although this signature was not associated with any viruses in our panel.

The *envfit* revealed that the percentage of time in freshwater, receiver network diameter, and condition factor of the fish were significant along NMDS 1 (Figure 4). Condition factor was ordinated opposite time in freshwater, suggesting that fish in higher condition spent less of their time in freshwater. Cross-validation with perMANOVA to test for associations between the ordinated infection metrics and putative predictors indicated that pathogen community was related to time in freshwater ($F = 3.61$, $P = 0.01$), and fjord of origin ($F = 2.86$, $P = 0.03$).

Discussion

The pathogen data described in this paper represents an important baseline evaluation for these northern Norwegian fjord communities that are anticipated to change in the near future. Indeed, infectious disease risk worldwide is expected to increase for wild animals and re-evaluation of the host-pathogen dynamics in this fjord may soon reveal changes (Harvell et al., 2002; Altizer et al., 2013). Fish in open net-pen aquaculture also represent important host reservoirs from which pathogens can spillback to wild populations and vice versa (Krkošek, 2017). Potential to intensify aquaculture operations in these northern regions of Norway will import a high density of potential hosts that could harbour pathogens relevant to wild salmonids; importantly, these will likely include those we found to be rare or absent from these populations at this time. Sea trout may be particularly vulnerable to the impacts of pathogens from aquaculture given that they spend much of the marine phase of their life history in coastal zones and fjords where aquaculture operations are sited (Thorstad et al., 2016).

Five pathogens had relatively high prevalence in our sample. The most common was *Ichthyobodo* sp., a group of flagellate fish parasites causing ichthyobodosis (Isaksen et al., 2010; Isaksen, 2013). Records of ichthyobodosis in fish farms exist for over a century and species from the complex have been recorded infecting brown trout (Isaksen et al., 2010, 2012). The two primary species are *I. necator*, a freshwater species, and *I. salmonis*, a euryhaline species that can affect salmonids in both the marine and freshwater environments (Isaksen et al., 2010, 2011). The ciliate *Ichthyophthirius multifiliis* is the etiological agent of white-spot disease and is more prevalent at warmer water temperatures (Bass et al., 2017). *Ichthyophthirius multifiliis* seems to proliferate at high host density such as on spawning grounds and in hatcheries (Bass et al., 2017). In Pacific salmon, *I. multifilis* can be a major cause of pre-spawning mortality (Traxler et al., 1998). *Flavobacterium psychrophilum* was one of the most prevalent pathogens infecting sea trout in the northern Norwegian fjords. This is a cosmopolitan fish pathogen that causes bacterial cold-water disease in salmonids with highest virulence at temperatures < 15 °C (Nematollahi et al., 2003). *Flavobacterium psychrophilum* seems to covary with senescence in adult salmon and be a predictor of mortality in migrating juvenile and adult salmonids (Furey 2016; Bass et al., 2017; Teffer et al., 2017). Bass et al. (2017) suggested a link between *F. psychrophilium* and *Candidatus* Branchiomonas cysticola, a bacterium first described in Norwegian farmed salmon (Toenshoff et al., 2012). Among sea trout in our sample, however, the two bacteria only co-occurred in 20% of individuals. *Ca.* B. cysticola is implicated in the formation of epitheliocysts in the gills and skin of salmon (Mitchell et al., 2013), recently also demonstrated in wild Chinook salmon [Di Cicco, unpublished data]). Twardek et al. (2019) identified high prevalence of both *Flavobacterium* and *Ca.* B. cysticola in steelhead (*Oncorhynchus mykiss*) returning to their spawning river in northern British Columbia, and found that the prevalence of both was

high among fish captured earlier in the migration below a natural barrier than above the barrier by recreational anglers. Therefore, these two pathogens may be implicated in premature mortality of migrating fish.

We anticipated that pathogens identified in sea trout in our sample would correlate to the migration of fish in the Tosenfjord and Skjerstadfjord as observed by acoustic telemetry. On the contrary, we found that the infections were inconsistently related to the movement patterns we observed. We did, however, identify the proportion of time spent in freshwater after release to be significantly related to the pathogen community based on NMDS and perMANOVA. Based on the ordinations, it seemed that Ichthyobodo was perhaps associated with time in freshwater. Ectoparasitic salmon lice (*Lepeophtheirus salmonis*) can alter behaviour of sea trout (Thorstad et al., 2015), causing them to move more frequently back to freshwater. Mechanistic details of the action of certain pathogens on the energy processing and swimming power/endurance is lacking except for Pacific salmonids, for which pathogens have been shown to influence the likelihood of en-route mortality of smolts leaving rivers (Miller et al., 2014; Jeffries et al., 2014; Furey, 2016) and adults returning to spawn (Teffer et al., 2017). There are also key interactions between pathogen communities and stressors, suggesting that pathogens increase susceptibility to disturbances (Teffer et al., 2017; Bass et al., 2019). Although this was beyond the scope of our study, it is relevant to note that such effects have been noted elsewhere.

Gene expression data revealed some potentially important details about each individual's acclimatization to freshwater and exposure to pathogens. The majority of the osmoregulatory (SMLT) genes up-regulated in fish portioning in the upper right corner of Figure 4a are expressed at higher levels in fish comfortable in freshwater (e.g. these are down-regulated in smolts). Our samples were predominantly taken from trout presumed to be migrating out of freshwater, which

had already been at sea the year before and had overwintered in freshwater. We did not know the history of these fish, but expect that they entered freshwater from August-October the previous year. We could speculate that fish in the upper right corner of Figure 4a have been in rivers for longer than those partitioning in the lower left. This is consistent not only with the genes being expressed, but also with the pathogens that are more (*I. hoferi* and *F. psychrophilum*), and less (*P. salmonis*), prevalent in these fish according to the NMDS plot.

We did not find significant activation of genes associated with stress and immunity in infected fish based on NMDS. Three genes with role in intracellular, largely but not exclusively viral (if a fuller range of VDD genes were not differentially expressed), responses were upregulated in the bottom of Figure 4a—Mx and GAL3. Smoltification and the physiological preparation for moving to sea are expected to coincide with a downregulation of the immune response (Houde et al., 2019). These gene signatures are clustering most strongly with *Tetracapsuloides bryosalmonae*, an agent transmitted from freshwater bryozoans that causes proliferative kidney disease. This is consistent with the freshwater affinity of the fish at the top right corner of the plot. Given that this parasite is only known to impact kidney tissue, differential immune stimulation in the gill is unlikely to be related to *Tetracapsuloides bryosalmonae* infection. Disease data could be prone to survivor bias given that most of the sampling was in the springtime and overburdened individuals could have died during winter. We did resolve a signature of viral disease development on NMDS3 that has been previously shown to predict fish that are responding to an RNA viral infection (Miller et al., 2017). Whereas none of the viruses on our panel were associated with this signature, it is possible that these fish were responding to a virus not on our panel; this panel of genes has, in fact, led to the successful discovery of several uncharacterized viruses (see Mordecai et al., 2019). Future research should

sample fish at sea or returning to rivers to spawn in the summer and autumn to more accurately reflect the influence of marine pathogens on sea trout, which were likely underrepresented in our study given sampling took place in freshwater.

We only had two fjords for comparison but found a significant difference between fjords in terms of the pathogen community composition and diversity, with significantly higher diversity in the more northerly Skjerstadfjord. The mechanisms for the differences are unclear, and a greater number of samples from different fjords could help elucidate what spatial and environmental factors contribute to the fish pathogen communities. Spatiotemporal pathogen dynamics are important to explore, particularly as these areas are facing climate change, which is expected to affect northern marine habitats more drastically (Burrows et al., 2011). Fjords in Norway are connected by marine species that migrate and disperse, which can be vectors for pathogens that generate local hotspots in the landscape. Human activities can influence the presence of pathogens and aquaculture operations, for example, can import pathogens or provide a reservoir in which pathogens can thrive and be transmitted to wild fish (Jones et al., 2015; Wiik-Neilsen et al., 2017). More research on the presence and prevalence of these pathogens in wild and farmed salmonids in other Norwegian fjords is important for comparison with our results to develop an understanding of the factors limiting the distribution of relevant salmonid pathogens. As conditions in these fjords continue to change with increasing human influence and climate change, sustained monitoring of these populations will be useful to track changes compared to our results that can act as a baseline for this area.

