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Interactions Between Sea Trout and Farmed Salmonids

Risk of pathogen exchange with special emphasis on Piscine orthoreovirus (PRV)

Master’s thesis in Ecology, Evolution, Behaviour, and Biosystematics
Supervisor: Jan Grimsrud Davidsen
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Norwegian University of Science and Technology
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Abstract

Understanding pathogen exchange between farmed and wild salmonids is important to determine ecological impacts from aquaculture on wild populations. A risk assessment of pathogen exchange was therefore conducted by investigating residency of anadromous brown trout (sea trout) around ten marine farm facilities with Atlantic salmon in three different fjord systems using acoustic telemetry, analysing spatiotemporal marine behaviour in combination with analyses of a hydrodynamic dispersal model in one of these fjord systems, and finally investigate fish pathology in wild juvenile brown trout from 15 freshwater systems draining to the three fjord systems. Heart and skeletal muscle inflammation (HSMI) and its causative agent Piscine orthoreovirus (PRV) were chosen as a model for this assessment.

In this study, there were no indication of sea trout being attracted to the salmon farms in Hemnfjorden, Tosenfjorden, or Skjerstadfjorden, in central and northern Norway. However, in Tosenfjorden, the sea trout resided within the fjord system during the main season of HSMI-disease outbreak in salmon farms and the hydrodynamic dispersal model indicated that local water currents could have transported pathogens up to 90 km from the source at surface and 450 km at ~30 m depth. Screening of PRV-1 in wild juvenile brown trout from 15 freshwater systems draining to the three fjord systems with aquaculture provided no evidence of PRV-1 reservoirs in the juvenile fish that hadn’t been to sea yet.

Based on brand-new knowledge about PRV-genotypes and host-susceptibility, the genotype PRV-1 is unlikely to infect sea trout. However, some genotypes could infect across salmonid species, and PRV-3 may infect between sea trout and farmed rainbow trout. The frequency of disease-outbreaks in Norwegian aquaculture is likely to increase with the annual increase in farmed salmonid production, further elevated by ongoing climate change, and the risk of transmission to sea trout is therefore likely to increase with it.
Sammendrag

Forståelse av patogenutveksling mellom oppdrett og ville laksefisker er viktig for å stadfeste økologiske påvirkninger fra akvakultur på ville populasjoner. En risikovurdering av patogenutveksling ble derfor utført ved å undersøke oppholdstiden til anadrom brunørret (sjøørret) rundt ti oppdrettsanlegg med atlantisk laks i tre forskjellige fjordsystemer ved bruk av akustisk telemetri, analyse av marin spatiotemporal adferd i kombinasjon med analyser av en hydrodynamisk spredningsmodell i et av disse fjordsystemene, samt undersøkelse av fiskpatologi i villedyrbrunørret fra 15 ferskvannssystemer som drenerer til de tre fjordsystemene. Hjerte- og skjelettmuskelbetennelse (HSMB) og dets smitteagens Piscine orthoreovirus (PRV) ble valgt som modell for denne vurderingen.

I denne studien var det ingen indikasjoner på at sjøørret ble tiltrukket av oppdrettsanleggene i Hemnfjorden, Tosenfjorden eller Skjerstadfjorden i Midt- og Nord-Norge. I Tosenfjorden oppholdte imidlertid sjøørreten seg i fjordsystemet under høysesong av HSMB-utbrudd i oppdrettsanlegg og den hydrodynamiske spredningsmodellen indikerte at lokale vannstrømmer kunne transportere patogener opp til 90 km fra kilden ved overflaten og 450 km på ~ 30 m dyp. Screening av PRV-1 i vill brunørrett-yngel fra 15 ferskvannssystemer som drenerer til de tre fjordsystemene med oppdrettsanlegg, ga ingen bevis på PRV-1 reservoarer i ungfisken som enda ikke hadde vært i sjø.

Basert på helt ny kunnskap om PRV-genotyper og vertsmottakelighet, så er det usannsynlig at genotypen PRV-1 kan infisere sjøørret. Noen genotyper kan derimot smitte på tvers laksefiskarter, og PRV-3 kan muligens smitte mellom sjøørret og oppdrettet regnbueørret. Frekvensen av sykdomsutbrudd i norsk oppdrettsnæring vil trolig øke med den årlige produksjonsøkningen av oppdrettslaks, ytterligere forhøyet av pågående klimaendringer, og risikoen for smitteoverføring til sjøørret vil derfor trolig øke med den.
Acknowledgements

First and foremost, I would like to express my deepest gratitude to my supervisor Jan Grimsrud Davidsen for giving me the opportunity to study this field of science of which I am so passionate about. Thank you for lending me your expertise and for your outstanding guidance.

This study would not have been possible without the contribution and collaboration of a wide collection of students, scientists, technicians, and aquaculture employees, of which I am very grateful. My gratitude goes to Sintef AS and Grim Eidnes for lending of equipment and software. A special thank you also goes to Aslak Sjursen, Lars Rønning, Marc Daverdin, and Sindre Eldøy for helping me during field work and for sharing their expertise. Also, thank you Anders Kolstad for your tremendous help in the statistical analysis.

Last, but not least, a wholehearted thank you to my friends, my family, and to my special someone. Thank you for the hugs, the encouragement, the laughs, the backrubs, and, of course, for all the wine. An especially deepfelt thank you goes to my dearest friend Trude Sofie Balsvik: your support and wonderfully disturbed sense of humour has been invaluable during my entire university experience.
Preface

In the current study, I have included several datasets from the research program “The secret life of sea trout”, spanning over several years. My part of the fieldwork included tagging of sea trout, downloading, and maintenance of acoustic receivers in Tosenfjorden and Skjerstadfjorden from spring 2017 to fall 2018. Additionally, I collected juvenile brown trout through electro-fishing in freshwater systems surrounding Hemnfjorden, Tosenfjorden and Skjerstadfjorden. I also extracted and conserved heart tissues from these juveniles for pathogen analysis.
# List of Abbreviations

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AIC</td>
<td>Akaike's Information Criterion</td>
</tr>
<tr>
<td>HSMI</td>
<td>Heart and skeletal muscle inflammation</td>
</tr>
<tr>
<td>ISA</td>
<td>Infectious salmon anaemia</td>
</tr>
<tr>
<td>ISAV</td>
<td>Infectious salmon anaemia virus</td>
</tr>
<tr>
<td>PCR</td>
<td>Real time polymerase chain reaction</td>
</tr>
<tr>
<td>RDCP</td>
<td>Recording Doppler Current Profiler</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>PRV</td>
<td>Piscine orthoreovirus</td>
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<tr>
<td>PRV-3</td>
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1. Introduction