There are some important limitations to our study that merit expansion and further investigation. Our screening was limited to 46 pathogens and we selected candidates of interest, which may have excluded some potential pathogens. Notably, we did not know the exact history

of the tagged fish with respect to their previous time spent at sea or area occupied although all were exposed to marine pathogens before based on scale analysis. Although Bass et al. (2017) suggested sex is an important factor contributing to the pathogen community infecting chinook salmon, we were unable to include this factor in our analysis because of incomplete information about sex from several individuals. We were unable to determine the precise age or prior history of enough fish that we sampled for this study, complicating some interpretations of our findings because modelling would exclude several individuals with uncertain age or sex and we opted for a simpler model with greater sample size. Larger individuals tend to be older, but body length was not significant in any analyses. This is somewhat counterintuitive given that larger individuals, if indeed they are older, should have had longer exposure to potential pathogens and more likely had previous marine exposure. However, there is survivor bias in this given that the large individuals sampled were non-random. Indeed, angling may be selective against pathogens; Twardek et al. (2019) found that angling selected for individuals with lower pathogen loads than net fishing, although there may have been spatial sampling bias.

Conclusions

The importance of host-pathogen dynamics in structuring ecosystems is increasingly recognized and the potential influence of pathogens on host populations is receiving attention as an avenue to understand population dynamics (Miller et al., 2014) yet limited research has been conducted on the pathogen profiles of fish in northern areas. We applied salmon Fit-Chips, recently developed in the Miller laboratory, for this study. Fit-Chips contain curated host biomarker panels predictive of shifts in immune status, specific and general stress responses,

smolt readiness, and imminent mortality (death within 24-72 hours) for application across salmonid species. Pairing individual data with pathogen and host biomarker data using Fit-Chips provides a generalizable method for rapidly assessing the status of individual salmonids to investigate whole animal "health" status and test hypotheses about population-level responses through the lens of the individual. Fit-Chips have been recently for salmonids in the Pacific, Arctic, and Atlantic Oceans and have great potential to improve our understanding of fish and food web ecology in these regions. Our research revealed relatively limited diversity of viruses, bacteria, and parasites among anadromous brown trout sampled from rivers in two fjords in northern Norway. We focus on the eleven pathogens that had positive tests in our sample but equally important is the large number of pathogens not present in the two fjords we sampled, but that have the potential to colonize as increasing human activity and climate add new stressors to these areas. Molecular signatures of pathogens including *Gyrodactylus salaris*, salmon gill pox virus, viral hemorrhagic septicemia, and *Yersinia ruckeri* (enteric redmouth) is important particularly as *G. salaris* causes substantial economic damage among wild Norwegian salmon. *Ichthyobodo*, *F. psychrophilum*, and *Ca.* B. cysticola were the most prevalent pathogens, all of which are relatively cosmopolitan and likely endemic. The baseline provided by this research should generate new opportunities for comparing pathogen communities of salmonids in other areas of Norway and in southern populations along the coast of Europe as well as temporal contrast in the future when changes to the pathogen community could precipitate from changing conditions in these northern regions of Norway.

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Data Availability

The data that support the findings of this study will be made publicly available through the Ocean Tracking Network database for animal telemetry data following publication of the data.

Conflict of Interest

The authors declare no competing interests.

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Tables

Table 1. High throughput qPCR screened for the following bacteria, parasites, and viruses from sea trout *Salmo trutta* in northern Norway. Agents are presented with their pathogen type, assay name, and primer codes.

Agent Name	Assay Name	Agent Type	Forward Primer	Reverse Primer	MGB-Probe-6fam
Aeromonas salmonicida	ae_sal	Bacterium	TAAAGCACTGTCTGTTACC	GCTACTTCACCCTGATTGG	ACATCAGCAGGCTTCAGAGTCACTG
Atlantic Salmon Calici Virus	ascv	Virus	ACCGACTGCCCGGTTGT	CTCCGATTGCCTGTGATAATACC	CTTAGGGTTAAAGCAGTCG
Atlantic salmon paramyxovirus	aspv	Virus	CCCATATTAGCAAATGAGCTCTATCTT	CGTTAAGGAACTCATCATTGAGCTT	AGCCCTTTTGTTCTGC
Candidatus Branchiomonas cysticola	c_b_cys	Bacterium	AATACATCGGAACG	GCCATCAGCCGCTCATGTG	CTCGGTCCCAGGCTTTCCTCTCCCA

			TGTCTAGTG		
Coronavirus (Nidovirus)	cov	Virus	GGATAATCCCAACCGAAAAGTTT	GCATGAAATGTTGTCTCGGTTTAA	CGATCCCGATTATC
Dermocystidium salmonis	de_sal	Parasite	CAGCCAATCCTTTCGCTTCT	GACGGACGCACACCACAGT	AAGCGGCGTGTGCC
Flavobacterium psychrophilum	fl_psy	Bacterium	GATCCTTATTCTCACAGTACCGTCAA	TGTAAACTGCTTTTGCACAGGAA	AAACACTCGGTCGTGACC
Gyrodactylus salaris	gy_sal	Parasite	CGATCGTCACTCGGAATCG	GGTGGCGCACCTATTCTACA	TCTTATTAACCAGTTCTGC
Ichthyobodo spp.	icd	Parasite	ACGAACTTATGCGAAGGCA	TGAGTATTCACTYCCGATCCAT	TCCACGACTGCAAACGATGACG

Ichthyophonus hoferi	ic_hof	Parasite	GTCTGTAC TGGTACG GCAGTTTC	TCCCGAAC TCAGTAGA CACTCAA	TAAGAGCAC CCACTGCCTT CGAGAAGA
Ichthyophthirius multifiliis	ic_mul	Parasite	AAATGGG CATACGTT TGCAAA	AACCTGCC TGAAACAC TCTAATTTT T	ACTCGGCCTT CACTGGTTCG ACTTGG
Infectious pancreatic necrosis virus	ipnv	Virus	GCAACTT ACTTGAG ATCCATTA TGCT	GAGACCTC TAAGTTGT ATGACGAG GTCTCT	CGAGAATGG GCCAGCAAG CA
Infectious salmon anemia virus	isav7	Virus	CAGGGTT GTATCCAT GGTTGAA ATG	GTCCAGCC CTAAGCTC AACTC	CTCTCTCATT GTGATCCC
Loma salmonae	lo_sal	Parasite	GGAGTCG CAGCGAA GATAGC	CTTTTCCTC CCTTTACTC ATATGCTT	TGCCTGAAA TCACGAGAG TGAGACTAC CC

Moritella viscosa	mo_vis	Bacterium	CGTTGCGAATGCAGAGGT	AGGCATTGCTTGCTGGTTA	TGCAGGCAAGCCAACTTCGACA
Myxobolus insidiosus	my_ins	Parasite	CCAATTTGGGAGCGTCAAA	CGATCGGCAAAGTTATCTAGATTCA	CTCTCAAGGCATTTAT
Nanophyetus salmincola	na_sal	Parasite	CGATCTGCATTTGGTTCTGTAACA	CCAACGCCACAATGATAGCTATAC	TGAGGCGTGTTTTATG
Neoparamoeba perurans	ne_per	Parasite	GTTCTTTCGGGAGCTGGGAG	GAACTATCGCCGGCACAAAAG	CAATGCCATTCTTTTCGGA
Oncorhynchus masou herpes virus	omv	Virus	GCCTGGACCACAATCTCAATG	CGAGACAGTGTGGCAAGACAAC	CCAACAGGATGGTCATTA
Parvicapsula pseudobranchicola	pa_pse	Parasite	CAGCTCCAGTAGTGTATTTCA	TTGAGCACTCTGCTTTATTCAA	CGTATTGCTGTCTTTGACATGCAGT

Paranucleospora theridion	pa_ther	Parasite	CGGACAGGGAGCATGGTATAG	GGTCCAGGTTGGGTCTTGAG	TTGGCGAAGAATGAAA
Piscichlamydia salmonis	pch_sal	Bacterium	TCACCCCCAGGCTGCTT	GAATTCCATTTCCCCCTCTTG	CAAAACTGCTAGACTAGAGT
Piscirickettsia salmonis	pisck_sal	Bacterium	TCTGGGAAGTGTGGCGATAGA	TCCCGACCTACTCTTGTTTCATC	TGATAGCCCCGTACACGAAACGGCATA
Piscine myocarditis virus	pmcv	Virus	AGGGAACAGGAGGAAGCAGAA	CGTAATCCGACATCATTTTGTGA	TGGTGGAGCGTTCAA
Piscine orthoreovirus	prv	Virus	TGCTAACACTCCAGGAGTCATTG	TGAATCCGCTGCAGATGAGTA	CGCCGGTAGCTCT
Renibacterium salmoninarum	re_sal	Bacterium	CAACAGGGTGGTTATTCTGCTTTC	CTATAAGAGCCACCAGCTGCAA	CTCCAGCGCCGCAGGAGGAC

Strawberry disease (Rickettsia-like organism)	rlo	Bacterium	GGCTCAA CCCAAGA ACTGCTT	GTGCAACA GCGTCAGT GACT	CCCAGATAA CCGCCTTCGC CTCCG
Salmon alphavirus 1, 2, and 3	sav	Virus	CCGGCCC TGAACCA GTT	GTAGCCAA GTGGGAGA AAGCT	TCGAAGTGG TGGCCAG
Salmon (Gill) chlamydia	sch	Bacterium	GGGTAGC CCGATAT CTTCAAA GT	CCCATGAG CCGCTCTCT CT	TCCTTCGGGA CCTTAC
Salmon Gill Pox Virus	sgpx	Virus	ATCCAAA ATACGGA ACATAAG CAAT	CAACGACA AGGAGATC AACGC	CTCAGAAAC TTCAAAGGA
Sphaerothecum destruens	sp_des	Parasite	GGGTATC CTTCCTCT CGAAATT G	CCCAAACT CGACGCAC ACT	CGTGTGCGCT TAAT
Spironucleus salmonicida	sp_sal	Parasite	GCAGCCG CGGTAAT TCC	CGAACTTTT TAACTGCA GCAACA	ACACGGAGA GTATTCT

Tetracapsuloides bryosalmonae	te_bry	Parasite	GCGAGATTTGTTGCATTTAAAAAG	GCACATGCAGTGTCCAATCG	CAAAATTGTGGAACCGTCCGACTACGA
Tenacibaculum maritimum	te_mar	Bacterium	TGCCTTCTACAGAGGGATAGCC	CTATCGTTGCCATGGTAAGCCG	CACTTTGGAATGGCATCG
Viral erythrocytic necrosis virus	ven	Virus	CGTAGGGCCCCAATAGTTTCT	GGAGGAAATGCAGACAAGATTTG	TCTTGCCGTTATTTCCAGCACCCG
Viral hemorrhagic septicemia virus	vhsv	Virus	AAACTCGCAGGATGTGTGCGTCC	TCTGCGATCTCAGTCAGGATGAA	TAGAGGGCCTTGGTGATCTTCTG
Vibrio anguillarum	vi_ang	Bacterium	CCGTCATGCTATCTAGAGATGTATTTGA	CCATACGCAGCCAAAAATCA	TCATTTCGACGAGCGTCTTGTTCAGC
Vibrio salmonicida	vi_sal	Bacterium	GTGTGATGACCGTTCCATATTT	GCTATTGTCATCACTCTGTTTCTT	TCGCTTCATGTTGTGTAATTAGGAGCGA

Yersinia ruckeri	ye_ruc_gln A	Bacterium	TCCAGCACCAAATACGAAGG	ACATGGCAGAACGCAGAT	AAGGCGGTTACTTCCCGGTTCCC

711

712 Table 2. Gene biomarkers, their biological function, primer sequences, and assay performance
713 metrics. MRS is the "mortality related signature" from Miller et al., 2011; VDD is a panel of
714 biomarkers predictive of a viral disease state from Miller et al. (2017); thermal biomarkers are
715 from Akbarzadeh et al (2018) and Houde et al. (2019a); hypoxia biomarkers are from Houde et
716 al. (2019a); stress-mortality is from Houde et al. (2019a); and Top smoltification biomarkers are
717 from Houde et al. (2019a,b).
718

Biomarker	Function	Forward Primer	Reverse Primer	MGB-Probe-6fam	R^2	Efficiency
HK_78d	Housekeep	GTCAAGACTGGAGGCTCAGAG	GATCAAGCCCCAGAAGTGTTTG	AAGGTGATTCCCTCGCCGTCCGA	0.99	107.36
HK_Coil-P84_R2_tm	Housekeep	GCTCATTTGAGGAGAAGGAGGATG	CTGGCGATGCTGTTCCTGAG	TTATCAAGCAGCAAGCC	0.99	104.00
HK_MrpL40_F1_tm	Housekeep	CCCAGTATGAGGCACCTGAAGG	GTTAATGCTGCCACCCTCTCAC	ACAACAACATCACCA	1.00	97.99

		GGGTCA				
		CACAGA	GCGCTCTA			
	General	AGCCAA	TAGCGTTG	AGACCAAGC		
GS_HSC70	Stress	AAG	ATTGGT	CTAAACTA	0.99	89.14
		TGGGCT				
		ACATGG	TCCAAGGT			
	General	CTGCCA	GAACCCA	AGCACCTGG		
GS_HSP90	Stress	AG	GAGGAC	AGATCAA	0.96	102.20
		TTGTTGC	CCTGTTGC			
		TGGTGA	CCTATGAA			
	General	GAAAAC	TTGTCTAG	AGACTTGGG		
GS_JUNB	Stress	TCAGT	T	CTATTTAC	0.99	105.17
		CGTGATT				
		CAGTGTT	TTCCTCCA			
		GTCATCT	GTGTTTTT	AAGTACATG		
HX_ALD_1	Hypoxia	TGA	TTCAGTCA	TGCCTTCTT	1.00	99.87
		GCCCCG				
		TGTGACT	TCGTCCCA	TCTACAAAT		
HX_COX6		GGTATA	TTTCTGGA	CACTGTGCC		
B1_19	Hypoxia	AG	TCCA	C	1.00	91.28

HX_ECE-2	Hypoxia	AGCAGACGCTGGGAGAGAAC	CACGCCTGGTACGCCTTATAG	CTGACAACGGAGGCC	1.00	92.82
HX_Enolase_2	Hypoxia	TGGCACAGAGAACAAGTCTAAGTTTG	CACCGGCCTTGCACACA	CCATCCTGGGCGTGTC	1.00	95.59
HX_GPX3	Hypoxia	AGGCCAGTCCTTCAGTGCAT	GGCAGGACCAGGAGGTAACA	TGGGCCTGGTAACC	0.99	87.16
HX_HIF1A_6	Hypoxia	AGAGGAGGCAGTGCTGTATTCAA	GGGACAAGGCCCTCCAAT	AGGGCCCTGACCATG	0.99	88.92
HX_MFHAS1	Hypoxia	CCGAGGCCTGGGTGAAC	TCAGCTGCTCCACAGAGAAGAA	TCAGTGGCTGCTAGTC	1.00	101.01