1.1 THE COMPLEX LIFE OF BROWN TROUT

The life-history of brown trout (Salmo trutta) consists of both fresh water residential and anadromous strategies (hereafter termed sea trout) (Jonsson, 1985; Berg & Berg, 1989), where the latter encompasses great variation in timing and duration of migration, and the spatial residency (Eldøy et al., 2015; Thorstad et al., 2016; Bordeleau et al., 2018). Both resident brown trout and sea trout spawn in freshwater, remaining there throughout their juvenile phase (Jonsson, 1985; Jonsson & Jonsson, 2011), while sea trout will after 1-8 years in fresh water undertake short or extended feeding migration to marine environments (Thorstad et al., 2016). In mid- and northern Norway, most sea trout has a yearly migratory route, where smolts and veterans usually migrate in spring and reside in coastal areas near their home river for 1-4 months before returning to the same watercourse to spawn and/or overwinter (Eldøy et al., 2015; Flaten et al., 2016). However, as in more temperate areas, veteran sea trout in mid- and northern Norway may also utilize the estuarine and marine environment during winter (Jensen & Rikardsen, 2012; Aldvén & Davidsen, 2017). Sea trout in central Norway have shown to be generally surface oriented, mainly residing in the first 5 m depths (Eldøy et al., 2017). Sea trout has a precise homing behaviour to natal rivers and spawning grounds (Jonsson & Jonsson, 2011), and it has been suggested that anadromy might be moderately influenced by epigenetics - meaning, anadromy could be genetically inherited and influenced by environmental variables (Jonsson & Jonsson, 2011). Growth and size are major determinants of reproductive success in fish; a larger female has a greater amount of energy to allocate for egg production consequently increasing its fecundity (Gross, 1987), whilst larger males have shown to be more competitive superior while spawning (Quinn & Foote, 1994; Alain et al., 2007). Sea migration in salmonids is an evolutionary strategy to
increase growth due to the greater feeding opportunities in the marine environment (Gross et al., 1988). Despite sea migration elevates mortality risk due to predation, pathogens, and parasites, the survivors typically experience an extensive increase in body size and consequently reproductive success (Fleming & Reynolds, 2004). However, this behaviour will only prevail if the benefits are greater than the costs (Sandlund & Jonsson, 2016). As an example of pathogens changing this balance, aquaculture-sourced salmon lice (Lepeophtheirus salmonis) epizootics has shown to probably shift this cost-benefit equation for local sea trout populations. Salmon lice epizootics could increase the marine mortality risk and induce behavioural adaptations in sea trout to such an extent that sea migration might no longer be beneficial (Thorstad et al., 2015; Halttunen et al., 2018).

1.2 INFECTIOUS DISEASES IN NORWEGIAN SALMON AQUACULTURE – A RISK ASSESSMENT

While salmon lice cross-infestation and its impacts between farmed and wild salmonids is well documented (Thorstad et al., 2015; Halttunen et al., 2018) infectious diseases have been neglected up until recent years (Johansen et al., 2011). In addition to salmon lice, infectious diseases represent the largest health effect on farmed salmonids in Norway and cause substantial economic losses for the Norwegian salmon industry (Hjeltnes et al., 2017; Madhun et al., 2018). The production species is dominated by Atlantic salmon (Salmo salar), and while it is commonly accepted that the industry’s economic sustainability depends on sound knowledge regarding fish pathology and epidemiology (Hjeltnes et al., 2017), there’s an increasing understanding that diseases in the marine production facilities of salmonids (hereby referred to as salmon farms) can impact wild fish populations (Pettersen et al., 2015; Garseth et al., 2017; Colquhoun et al., 2018b). Risk assessments can be used to evaluate potential needs and procedures for preventive measures within aquaculture and hence prevent detrimental economic losses due to diseases (Colquhoun et al., 2018b). Such preventive measures may also have a conservational purpose in protecting wild fish.
populations that are interacting with the farmed salmonids (Garseth et al., 2017; Colquhoun et al., 2018b).

Creating a risk assessment regarding diseases requires knowledge on how infection causes disease, and how the infection itself occurs (Snieszko, 1974). Snieszko (1974) identified that an infection occurs when a susceptible host is exposed to a virulent pathogen under the right environmental conditions – and whether a disease outbreak will happen depends on the relationship between these factors. Hence, knowledge about the pathogen itself, the host, and the environment of which the pathogen and the host interact with, are needed to create a risk assessment about pathogen exchange. Such knowledge includes, among others, the virulence of the pathogen, the pathogens’ host specificity, the hosts’ susceptibility, potential reservoirs of the pathogen, and variables on what affects the pathogens’ contagiousness (Colquhoun et al., 2018b). Focusing on the marine stage of salmon farming introduces additional elements for the risk assessment, mainly how the open design of marine production permits dispersal of farm-sourced pathogens with local water currents (Johansen et al., 2011; Kristoffersen et al., 2013; Pettersen et al., 2015) and aggregation and attraction of wild fish (Dempster et al., 2009; Uglem et al., 2014).

1.2.1 Piscine orthoreovirus

A common disease which is considered a serious problem in Norwegian salmon farming is heart and skeletal muscle inflammation (HSMI) (Taksdal et al., 2017). The virus Piscine orthoreovirus (PRV; family Reoviridae) was first identified in 2010 as the causative agent for HSMB in Atlantic salmon (Salmo salar) (Taksdal et al., 2017; Dahle & Olsen, 2019). A new genotype, Piscine orthoreovirus -3 (PRV-3), was identified in 2015 as causing a HSMI-like disease in farmed rainbow trout (Onchorhynchus mykiss) (Olsen et al., 2015), and the PRV-genotype causing HSMB in Atlantic salmon was very recently determined to be genotype PRV-1 (Colquhoun et al., 2018a; Dahle & Olsen, 2019). HSMI causes inflammation and specific lesions in the heart and skeletal muscle, and affected salmonids may
develop anorexia and abnormal swimming behaviour (Taksdal et al., 2017). In Atlantic salmon, the disease is connected to seawater and arise 8-10 weeks post-infection (Morton et al., 2017). Outbreaks mainly occur 5–9 months after sea-transfer, commonly causing 90-100 % morbidity in farmed Atlantic salmon with associated mortality between 0-20 % (Taranger et al., 2015). According to the Norwegian veterinary institute (www.vetinst.no; accessed 20.04.2019), Atlantic salmon smolts that are transferred to the sea in autumn are of twice the risk of developing HSMI, compared to smolts that are sea-transferred in spring. It has also been detected PRV-positive farmed pre-smolts prior sea-transfer, both in Atlantic salmon (Dahle & Olsen, 2019) and rainbow trout (Olsen et al., 2015), indicating that PRV genotypes 1 and 3 can also infect in fresh water and/or be transmitted vertically (from mother to offspring). Sea trout have shown to be only rarely (1.9–3%) infected by PRV-1 in Norway (Garseth et al., 2013a), but the species’ persistence in coastal areas elevates vulnerability to ecological impacts from salmon farms (Thorstad et al., 2015). This increases the possibility that sea trout can serve as intermediary hosts for farm-source PRV-1 between anadromous and residential individuals.