		TGTAGG	TCTTAACA			
		AGATGC	GAGCGAT	TGCTAAAGT		
		AGCCAC	GTTCAGCT	TCTCCTCTG		
HX_PAM	Hypoxia	AGA	T	AC	1.00	90.69
		CGAACC				
		AAGTGG	CCGGACAT			
HX_RAMP		TGCAAG	GCCTGGA	CTTCATCCA		
1	Hypoxia	ACT	AGA	GATCCATTC	1.00	95.15
		GAGAGT				
		ACAAGG	GCCCGCCG			
		CCATTAT	AGGACAA	CGGCAGGAG		
HX_SOX-5	Hypoxia	GAGGAA	G	ATGAG	0.97	83.74
		CTTCAA				
		GGTGCC	CGAGTGCT			
		TGAGAC	TCTCTCCA	CCGCCAGTC		
HX_glu1	Hypoxia	CAA	CCAGTAC	GGCT	1.00	106.04
		TGGAGG	GAAACAC			
		CGTTTGT	AGCAGGA			
		AGCTGA	AGGAACA	CCACCCTCA		
HX_PgK	Hypoxia	A	TAA	CATGCA	1.00	94.30

		TTCCAC	GTTTGTGT			
		ACGGAG	TGTAGTGA	TCCAGACTG		
		TCATCAT	AAGAGGT	TTTGAACTA		
HX_Ngb1	Hypoxia	GTT	TGAG	G	1.00	87.31
		AACTGT				
		CAGAAA	GAAGTATT			
		GCAGAA	CTCACACC	AAGTTTTTG		
		CTACTTC	GAGTCCTA	TCACCACTG		
HX_VEGFa	Hypoxia	CT	TCT	TAT	0.99	99.18
		ATTGGC	AGCTTCAG			
		CTGTCC	ATCAAGG	TGGAATCTG		
	Immune	AAAACA	AAGAAGT	TGTGTCTGA		
Im_C3	stimulation	CA	TC	ACCCC	0.99	98.16
		ACGCAC	CAGTGGA	TTGCCGTGT		
	Immune	CTTGAG	AACCAGC	CGCTGAGCT		
Im_C5aR	stimulation	GGTCATT	ACAGG	TCTT	0.99	98.20
		GTGGCG	CTTGTGGA			
		GCATTG	TACTTCTT	CACCATCAG		
	Immune	CTGATAT	ACTCCTTT	CTATGTCAT		
Im_CD83	stimulation	T	GCA	CC	1.00	97.20

		CGTCAT				
		CTGCAA	GGGCGTA	TGCAGCACA		
	Immune	AGATTG	GCTTCTGA	GATGTACTG		
Im_IFNa	stimulation	GA	AATGA	ATCATCCA	0.99	104.45
		CTTGGCT	GGCTAGTG	TGGAGAGAA		
	Immune	TGTTGAC	GTGTTGAA	CGAGCAGTT		
Im_IGMs	stimulation	GATGAG	TTGG	CAGCA	1.00	100.90
		AGGACA				
		AGGACC	CCGACTCC	TTGCTGGAG		
	Immune	TGCTCA	AACTCCAA	AGTGCTGTG		
Im_IL1B	stimulation	ACT	CACTA	GAAGAA	1.00	109.78
		ATCATC				
		CTGTCA	TCTGGTGC	TGCATCCCC		
	Immune	GCCCAG	AGTGGTA	TCTACACCC		
Im_ILIR	stimulation	AG	ACTGG	CAAA	1.00	92.96
		GCGACA				
		GGTTTCT	TGTCAGGT	TGGTGTCCT		
	Immune	ACCCCA	GGGAGCTT	GGCAGAAAG		
Im_MHCI	stimulation	GT	TTCTG	ACGG	0.99	93.19

		GGGAGA				
		TGATTCA	TTACGTCC	TCGAGGACA		
	Immune	GGGTTC	CCAGTGGT	CGAGGACTC		
Im_SAA	stimulation	CA	TAGC	AGCA	0.99	91.98
		GCCAGC	AGTCACCT			
	Inflammatio	GGAGCA	GGAGGCC	TCAGCGAGA		
Inf_MMP13	n	GGAA	AAAGA	TGCAAAG	0.99	108.18
		TGCAGT				
		CTTTTCC	TCCACATG			
	Inflammatio	CCTTGG	TACCCACA	AGGATTGGC		
Inf_MMP25	n	AT	CCTACAC	TGGAAGGT	0.98	113.64
		GATGCT				
		GACCAC	ACCTCTGT			
		ATCAAA	CCAGCTCT	AACTACCAG		
MRS_C7	MRS	CTGC	GTGTC	ACAGTGCTG	1.00	95.04
		CAAAGC		ACCTGATCG		
		CAGTAT	TTGTTTTC	CCAGTAGCA		
MRS_COM		GGACTG	TGCTGCCC	TGAGCATGT		
MD7	MRS	TTTCAG	CTCTA	AC	1.00	93.32

		TGCAGA				
		TGAGCTT	GCAGTAA	CTCAACGAT		
		GTTGTCT	AGATCTGC	GACATCCAC		
MRS_FYB	MRS	ACAG	CGTTGAGA	AGTCTCCCC	0.99	82.96
		CTTGTAA	TGGTGAA	TCTGTACTG		
		CAGTTC	GCATTTCT	AGCATCCCC		
		GACATG	GTATGTCA	GCACATTAC		
MRS_HTA	MRS	GCTTATT	A	A	1.00	96.68
		CGTCAA				
		GCTGAA	CCTCAGGG			
MRS_NKA		CAGGAT	ATGCTTTC	CCTTGGCCT		
_B1	MRS	CGT	ATTGGA	GAAGTTG	0.99	106.06
		CGCCAC	TCCTCAGC	AGATCCCCA		
		CACAAC	CTCTTCTT	AGACTCTGT		
MRS_RPL6	MRS	CAAGGT	CTTGAAG	CAGACGCCT	1.00	111.92
		GATGCC				
		GGAGGG	CCGACTGG			
		AAAAGA	CTCTTGGA	TCCAAGATG		
Os_PRLR	Osmotic	C	CTTG	TTGGCTGC	0.97	115.72

Os_RGS21	Osmotic	TCCCGACTACAGCGCAGAT	TCCTCAGGGCTAAGTCGTTCA	TTCCCAATCCCCC	0.99	99.82
SM_hsp90a_15_v2	Stress-Mortality	GACACGGTGTTGGGTTGGTT	TTGCAGTCAACTCTCCATGCA	TCATGTGCAACATAACAT	1.00	93.69
Tm_EEF2_ssa14	Thermal	AGGTCACAGCCGCCCTTAG	ACACAGTCTCTGTCTGCACACAC A	CGACTGCGTCTCAGGT	0.97	102.40
Tm_FKBP10_ssa3-6	Thermal	ACTATGAGAATGCCCCCATCAC	CTCGTCCAGACCCTCAATCAC	CCTGGGAGCCAACAA	0.99	109.21
Tm_FKBP10_ssa19	Thermal	CCTGAAGAGATCATTGCTGACATG	GACGATGACCCCATCCTTGT	TCAGGAACCAGGACCG	1.00	99.50

		ATGACC				
		CTCAGA	CCTCATCA			
Tm_HSP90		CACACT	ATACCCAG	CGCATCTAC		
AA1	Thermal	CCAA	TCCTAGCT	AGAATGA	0.99	89.44
		TTGGAT				
		GACCCT	CGTCAATA			
Tm_HSP90		CAGACA	CCCAGGCC	CCGAATCTA		
alike	Thermal	CACT	TAGCT	CCGGATGAT	1.00	87.49
		GCTCCCT	GCCTCCCT			
Tm_Map3k		GGGTTC	TCAGCAG	CCAGCAATA		
14	Thermal	ATGGAT	AGACA	GCTTATG	0.99	91.78
		GGTCATT	CCTAGATA			
		TTGGTTT	TAGCTATC	TGATACGTG		
Smlt_CA4_	Smoltificatio	TGTACA	CACGTACT	GTATAGAAA		
v1	n	CAGTCT	CACCTA	AG	0.97	105.46
		ACCTGG				
		GTTACA	TGGTTTCG			
Smlt_CCL1	Smoltificatio	GACCTG	TGGCATTT	CTCATGGAC		
9_v1	n	ATGAA	CTTG	CGCCTCA	0.99	96.08