Therefore, the primary objective of this study was, by use of acoustic telemetry in combination with a hydrodynamic model and fish pathology, to investigate how sea trout’s temporal (seasonal) and spatial (proximity to salmonid farms) marine migratory behaviour affected the risk of pathogen exchange with farmed salmonids. Due to its resilient nature and documented omni-presence in both farmed and wild Norwegian Atlantic salmon (Garseth et al., 2013a; Garseth & Biering, 2018), PRV was used as a model in developing this risk assessment. The secondary objective was to investigate possible freshwater reservoirs of PRV-1 by screening wild juvenile brown trout from rivers draining to fjord systems with aquaculture.
2. Materials and Methods

2.1 STUDY AREAS

Data was collected from the fjord systems Hemnfjorden and Snillfjorden (63°20’N, 9°10’E) in Sør-Trøndelag County, central Norway; Tosenfjorden and Bindalsfjorden (65°12’N, 12°13’E) in Nordland County, northern Norway; and Skjerstadfjorden (67°14’N, 14°44’E) in Nordland County, northern Norway.

2.1.1 Hemnfjorden and Snillfjorden

In 2011 – 2014, data about sea trout migratory behaviour in Hemnfjorden and Snillfjorden was collected. Located in central Norway, the inner part of the fjord system is divided in two interconnected fjord arms, the Hemnfjorden and Snillfjorden. In total, the two inner fjords cover >60 km² of sea surface and have a 65 km long shoreline (Figure 1). The fjord system connects to the open sea through a 36 km long strait. Water column depths in the study area range from ~ 0–100 m in the near shore areas, to a maximum of ~ 400 m in the deepest parts.

Sea trout was tagged in the Søa watercourse and River Snilldalselva. In the Søa watercourse, the freshwater section accessible to sea trout is 10.2 km long and includes Lake Rovatnet (surface area 7.65 km²). The Søa watercourse drains out into the Hemnfjorden fjord arm by Krokstadøra area (Figure 1). River Snilldalselva is ~ 7 km long, is not connected to a lake and drains out into the Snillfjorden-arm of the fjord system, close to Kyrksæterøra (Figure 1).

2.1.2 Tosenfjorden and Bindalsfjorden

In 2015-2017, data was collected from Tosenfjorden and Bindalsfjorden, a two inter-connected marine fjord system, located in Nordland County (Figure 2). The deepest part of the fjord system, 741 m, is situated at the south-eastern part of Bindalsfjorden.
Sea trout were captured and tagged from Storelva, Leirelva, Åbjøra, and Urvold. All three watercourses drain into Tosenfjorden (surface area ~97 km²) which then leads to Bindalsfjorden (surface area ~91 km²) and finally to the Atlantic Ocean, located at ~33 km from Flostrømmen and Urvold estuaries.

At the lowest part of Åbjøra watercourse, in Åelva, the watercourse drains into Tosenfjorden east of the municipality Terråk via two outlets: 1) via Flostrømmen into Floet, which is a large brackish pool of water, and 2) directly into the fjord via Osan. The Urvold watercourse drains out into Ørnhaugbukta in Tosenfjorden. At the outlet of the watercourse at the outmost part of Tosenfjorden there is an ~200 m long river stretch up to Urvoldvatnet 8 m above sea level.

Hydrodynamic data was collected in Tosenfjorden in 2017, by means of a Recording Doppler Current Profiler (RDCP) (see section 2.3).

2.1.3 Skjerstadfjorden

In 2016-2018, data was collected from the ~32 km long Skjerstadfjorden, located in Nordland County. Skjerstadfjorden is a continuing of Saltfjorden and has two fjord-arms: Misvær fjorden and Valnesfjorden (Figure 3). Skjerstadfjorden has a maximum depth of ~500 m, and highly geographic restricted in- and outlet, where most of the water flows through Saltstrømmen. The topography is relatively uniform, with steep sides ending on a flat surface in the middle of the fjord. Adjacent water systems (Misvær fjorden, Saltdalsfjorden, Fauskevika, Klungetvika, and Valnesfjorden) have varying in- and outlet depths.

The tagging locations was situated in river Lakselva (draining to Valnesfjord), River Laksåga (draining to Sulithjelma), Botnvassdraget, and river Saltdalselva.
2.2 TELEMETRY – MIGRATORY BEHAVIOUR

2.2.1 Study Populations

To investigate residency around salmon farms, sea trout from Rovatnet and Snilldalselva in Hemnfjorden and Snillfjorden; Lakselva, Laksåga, Botnvassdraget, and Saltdalselva in Skjerstadfjorden; and Leirelva, Åbjøra, and Urvold in Tosenfjorden and Bindalsfjorden was caught and tagged with acoustic tags. Further, data on the tagged sea trout from Tosenfjorden and Bindalsfjorden were used as a case study to evaluate the seasonal timing of the marine feeding migration in relation to the annual timing of HSMI-outbreaks in salmon farms.

Detailed descriptions of the populations used for this study are found in Davidsen et al. (2014), Davidsen et al. (2018) and Meyer (2018).

2.2.1.1 Temporal migratory pattern in Tosenfjorden

In order to assess the risk of pathogen exchange between wild sea trout and farmed salmonids, data on seasonal migratory pattern from sea trout in Tosenfjorden investigated by Davidsen et al. (2018) were used in this study. A total of 74 acoustic receivers (Vemco Inc., Canada, models VR2W and VR2-AR) were used to track the study populations during the time period 2015-2017. The number of operational receivers varied during the study and the different time periods are listed in Figure 2.

2.2.2 Fish Capture and Tagging

The fish used for the analyses in the study, were all tagged using the same protocol. Rod fishing, gill nets or dip nets was used to capture fish. Gill nets were regularly checked and, to avoid harm, fish were gently removed by cutting the net with scissors. After capture the fish were stored in holding nets, for a maximum of four hours, until tagging. The fish were then transferred to containers with 2-phenoxy-ethanol (EEC No 204 589-7, 0.5 ml per L water) solutions for four minutes until fully anesthetised. Fully anesthetised fish was
then placed in a cylindrical plastic tube containing water and a ~1.5 cm long incision was made adjacent to the linear alba. A disinfected acoustic transmitter was then inserted into the body cavity and the incision was closed with two or three sutures (Resolon 3/0 for veteran fish; Resolon 5/0 for smolts). The size of the transmitter was determined based on the fish size (see Davidsen et al. (2014), Davidsen et al. (2018) and Meyer (2018) for more details). A modified carlin tag was attached on the veteran sea trout’s just below the dorsal fin using two cannulas. Water was continuously poured over the fish’ gills during the tagging procedure. After tagging, DNA-sample from the adipose fin and scale samples were collected, before recording the fish’ length and finally its weight. The fish was then placed in a darkened holding tank until deemed fit enough to be released into a calm area near the tagging site.

The procedures for fish capture and tagging is described in more detail in Davidsen et al. (2014), Davidsen et al. (2018), Eldøy et al. (2015) and Meyer (2018).

2.2.3 Acoustic Transmitters

The acoustic tags, from Thelma Biotel AS and Vemco INC, emitted unique acoustic signals (69kHz) which would be registered if located within the receiver’s detection range. Tag size and other specifications differed within and between the tagging groups. See Davidsen et al. (2014), Davidsen et al. (2018) and Meyer (2018) for further details.

2.2.4 Tracking of Tagged Fish

The detection range of receivers ranges from 200-400 m radius (see Bordeleau et al. (2018) and Eldøy et al. (2015) for more details). Most receivers were deployed at 5 m depth, either being chained to floating structures at the salmon farm facilities or by being moored to anchored buoys and used in investigating the residency around salmon farms and their corresponding control locations. Some receivers were immersed and moored with on-board acoustic release system (Vemco model VRW-2 AR) or an external acoustic release (Subseasonic modell
AR-60- E). Few receivers were moored at 50-150 m depth and only used in this study for investigation of temporal migratory pattern in Tosenfjorden (see Davidsen et al. (2018) for more details).

2.2.4.1 Residency of Sea Trout around Salmon farms and Control Locations

Tracking data from receivers deployed at marine semi-open salmon production facilities (referred to as salmon farms) and adequate control locations in the three fjord systems used in this study, was extracted for further analyses. The control locations were placed 800-1000 m away from the actual salmon farm in a similar near-shore environment. For each location, total number of individual fishes visiting the salmon farm versus the control location and the residency of the stays were paired.

A total of 20 acoustic receivers (Vemco Inc., Canada, models VR2, VR2W and VR2-AR) were used for registration of number of visits and subsequently calculating the mean residency. Registrations were conducted for three years in each fjord, over a time period from 2012 to 2018 (Table 1).

In Hemnfjorden, three salmon farms with control locations, adding up to a total of six acoustic receivers, were used in this study (Figure 1). In Tosenfjorden, three salmon farms and their control locations, a total of six acoustic receivers, were used in this study (Figure 2). In Skjerstadfjorden, data from four salmon farms with control locations, eight acoustic receivers in total, were used (Figure 3).

The number of operational receivers differed during the study, and the different time periods for Hemnfjorden, Tosenfjorden, and Skjerstadfjorden, are listed in Figure 1, Figure 2, and Figure 3, respectively.
Figure 1: Map of Hemnfjorden and Snillfjorden, showing the locations of receivers. The salmon farm-located receivers with their corresponding controls selected for this study was station number 128 and 16 (Stokkvika), 1 and 123 (Troan), and 119 and 115 (Hafsmo). All receivers were operational from year 2012 to 2014, except receiver 1 and 16 which were only operational in 2014.
Figure 2: Map of Tosenfjorden and Bindalsfjorden, showing the locations of receivers. The salmon farm-located receivers with their corresponding controls selected for this study was as following: station number 51 and 52 (Tosbotn), 39 and 37 (Mullinga), and 77 and 78 (Oksbåsen).
Figure 3: Map of Skjerstadfjorden, showing the locations of receivers. The salmon farm-located receivers with their corresponding controls selected for this study was as following: station number 13 and 14 (Øksengård), 20 and 21 (Daumannsvika), 34 and 30 (Leivsethamran), and 36 and 35 (Storvika).
2.3 MONITORING LOCAL WATER CURRENTS IN TOSENFJORDEN

The monitoring of water flow conditions was measured with a Recording Doppler Current Profiler (RDCP) instrument, a multiparameter platform moored at the bottom in a fixed frame at 35 meters depth in Tosenfjorden near Mullingen (coordinates: 65° 6.8344' N and 12° 27.2678' E, Figure 3) from 4\textsuperscript{th} of May to 31\textsuperscript{st} of August 2017.

The instrument was configured into two columns. Column 1 consisted of 15 measurement cells, each cell being 3 m wide and configured having a 50 % overlap with adjacent cells. The data from Column 1 was collected from these cells with centres at 3.5 – 5.0 – 6.5 – 8.0 … 23.0 and 24.5 m distance from the instrument. Since the instrument was located at 35 m depth, the data provided measurements from 10.5 - 12 - 13.5 - 15 … 30 and 31.5 m depth, a total of 15 measurements. Column 2 was configured to measure at surface level with 1 m wide cells, starting at 2 m depth with cell centres at 2.5 – 3.0 – 3.5 – 4.0 and 4.5 m distance from the surface and a 50 % overlap. Column 2 thus provided measurements from 2.5 – 3.0 – 3.5 – 4.0 and 4.5 m depths.

2.4 PRV-1 SCREENING OF JUVENILE BROWN TROUT

2.4.1 Study Populations

All specimen included in PRV-1 screening were sampled from fresh water sources that are accessed by, and in contact with, seawater migrating salmonids, but the specimen themselves had not been in seawater. The fresh water sources were all rivers draining out to fjords with aquaculture. The collection of the specimen was conducted in 2017-2018 through electro-fishing, where brown trout from 71 mm to 570 mm length (from tip of snout to tip of longest caudal fin) were captured for heart tissue extraction. Most specimen was of minimum 127 mm length to ensure that the specimen was large enough for heart tissue extraction.
A total of 171 trout was sampled from a total of 15 different freshwater systems around Hemnfjorden and Snillfjorden, Tosenfjorden and Bindalsfjorden, and Skjerstadfjorden and Saltalsfjorden. A more detailed description of the sampling sites can be found in Davidsen et al. (2014), Davidsen et al. (2018) and Meyer (2018).

2.4.2 Tissue Extraction and PCR-analysis for PRV-1

Tissue extraction and conservation of tissue was done according to Fish Vet Group protocols (www.fishvetgroup.no; accessed 15.08.2017).

Two tissue samples from the heart of 2 mm³ in size per specimen was extracted and placed in one RNAlater-containing test tube for each respective specimen. Test tubes containing tissue samples was placed on ice after each sampling. The equipment was sterilized with 70 % alcohol and fire between each sampling to prevent contamination. The tissue samples were then stored in a refrigerator at 4 °C during the first night and then stored in -20 °C until shipment. The tissues were shipped to PatoGen AS which conducted the PRV detection through PCR-analysis.

2.5 DATA ANALYSIS

2.5.1 Migratory Behaviour

2.5.1.1 Filtering of Telemetry Data for Salmon Farm Residency Analyses

Telemetric data used in this study, for analyses of residency around salmon farms and their corresponding control locations, were collected during the time period 12.04.2012 - 02.08.2018 (Table 1). The data was stored and processed in the program VUE [version 2.3.0, VEMCO, 09.2016].
2.5.1.2 Removal of Registrations and Residency Search

First step of the data processing was to exclude receivers that were not used in this study, giving 20 receivers. Thereafter number of detections at each receiver were identified using “Residency search” in VUE.

When analysing residency time at salmon farms and their corresponding control locations, tagged sea trout which only passed through the localities were not included in the calculations. This was done using the function “Residency search” in the program VUE. A residency was approved if it were minimum two registrations within 60 minutes. If the sea trout was absent for 60 minutes or more after an approved residency, two more registrations within 60 minutes timespan had to happen for it to be registered as a new residency.

The “Residency search” thus consider that fish individuals \((n)\) could have been detected at the receivers several times during the entire study period, giving a total of 210 103 number of registered detections in the dataset. Three transmitter-ID’s were pinger tags and thus removed, giving a total of 108 271 detections, a 48% reduction of the original dataset.