		TCTCTTC ATTGCA	ACAGCAG			
Smlt_CCL4_v1	Smoltification	ACAATC TGCTT	TCCACGGG TACCT	CTACGCAGC AGCATT	1.00	93.50
		GGAATT TAGTGG	TCCCATCC			
Smlt_EEF2_v1_ssa23	Smoltification	ATGTCTG ACCATT	CTCACTCG TACAG	CCCATTCCTT CTATTCCT	0.99	98.93
		GGGCGT	GCATGCA			
Smlt_FKBP5_v1	Smoltification	TCCTCTG GGTGTA	GCATTCTC CTTTCT	ACAGGGCCA TGGAGA	0.99	111.81
		GTTTGG ATGTACT	GCACCCTC			
Smlt_FMNL1_v1	Smoltification	GGTGGA TTACCT	CAAGTCA AACGA	CTACGCCCA GTGTGAC	0.98	99.29
		GGAGCC TCCCAT	TGGCGTGG			
Smlt_IL12B_v1	Smoltification	GCTCTTA CT	ACCACTTT GAC	CCCCTCACA TTCCA	0.99	110.54

Smlt_NKAa1-a_v2	Smoltification	TGGAATCAAGGTTATCATGGTCACT	CCCACACCCTTGGCAATG	ATCATCCCATCACTGCGA	0.99	105.26
Smlt_NKAa1-b	Smoltification	TGAAGAAGTGGTGGTTGGAGATC	GGCAGAGACAATACGCAAATCA	TGAAAGGAGGAGATAGAAT	1.00	113.83
Smlt_NKAA1C	Smoltification	AGGGAGACGTACTACTAGAAAGCAT	CAGAACTTAAAATTCCGAGCAGCAA	ACAACCATGCAAGAACT	0.99	106.74
Smlt_WAS_v1	Smoltification	GCAGGAGCTCTATAACCAAATGGT	CAGCAAATGCGTGGAAGAAG	TACCACAGCCCCCGAC	1.00	102.47
VDD_DEXH_MGL3	Viral Disease	TGGAGAAGAAGGGTGTGACAGA	CGCAGGTGGAGAGCACACT	AGGAACAGACTGCTGGC	1.00	90.91

		AGGGAC				
		AACTTG				
		GTAGAC	TGACGCAC			
VDD_HER	Viral	AGAAGA	ACACAGCT	CAGTGGTCT		
C6	Disease	A	ACAGAGT	CTGTGGCT	1.00	97.59
		CCGTCA				
		ATGAGT	CACAGGC			
VDD_IFIT5	Viral	CCCTAC	CAATTTGG	CTGTCTCCA		
_MGL	Disease	ACATT	TGATG	AACTCCCA	1.00	95.17
		AGATGA				
		TGCTGC	CTGCAGCT	ATTCCCATG		
VDD_Mx_o	Viral	ACCTCA	GGGAAGC	GTGATCCGC		
nts	Disease	AGTC	AAAC	TACCTGG	1.00	106.17
		CCACTT				
		GCCAGA	CGTAACTG			
VDD_NFX	Viral	GCATGG	CCCAGAGT	TGCTCCACC		
_MGL2	Disease	T	GCAAT	GATCG	1.00	95.74
		TTGTAGC				
		GCCTGTT	TACACTGC			
VDD_GAL	Viral	GTAATC	TGAGGCC	CTTGGCGTG		
3_MGL2	Disease	ATATC	ATGGA	GTGGC	0.99	103.71

		GCTCTC				
VDD_VHS		GTAAAG	GGGCGAC			
VIP4_MGL	Viral	CCCCAC	TGCTCTCT	AAACTGCAC		
3	Disease	ATC	GATCT	GTCGCGC	1.00	95.52
		GCAAAC				
VDD_VHS		TGAGAA	CCGTCAGC			
V-	Viral	AACCAT	TCCCTCTG	TGTGGAGAA		
P10_MGL2	Disease	CAAGAA	CAT	GTTGCAGGC	1.00	97.19

719

720 Table 3. Summary of eleven pathogens detected in our samples from the Tosenfjord and
721 Skjerstadfjord complexes in northern Norway. Note that the designation of origin includes
722 information from >28,000 salmon surveyed for microbes in Canada; detections of microbes in
723 smolts prior to leaving freshwater have led to the identification of many agents previously only
724 studied in the marine environment being deemed freshwater and saltwater origin.
725

Code	Pathogen	Key Etiological Associations	Taxonomy	Origin	Description	Further Reading
pisck_sal	*Piscirickettsia salmonis*	Piscirickettsiosis	Bacterium	Marine	A bacterium of marine origin described from affected farmed coho salmon in Chile. Has been recorded affecting many salmonid species including farmed Atlantic salmon in Norway.	Fryer et al., 2003

ic_hof	*Ichthyophonus hoferi*		Protist	Freshwater and marine	A parasite of marine fishes shown to transmit vertically from herring to chinook salmon consumers. Rapidly increased in prevalence in the Yukon River causing pre-spawn mortality and spoilage of meat.	Zubchenko and Karaseva 2002; Kocan et al., 2004
pa_pse	*Parvicapsula pseudobranchicola*		Parasite	Freshwater and marine	A parasite first described in Norwegian Atlantic salmon farms with particularly high prevalence in northern regions of Norway with detection in chinook salmon in British Columbia. Affected fish appear lethargic and may develop ocular impairments.	Nylund et al., 2018

pch_sal	*Piscichlamydia salmonis*	Gill epitheliocystis	Bacterium	Marine	A chlamydia-like bacterium causing gill epitheliocystis in farmed Atlantic salmon	Draghi et al., 2004
ic_mul	*Ichthyophthirius multifiliis*	White-spot disease	Protozoan	Freshwater	Ciliate protozoan that is the etiological agent of white-spot disease in fish with greater virulence at warmer water temperatures. Suggested as a threat to chinook salmon runs in British Columbia.	Bass et al., 2017
aspv	*Atlantic salmon paramyxovirus*	Proliferative gill inflammation	Virus	Freshwater and marine	Isolated from gills having proliferative gill inflammation, an important cause of mortality in farmed Atlantic salmon.	Kvellstad et al., 2005

te_bry	*Tetracapsuloides bryosalmonae*	Proliferative kidney disease	Myxozoan	Freshwater	Myxozoan with an intermediate life stage in freshwater bryozoans and infects multiple species of salmonids. Virulence increases with water temperature.	Bass et al., 2017
fl_psy	*Flavobacterium psychrophilum*	Cold water disease	Bacterium	Freshwater	Common, globally-distributed species causing cold water disease. Prevalent in hatcheries and among many species, particularly at cold water temperatures. Load seems to increase with senescence in chinook salmon during the spawning migration.	Bass et al., 2017

sch	Gill chlamydia		Bacterium	Freshwater and marine	Recently described among chinook salmon in British Columbia, exists in relatively low prevalence among sampled farmed Atlantic salmon.	Laurin et al., 2019
c_b_cys	*Candidatus* Branchiomonas cysticola		Bacterium	Freshwater and marine	Common pathogen first described in Norway and found to be highly prevalent among farmed and wild salmon in British Columbia. Associated with gill epitheliosis in Norway and recently in BC.	Bass et al., 2017; Twardek et al., 2019
IcD	*Ichthyobodo* sp.	Ichthyobodosis	Flagellate	Euryhaline	A group of flagellate parasites causing ichthyobodosis in fish including salmonids. Known to occur among sea trout and several	Isaksen et al., 2010, 2011, 2012

					other species. Ichthyobodosis is an economically important disease in aquaculture. Two species are likely to occur here, *I. salmonis* and *I. necator*.	

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727

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Figures

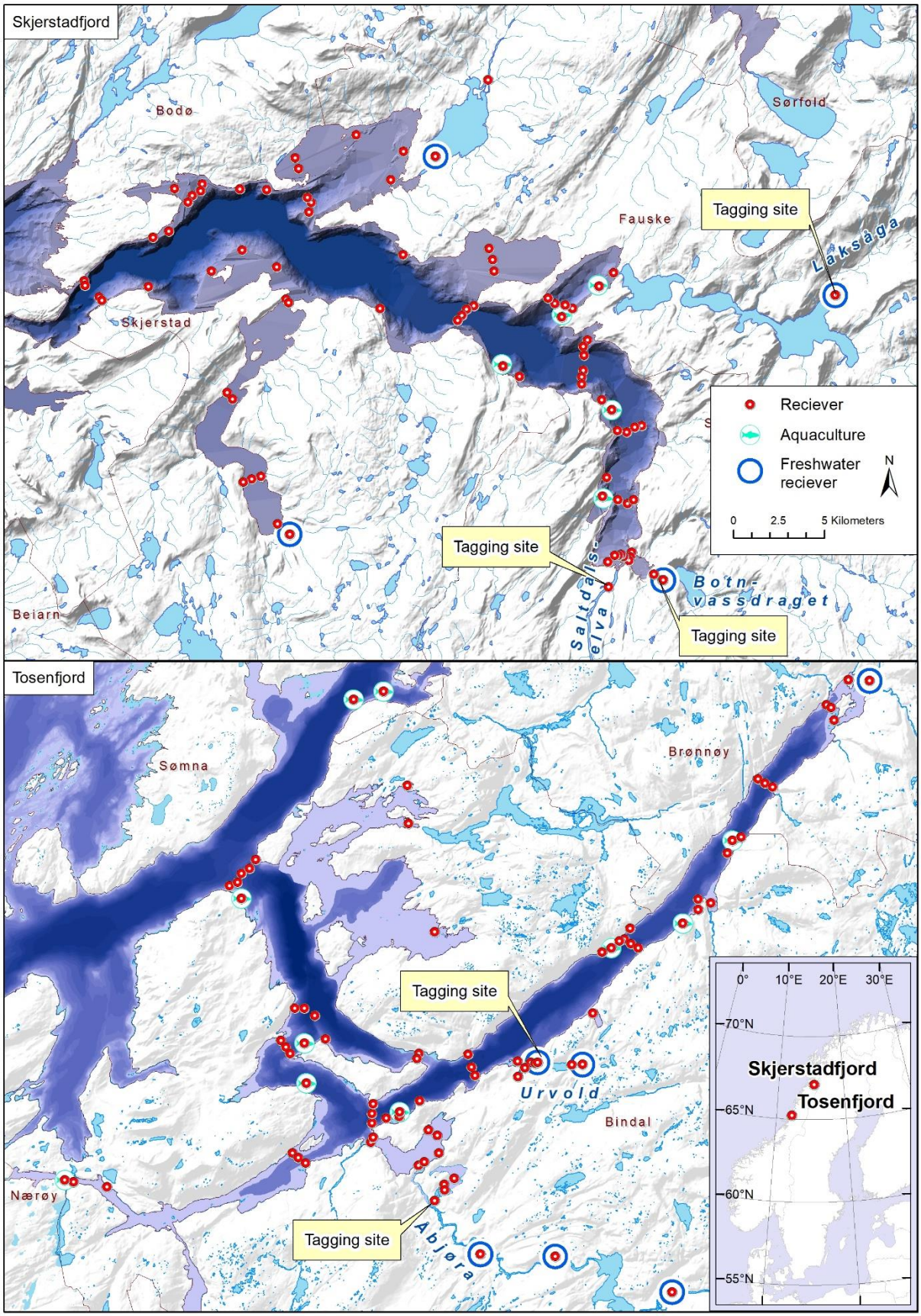
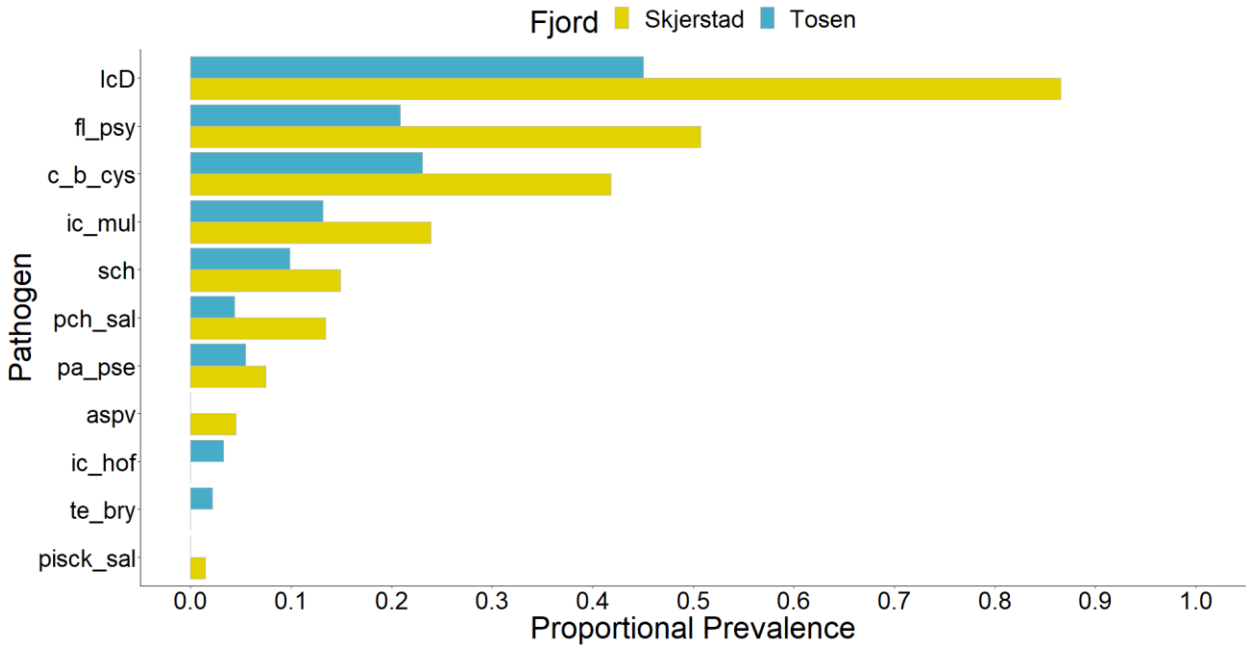

Skjerstadfjord
Bodø
Sørfold
Fauske
Tagging site
Laksåga
Skjerstad
Reciever
Aquaculture
Freshwater reciever
N
0 2.5 5 Kilometers
Tagging site
Saltdals-elva
Botn-vassdraget
Tagging site
Beiarn
Tosenfjord
Sømna
Brønnøy
Tagging site
Urvold
Bindal
0° 10°E 20°E 30°E
70°N
Skjerstadfjord
Tosenfjord
65°N
60°N
55°N
Nærøy
Tagging site
Abjøra

Figure 1. Map of the Skjerstadfjord and Tosenfjord in Norway. Each fjord is a drainage basin for rivers used by sea trout (*Salmo trutta*) that are returning to spawn. Fish were tagged in the rivers from March-May (a small subset tagged in September 2017) 2016 and 2017. Each fjord is covered by an array of acoustic receivers, which is noted in the map. The locations of active aquaculture sites are also indicated for reference.