Through visual inspection, two transmitter-ID’s showed a detection pattern indicating the tags had been immobile for minimum one week and were consequently removed from the dataset since this is unlikely behaviour for live fish. This removed 6488 detections, resulting in 101 783 detections in the dataset, a reduction of 51% from the original dataset.

Minimum residency time for infection risk was defined as 30 minutes. Thus, after an approved residency was identified, only individuals with a minimum residency of 30 minutes were included in the subsequent calculations. Eliminating residency below 30 minutes resulted in a total of 86 643 detections of tagged sea trout, including 522 individuals (Table 2). The total reduction of detections from the original dataset was 57%.
2.5.1.3 Calculating Mean Residence around Salmon farms and Control Locations

Some individual fish might have had very few detections but extremely long residency at a receiver. To account for this, mean residency was calculated in two steps: 1) the mean residency for each individual fish at each receiver were calculated; and 2) the results from these calculations were then used to calculate the average of all mean residency at each receiver. The first step provided the mean residence necessary for statistical analyses (see section 2.5.1.2). The second step provided the mean residency time around salmon farms and controls for presentation in Table 2.

2.5.1.3.1 Defining a Visit

A residency were further identified as a visit by the mixed effect model for residency (see section 2.5.1.2.1), where it also considered that individual fish (n) could have been detected at and within other sites (a 'site' is a salmon farm and its corresponding control location), both within each year and during the whole study period. An identified number of visits (Table 2) is therefore not the sum of all detections, but a number of which the non-independence of the residuals had been considered (hence a much lower number of visits than detections).

The model definition of visits resulted in a total of 1860 identified visits of tagged sea trout, including 522 individuals (Table 2).

2.5.1.3.2 Defining the Moment of Infection Risk

The receivers used in this study had a detection rage between 200 – 400 m radius (see 2.2.3). Therefore, after an approved residency was identified, tagged sea trout which resided for a minimum of 30 minutes (see 2.5.1.1) within 200 – 400 m radius of an assumed infected salmon farm was defined as being at risk of infection.
2.5.1.4 Mapping of Temporal Migratory Pattern in Tosenfjorden

In order to assess the risk of pathogen exchange between wild sea trout and farmed salmonids, data on seasonal migratory pattern from sea trout in Tosenfjorden investigated by Davidsen et al. (2018) were used in this study. The maps show the monthly sea trout usage of areas in Tosenfjorden and Bindalsfjorden from November 2016 to October 2017. The maps show how many different acoustic tagged sea trout were registered per station per month. See Davidsen et al. (2018) for more details.

2.5.1.2 Statistical Analyses

2.5.1.2.1 Residency

All statistical analyses were done in R version 3.4.4 (R Core Team, 2013). Packages lme4 (Bates et al., 2015) and glmmTMB (Brooks et al., 2017) provided the platform for all data analyses.

To investigate whether there were any differences in mean residency between salmon farms and their corresponding control locations, a generalized linear mixed effect model fitted with gamma distribution (log link) were developed.

Each salmon farm with its corresponding control location was treated as a site, to a total of ten sites (Table 2), and differences in residency and number of visits where analysed within each site. “Salmon farm” and “Control” was presented as two levels within one treatment and included in the model together with the variables “Fjord system” and “Year of sampling”. The treatment effect on each site was then tested using the relevel function.

In accordance with the hypothesis, the interaction between the treatment and the site was analysed as the explanatory variable (fixed factor). Including individual fish as a random factor accounted for non-independence of the observations within the site. The simplest and final model was identified and selected using Akaike’s Information Criterion (AIC) values.
2.5.1.2.2 Number of Fish Individuals, Visits and Detections

Within each pair, significant higher number of fish individuals \((n)\) and significant higher number of visits were identified using chi-square t-test (see section 4.1.1).

For Hafsmo salmon farm and its control location in Hemn fjorden, the highest number of fish individuals \((n)\) and number of detections per year were identified using chi-square t-test (Table 3).

2.5.2 MONITORING LOCAL WATER CURRENTS IN TOSENFJORDEN

2.5.2.1 Computer Software and Estimating Particle Movement

Analysis was performed by the software RDCP Studio, version 5.1 [Windows CE Application RDCPMS2].

The data was used to create current-profiles showing horizontal speed and direction, and Progressive vector plots. Scatter graphs was created to illustrate the speed and horizontal direction of movement of particles within each individual cell depth. The Progressive vector plot is based on a probability model estimating movement of a particle from point zero, founded solely on horizontal current measurements from each respective cell with increasing residuals with increasing distance from zero. Progressive vector plots do not consider the fjord topography which further increases the error of estimation.

2.5.3 PRV-SCREENING OF JUVENILE BROWN TROUT

Screening for PRV-1 was conducted through PCR analysis by PatoGen AS, a company which offers PCR services (www.patogen.no; accessed 15.08.2018).

2.5.4 DEFINING LOW, INTERMEDIATE, AND HIGH RISK

In this study, the risk of pathogen exchange between farmed salmonids and wild sea trout is assessed in qualitative terms, meaning the assessment is based on rough estimates of “low”, “intermediate” and “high” risk. The cornerstone of the
risk assessment builds on the relationship between hosts and pathogens, and the environment in which they interact with, in relation to literature search regarding previous studies on fish epidemiology and pathology both in farmed and wild populations.

3. Results

3.1 TELEMETRY – MIGRATORY BEHAVIOUR

3.1.1 Residency of Sea Trout around Salmon farms and Control Locations

A total of 1860 visits of tagged sea trout from three fjord systems was detected and used in determining mean residency at salmon farms and their corresponding control locations (Table 1).

<table>
<thead>
<tr>
<th>Fjord</th>
<th>Year</th>
<th>2012</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2018</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemnfjorden</td>
<td></td>
<td>87</td>
<td>110</td>
<td>253</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tosenfjorden</td>
<td></td>
<td>88</td>
<td>99</td>
<td>681</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skjerstadfjorden</td>
<td></td>
<td>207</td>
<td>95</td>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In Hemnfjorden, a longer mean residence time and fewer visits was registered around the salmon farms Stokkvika and Trøan than their corresponding control locations but none were significant. The salmon farm Hafsmo had a longer mean residence and fewer visits than its control location (Table 2).

In Tosenfjorden, fewer fish and a shorter residence time was registered around the salmon farms Tosbotn and Mullingen than their corresponding control locations, whilst the opposite was registered for Oksbåsen (Table 2). Two individuals around Oksbåsen had an extra long residence and thus affected the results by greatly increasing the mean residence time and the standard error.
(mean residence time (S.E.): 171 (42); range: 30 – 1121) but removing them from the dataset still resulted in a higher mean residence around the salmon farm than around its control location (Table 2).

In Skjerstadfjorden, fewer fish and a lower residence time was registered around the salmon farms than their corresponding control locations but none of the differences showed to be significant (Table 2). Too few fish ($n = 3$) had been registered around Øksengård for a statistical test to be conducted.
Table 2: Number of individual visiting sea trout and their paired period of residency around salmon farms in Hemnfjorden, Tosenfjorden, and Skjerstadfjorden. P-value shows if there was a significant difference in length of residency of sea trout around the salmon farm and its corresponding control location. Significant P-values are shown in bold, and a significant longer residence at salmon farm or control locations within a pair are represented in yellow or green, respectively. A significant higher number of fish individuals (n) and visits within a pair are shown with * and **, respectively.