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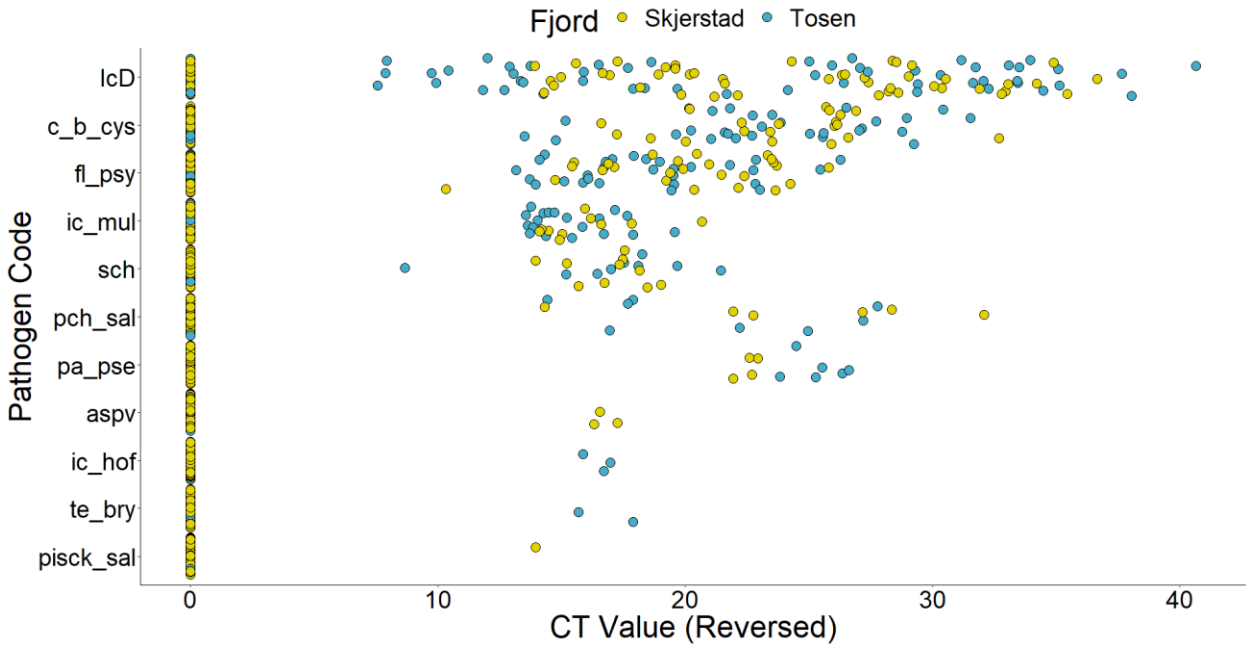

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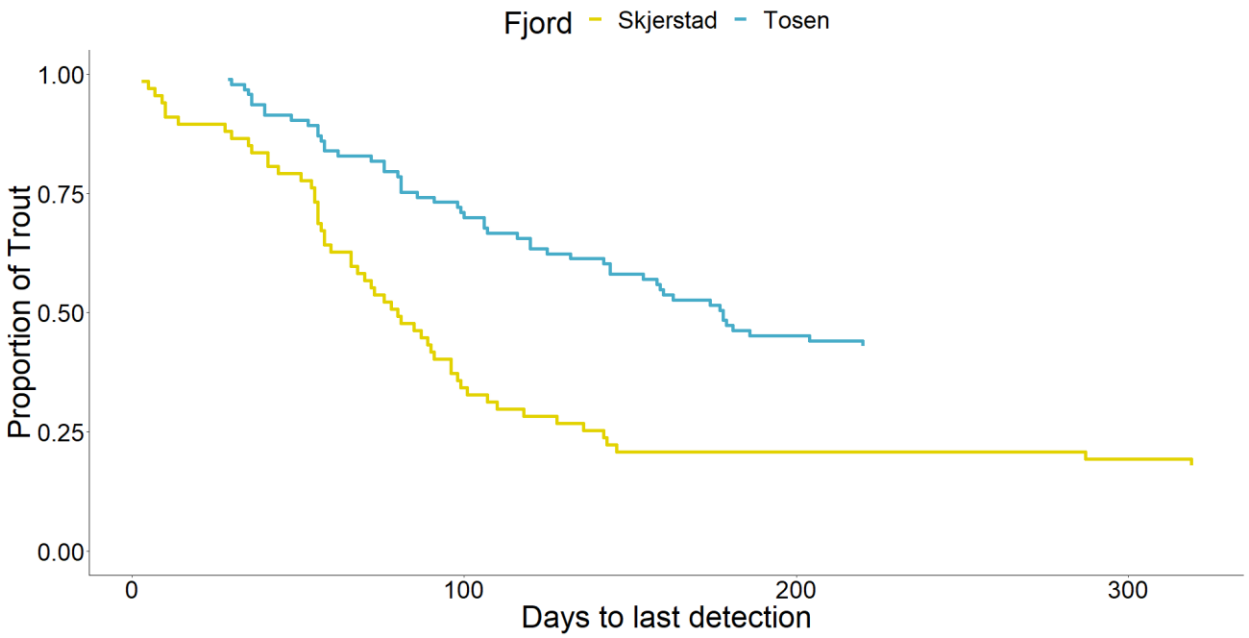
739

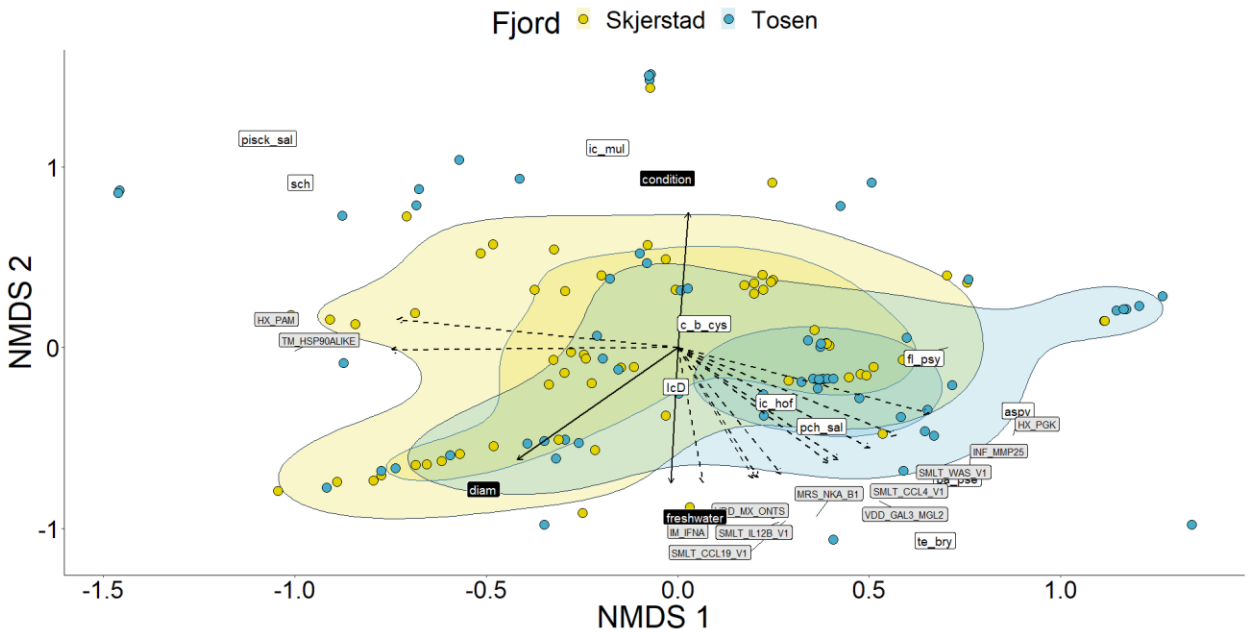
740 Figure 2. Pathogen prevalence and co-infections in sea trout (*Salmo trutta*) from the Skjerstadfjord
741 and Tosenfjord, Norway. Panel 1 shows the proportional prevalence of each pathogen in the
742 sample after excluding fish with no pathogens present. Panel 2 shows a measure of relative
743 pathogen load, depicted as 45 - raw copy numbers C_T (cycle threshold) of pathogens in each fjord,

such that null values are zero and high values approach the maximum possible value of 45 (points jittered to avoid overlap). Available in colour online only.