<table>
<thead>
<tr>
<th>Fjord</th>
<th>Salmon farm/control location</th>
<th>Number of fish individuals (n)</th>
<th>Number of visits</th>
<th>Mean residence in minutes (S.E.)</th>
<th>Range (minutes)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemnfjorden</td>
<td>Stokkvika (st.128)</td>
<td>19</td>
<td>75</td>
<td>90 (15)</td>
<td>31 - 889</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Stokkvika Control (st. 16)</td>
<td>18</td>
<td>122**</td>
<td>70 (6)</td>
<td>31 - 270</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trøan (st.1)</td>
<td>20</td>
<td>56</td>
<td>79 (12)</td>
<td>31- 402</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Trøan Control (st.123)</td>
<td>17</td>
<td>70</td>
<td>74 (11)</td>
<td>30 - 205</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hafsmo (st.119)</td>
<td>21</td>
<td>45</td>
<td>159 (39)</td>
<td>31 - 697</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Hafsmo Control (st.113)</td>
<td>20</td>
<td>82**</td>
<td>67 (6)</td>
<td>30 - 263</td>
<td></td>
</tr>
<tr>
<td>Tosenfjorden</td>
<td>Tosbotn (st.51)</td>
<td>27</td>
<td>71</td>
<td>103 (14)</td>
<td>30 - 708</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Tosbotn Control (st.50)</td>
<td>40</td>
<td>188</td>
<td>160 (19)</td>
<td>30 - 1047</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mullingen (st.39)</td>
<td>28</td>
<td>207</td>
<td>79 (8)</td>
<td>30 - 425</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Mullingen Control (st.37)</td>
<td>48*</td>
<td>285**</td>
<td>99 (11)</td>
<td>30 - 1310</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oksbåsen (st.77)</td>
<td>23</td>
<td>72**</td>
<td>115 (5)</td>
<td>30 - 585</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Oksbåsen Control (st.78)</td>
<td>32</td>
<td>45</td>
<td>58 (17)</td>
<td>31 - 188</td>
<td></td>
</tr>
<tr>
<td>Skjerstadfjorden</td>
<td>Øksengård (st.13)</td>
<td>3</td>
<td>4</td>
<td>84 (13)</td>
<td>53 - 124</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Øksengård Control (st.14)</td>
<td>25</td>
<td>34</td>
<td>86 (12)</td>
<td>31 - 212</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daumannsvika (st.20)</td>
<td>21*</td>
<td>32</td>
<td>100 (18)</td>
<td>30 - 698</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Daumannsvika Control (st.21)</td>
<td>6</td>
<td>43</td>
<td>100 (43)</td>
<td>30 - 433</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leivsethamran (st.34)</td>
<td>13</td>
<td>21</td>
<td>82 (8)</td>
<td>31 - 166</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Leisethamran Control (st.30)</td>
<td>23</td>
<td>51**</td>
<td>87 (16)</td>
<td>30 - 351</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Storvika (st.36)</td>
<td>67</td>
<td>206**</td>
<td>107 (11)</td>
<td>30 - 1238</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Storvika Control (st.35)</td>
<td>51</td>
<td>151</td>
<td>100 (7)</td>
<td>30 - 687</td>
<td></td>
</tr>
</tbody>
</table>
In Hemnfjorden, a higher number of detections were registered at Hafsmo salmon farm in year 2013, while a higher number of detections were registered at the control location in 2014 (Table 3).

Table 3: Number of fish individuals (n) and detections at Hafsmo salmon farm and its control location per year. A significant higher number of fish individuals (n) and detections are shown with *.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of fish individuals (n)</th>
<th>Number of detections</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hafsmo</td>
<td>Control</td>
</tr>
<tr>
<td>2012</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>2013</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>2014</td>
<td>7</td>
<td>5</td>
</tr>
</tbody>
</table>

3.1.2 Seasonal Migratory Pattern in Tosenfjorden

The timing of outward migration varied between sea trout from different watercourses. Sea trout from Urvold watercourse mainly migrated out into Tosenfjorden during May (Figure 6), whilst the outward migration from Flostrømmen estuary in Åbjøra watercourse spanned over a three-month period from March to June (Figure 5 and Figure 6). The Osan area, near Floet (Figure 2), appeared as a wintering area (Figure 4 and Figure 5).

The usage of the fjords different parts varied with seasons and the sea trout predominately resided in the fjord from mid-May to mid-July (Figure 6). The sea trout mainly used the estuary areas around Flostrømmen, Urvold and Storelva from October to March, with a peak in March (Figure 5). Sea trout were registered at the Storelva estuary throughout the whole year, although few (Figure 4, 5, 6 and 7). In April, sea trout from Åbjøra watercourse began to leave the estuary area in Flostrømmen to quickly spread out into the fjord system, followed with some sea trout registered at Mullingen (st. 39) in April (Figure 5). Some sea trout were also registered around Tosbotn (st. 51) in April. Sea trout from Urvold watercourse and Leirelva entered the fjord in May (Figure 6), followed with a peak in registered sea trout around Oksbåsen (st. 77) and
Tosbotn (st. 51) during the same month. Most sea trout were also around Mullingen (st. 39) in May. Most sea trout had returned to their watercourses by mid-July (Figure 6), thus fewer sea trout resided around salmon farms from mid-July onwards as well. Few sea trout were registered in the fjord from August till October (Figure 4), and no sea trout were in the vicinity of Mullingen (st. 39), Oksbåsen (st 77.), or Tosbotn (st. 51) salmon farms from August to February (Figure 4 and Figure 5).
Figure 4: The sea trout usage of the area in Tosenfjorden and Bindalsfjorden during the period from November 2016 to January 2017. The figure shows how many different acoustic tagged sea trout were registered per station per month.
Figure 5: The sea trout usage of the area in Tosenfjorden and Bindalsfjorden during the period from February to April 2017. The figure shows how many different acoustic tagged sea trout were registered per station per month.
Figure 6: The sea trout usage of the area in Tosenfjorden and Bindalsfjorden during the period from May to July 2017. The figure shows how many different acoustic tagged sea trout were registered per station per month.
Figure 7: The sea trout usage of the area in Tosenfjorden and Bindalsfjorden during the period from August to October 2017. The figure shows how many different acoustic tagged sea trout were registered per station per month.
3.2 MONITORING LOCAL WATER CURRENTS IN TOSENFJORDEN

The RDCP-instrument which conducted the measurements were configured into two columns, where column 1 (Figure 8, 10, 11, and 12) provided measurements from depths of 10.5 – 31.5 m below the surface and column 2 (Figure 9 and Figure 13) from depths of 2.5 – 4.5 m below surface.

The Progressive vector plot for column 1 (Figure 8) estimated a north-eastward particle transfer for all 15 cells (each cell being 3 m wide and configured having a 50 % overlap with adjacent cells). The current direction in the cells closest to the surface, starting with Cell 15, was much more in the north-westwards direction than the current direction of the water closer to the seabed. The bottommost cell, Cell 1, had a greater scattering, indicating upwelling of water masses from the seabed. The particles propagation distance was between 50 to 450 km.
Figure 8: Progressive vector plot for column 1 (instrument referenced), showing estimated particle movement for each respective cell. The plot shows estimated particle movement in km along the x-axis and direction of movement, from point zero, in degrees represented by the outermost circle.

The Progressive vector plot for column 2 (Figure 9) estimated a great scattering of particles for all cells (each cell being 1 m wide and configured having a 50% overlap with adjacent cells) with a north-eastward and south-westward current direction. In Cell 1, 2.5 m below the surface, the particle had been greatly scattered and transported up to ~70 km in ~235-degree direction. The current direction in the cells closest to the surface was much more in the south-westward direction than the current direction of the water closer to the seabed. The particles propagation distance for all cell depths was between 40 to 90 km.
Figure 9: Progressive vector plot for column 2 (surface referenced), showing estimated particle movement for each respective cell. The plot shows estimated particle movement in km along the x-axis and direction of movement, from point zero, in degrees represented by the circles.

The horizontal speed and direction of the water currents in column 1 and column 2 are presented in Scatter graphs (Figure 10, 11, 12, and 13). In column 1 (Figure 10, 11 and 12), measurement points from all cells (cell centres of 10.5 - 31.5 m below surface), shows that the current was in the southward direction, with an equal amount of measurement points at southwest and southeast, but a greater concentration of points with a stronger average current speed at the 285-degree direction (average of 30 cm/s in the south-west direction versus average 25 cm/s in the north-eastward direction), indicating a net-flow of water out of the fjord. There was registered a great scattering of measurement points in the cell closest to the instrument (Figure 10 a), that is, cell centre at 31.5 m below the surface.
Figure 10: Scatter graphs from column 1 (instrument referenced), showing measurements with cell depths of 2.0 – 5.0 m (a), 3.5 – 6.5 m (b), 5.0 – 8.0 m (c), 6.5 – 9.5 m (d), 8.0 – 11.0 m (e), and 9.5 – 12.5 m (f) distance from the instrument. The Scatter graphs show the estimated speed of particle movement in cm/s given by the x-axis, and the degree of movement from point zero represented by the circles.
Figure 11: Scatter graphs from column 1 (instrument referenced), showing measurements with cell depths of 11.0 – 14.0 m (g), 12.5 m – 15.5 m (h), 14.0 – 17.0 m (i), 15.5 – 18.5 m (j), 17.0 – 20.0 m (k), and 18.5 – 21.5 m (l) distance from the instrument. The Scatter graphs shows the estimated speed of particle movement in cm/s given by the x-axis, and the degree of movement from point zero represented by the circles.
Figure 12: Scatter graphs from column 1 (instrument referenced), showing measurements with cell depths of 20.0 – 23.0 m (m), 21.5 – 24.5 m (n), and 23.0 – 26.0 m (o) distance from the instrument. The Scatter graphs shows the estimated speed of particle movement in cm/s given by the x-axis, and the degree of movement from point zero represented by the circles.

In column 2 (Figure 13), the measurement points in the 60-degree direction had a greater speed than the ones in the 240-degree direction, but the number of measurements points in the 60-degree direction decreased towards the seabed. The measured speed ranged from ~80 to ~60 cm/s as descending towards the seabed.
Figure 13: Scatter graphs from column 2 (surface referenced), showing measurements with cell depths of 2.0 – 3.0 m (a), 2.5 – 3.5 m (b), 3.0 – 4.0 m (d), and 4.0 – 5.0 m (e) below surface. The Scatter graphs shows the estimated speed of particle movement in cm/s given by the x-axis, and the degree of movement from point zero represented by the circles.
The Progressive vector plots and Scatter graphs for both columns did not account for the fjord topography and assumed that the particles stayed within the depth range of their respective measurement cells while the measurements were conducted.

3.2 PRV-SCREENING OF JUVENILE BROWN TROUT

The PCR-analyses conducted by PatoGen AS did not detect PRV-1 in 169 of 171 specimen, whereas the 2 remaining specimens were not approved by their in-house quality check for screening.

4. Discussion

The main results in this study showed no indications of sea trout being attracted to salmon farms but did however show that most of the fish resided within Tosenfjorden during the main season (spring and early summer) of HSMI-outbreak in salmon farms. The hydrodynamic modelling indicated that local water currents in Tosenfjorden at depths commonly occupied by sea trout (2.5 – 4.5 m) could transport pathogens up to 90 km from an infected farm, and hence still affect the fish even if they were not attracted to the salmon farms. The PRV-1 screening of wild juvenile sea trout from the 15 rivers used in this study provided no evidence of PRV-1 reservoirs in juveniles that hadn’t been to sea yet.

4.1 RESIDENCY AROUND SALMON FARMS AND CONTROL LOCATIONS

Increased residence time around salmon farms may increase the risk of disease transfer between wild and farmed fish, and among adjacent farm facilities (Dempster et al., 2009; Johansen et al., 2011). In the current study, tagged sea trout did not reside for a longer time around eight of ten salmon farms. This indicates that the sea trout were not attracted to surplus feed or other organic materials from the marine production of farmed salmon.
The two salmon farms where sea trout had prolonged residence time were Hafsmo in Hemnfjorden and Oksbåsen in Tosenfjorden.

At Hafsmo, sea trout resided for a longer time around the salmon farm than at its control location, but fewer visits were detected at the farm facility. Equal number of fish individuals \((n)\) visited at the farm and control location, both within each year and during the whole study period.

At Oksbåsen, both a greater number of visits and a longer mean residence time were observed than at its control location. However, the Oksbåsen facility was only surveyed with acoustic receiver in 2017, while the other two salmon farms in Tosenfjorden (Mullingen and Tosbotn) had receivers during the whole period of 2015-2017.

Within each of the two fjord systems, all facilities are managed by the same principles (Aqua Gen in Hemnfjorden; Sinkaberg-Hansen in Tosenfjorden) and it is therefore unlikely that there were differences in e.g. feeding strategies or the general management which could have attracted sea trout. Despite attempting to pair-up farm facilities with control locations that have similar near-shore habitats and similar distance to land, there could have been special conditions at both Hafsmo and Oksbåsen which attracted sea trout. For Oksbåsen in particular, one should be careful in drawing conclusions about indications of attraction since this facility were only monitored for one season.

Previous studies have shown that marine fish can be attracted to surplus feed from the semi-open salmon farms (Dempster et al., 2009; Uglem et al., 2014), and thus reside closer and for a longer time around farm facilities. Reasons to why the sea trout did not show a greater residency around eight of the ten salmon farm facilities, may be due to that the species primarily resides in the top five meters of the water column (Eldøy et al., 2017; Kristensen et al., 2018) whilst surplus feed are found closer to the bottom. Therefore, the food is more accessible for fish such as cod \((Gadus morhua)\) and saithe \((Pollachius virens)\) that prefer deeper waters (Uglem et al., 2014).
4.1.1 Model Limitations

The statistical model for residency investigated whether there was a significant difference in residency time between the salmon farm and its corresponding control, and it assumed that minimum residency time for infection risk was 30 minutes. Hence it did not consider possible accumulating infection risk with increased residency nor possible accumulating infection risk with increasing number of visits.