747

748 Figure 3. Detections of sea trout (*Salmo trutta*) tagged in the Skjerstadfjord and Tosenfjord,
749 Norway. Steps are made when a fish in each group was last detected. From the Tosenfjord, 54%
750 of sea trout were considered survivors compared to 30% of fish from the Skjerstadfjord. Available
751 in colour online only.


Fjord
Skjerstad
Tosen
pisck_sal
sch
ic_mul
condition
HX_PAM
TM_HSP90ALIKE
c_b_cys
fl_psy
lcD
ic_hof
pch_sal
aspv
HX_PGK
INF_MMP25
SMLT_WAS_V1
MRS_NKA_B1
SMLT_CCL4_V1
VDD_GAL3_MGL2
te_bry
diam
freshwater
IM_IFNA
SMLT_IL12B_V1
SMLT_CCL19_V1
NMDS 2
NMDS 1
1
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-1
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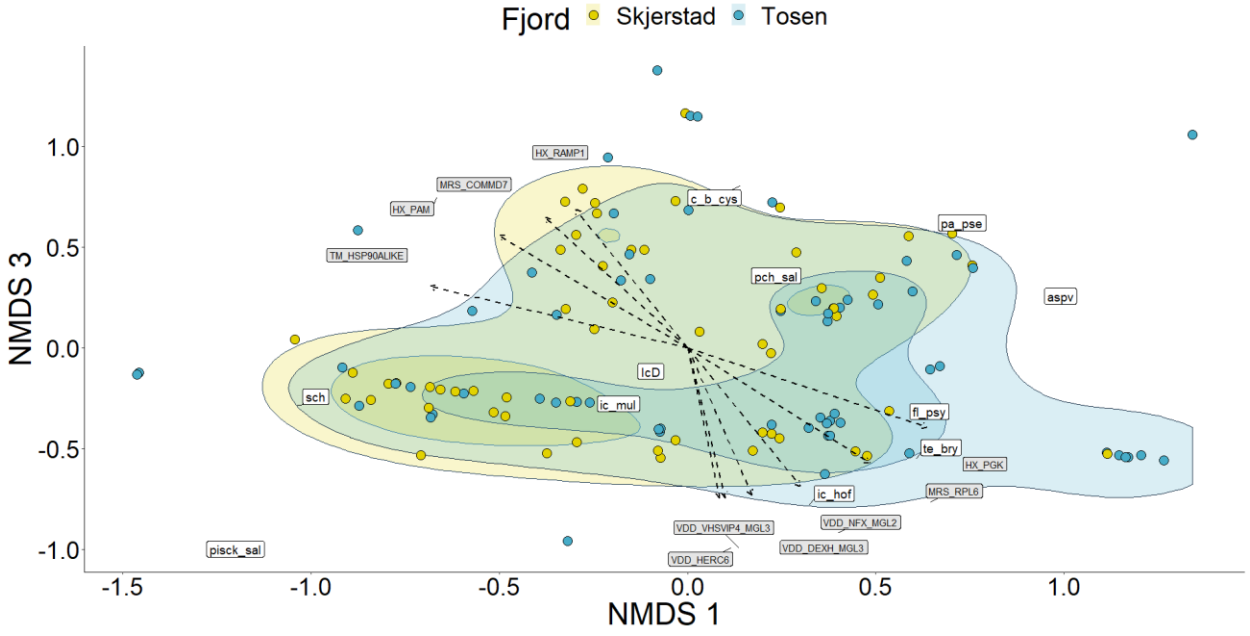
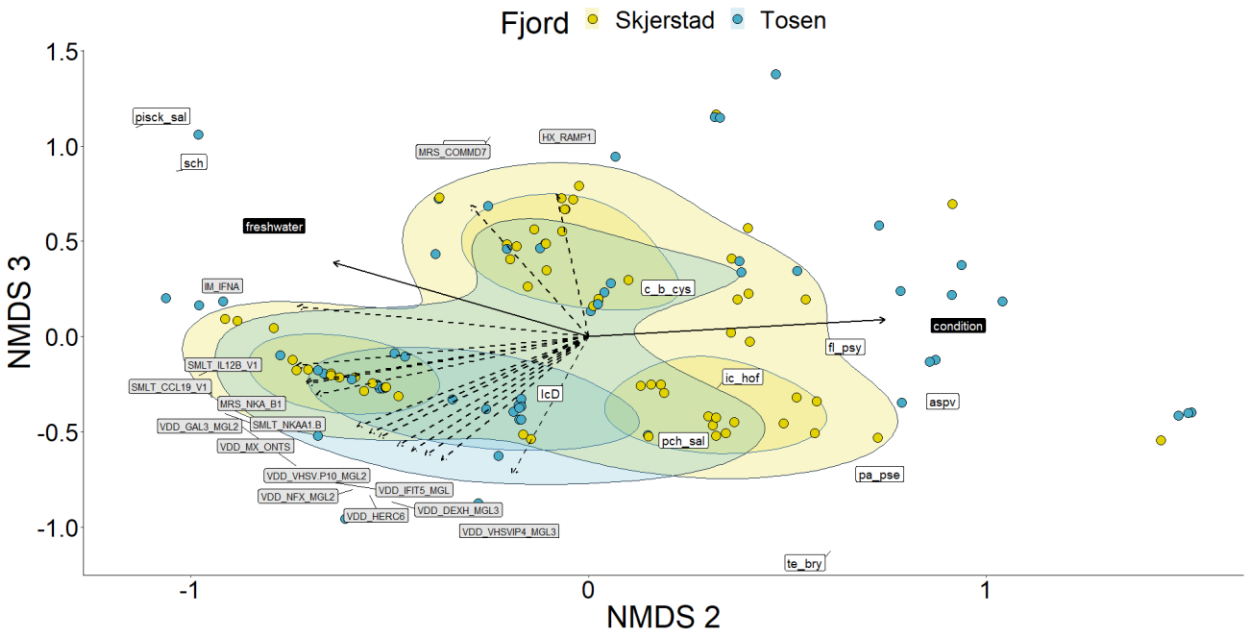
-1.0
-0.5
0.0
0.5
1.0

752


Fjord
Skjerstad
Tosen
HX_RAMP1
MRS_COMMD7
HX_PAM
c_b_cys
pa_pse
TM_HSP90ALIKE
pch_sal
aspv
lcD
sch
ic_mul
fl_psy
te_bry
HX_PGK
MRS_RPL6
ic_hof
VDD_VHSVIP4_MGL3
VDD_NFX_MGL2
VDD_DEXH_MGL3
VDD_HERC6
pisck_sal
NMDS 3
NMDS 1
1.0
0.5
0.0
-0.5
-1.0
-1.5
-1.0
-0.5
0.0
0.5
1.0

753



754

755 Figure 4. Biplots of non-metric multidimensional scaling (NMDS) results for sea trout (*Salmo*
756 *trutta*) sampled from the Skjerstadfjord (red) and Tosenfjord (blue), Norway. The NMDS was fit
757 with three axes, so three plots are shown to display all combinations. Pathogens (white text boxes),
758 gene expression (grey text boxes with dashed lines from origin), and individual metrics (black
759 boxes with black arrows from the origin) are shown in ordinated space. Density contours show the
760 positions of fish from each fjord (according to contour colour) in ordinated space. Note that only
761 genes (grey) and individual metrics (black) deemed significant by the *envfit* call are included on
762 the plot. Refer to Table 1 for pathogen codes. Available in colour online only.