4.2 SEASONAL MIGRATORY BEHAVIOUR AND INFECTION RISK

Several different diseases in Norwegian aquaculture are associated with certain seasons, many of which are linked to water temperature and weather conditions (Poppe, 1999). Information about the seasonal behaviour of the marine feeding migration is therefore crucial in order to evaluate the risk of pathogen exchange between wild and farmed salmonids. In this study, the tagged sea trout predominately resided in Tosenfjorden from mid-May to mid-July which is during the main season of HSMI-outbreak in salmon farms (Taranger et al. (2015); Morton et al. (2017); www.vetinst.no; accessed 20.04.2019).

Sea trout from Åbjøra watercourse resided for a longer time in the fjord system than sea trout from Urvold watercourse. Previous study have showed that an elevated time spent in coastal areas in fjord systems with aquaculture has the potential to increase the risk of salmon lice encounters (Moore et al., 2018). Likewise, so probably does the risk of encountering farm-sourced microparasites (pathogenic microorganisms; viruses, bacteria, fungi). The sea trout from Åbjøra watercourse could therefore have been at a greater risk of encountering farm-sourced PRV-1, compared to individuals from Urvold which conducted their feeding migrating over a shorter time-period.

The duration of marine residence has shown to be positively correlated with higher sea temperatures (Berg & Berg, 1989), and populations further south of
Norway might therefore be of greater infection risk than those at higher latitudes. However, there are great differences between populations within latitudinal regions of Norway on the duration of marine residency (Jensen & Rikardsen, 2012; Eldøy et al., 2015; Bordeleau et al., 2018), and the risk of being infected can therefore vary.

Sea trout from Åbjøra were found to migrate earlier to the marine environment compared to sea trout from Urvold. As the risk of HSMI-outbreak in salmon farms is greatest in spring (Taranger et al. (2015); Morton et al. (2017); www.vetinst.no; accessed 20.04.2019), population differences in marine entry may result in difference in infection risk between these populations. Disease-outbreaks during the marine production in Norwegian salmon industry commonly happen together with sudden temperature changes, often in combination with extreme weather conditions (Poppe, 1999). Temperature, especially rapid changes, are of great significance in disease development in fish, even if the changes happen within the optimal temperature range of the fish (Poppe, 1999). Poppe (1999) explains this to likely be because changes in temperature stresses the fish and impair its immune response to damage and/or infections, and that aquatic microorganisms are often activated in connection with stirring of the water masses (which are mixed because of differences in temperature and salinity). Additionally, many aquatic pathogens have temperature thresholds, where temperatures above or below triggers infection or disease development (Peeler & Taylor, 2011). Sea trout which conducts their marine feeding migration at the onset of a new season might therefore be of greater transmission risk from farm-sourced pathogens than those that migrate out during more stable environmental conditions.

Higher water temperatures can also provide better living conditions for aquatic bacteria: The Norwegian veterinary institute (Zerihun et al., 2019) reported an increase of mycobacteriosis outbreaks in marine farmed Norwegian Atlantic salmon, and speculates whether there is a connection between the outbreaks and
the previous warm summer of 2018. It is therefore likely that sea trout populations at more temperate areas in southern Norway are at higher transmission risk of farm-sourced bacterial infections compared to populations at higher latitudes.

Seeing that climate change increases water temperatures and the frequency of extreme weather conditions (IPCC, 2014; Ornes, 2018), and that the Norwegian aquaculture has an annual 10 – 20 % increase of production (Lillehaug et al., 2017), one can suspect that the frequency of disease-outbreaks in salmon farms is likely to increase. Consequently, the risk of farm-sourced pathogen transmission to sea trout is likely to increase with it.

However, brand-new discoveries about PRV-genotypes reveal that sea trout is less susceptible to PRV-1, or PRV-1 is less adapted to sea trout (Garseth & Biering, 2018). Despite that the timing of outward migration and an elevated time spent in coastal areas has the potential to increase the risk of encountering PRV-1, the virus is unlikely to infect sea trout. Nevertheless, extreme weather conditions and especially high and fluctuating water temperatures could impair the fish’ immune system and make it more susceptible for PRV-1 infection, but the risk would probably only increase up to an intermediate even under such extreme conditions.

4.3 FARM-SOURCED PATHOGEN DISPERSAL AND PRODUCTION OF VIRULENCE

Local water currents is a contributing risk factor when estimating aquatic pathogen dispersal, and consequently the risk of local infection pressure, in fjord systems with farmed and wild salmonids (Kristoffersen et al., 2009; Johansen et al., 2011; Taranger et al., 2015). The hydrodynamic dispersal modelling in this study indicated that local water currents in Tosenfjorden at 2.5 – 4.5 m depth could have transported particles, and potentially pathogens, between 40 – 90 km at a speed of ~60 to ~80 cm/s as ascending towards the surface. The northwest-
and southeast-ward direction of movement indicated that particles, and potentially pathogens, were transported with tidal currents.

It is unknown for how long and in what quantities PRV is shed from infected fish nor viral survival in seawater (Taranger et al., 2015). However, the estimated 90 km dispersal at surface depths in this study are strengthened by modelling conducted by Kristoffersen et al. (2013) which indicate that PRV can spread 50-100 km radius from one infected farm and persist over a longer time in the environment. The Kristoffersen et al. (2013) model was created prior to new discoveries of PRV-genotypes (Olsen et al., 2015; Dahle & Olsen, 2019), and therefore applies to PRV-1 which is the causative agent for HSMI in Atlantic salmon. Future modelling of PRV-genotypes which are associated with disease in sea trout is therefore needed.

Previous studies has shown that sea trout commonly occupy the first 5 m of the water column (Eldøy et al., 2017; Kristensen et al., 2018). In the current study, measurements were done from 2.5 – 31.5 depth. The depths from 0-2.5 m were not included due to a too deep placement of the instrument and turbulent wave actions at the surface. The bottommost measurements, from 10.5 – 31.5 m depth, indicated that particles, and potentially pathogens, could have been transported up to 450 km at ~30 cm/s. However, since these depths are more rarely used by sea trout (Eldøy et al., 2017; Kristensen et al., 2018) the possible longer transportation of pathogens at such depth is expected to be less important. Failure to find previous studies that indicate that particles, and potentially pathogens, can be transported from the bottom of salmon farms and up to 5 m depth further supports this conclusion.

High host densities can increase both the production and the virulence of pathogens above levels found in natural populations (Mennerat et al., 2010) and in the salmonid farming industry the salmon farms contain salmonids that greatly exceeds natural populations (Johansen et al., 2011; Pettersen et al., 2015). One example of farming-induced heightened virulence is that of Infectious
salmon anaemia virus (ISAV; family *Orthomyxoviñdae*), where it is believed that non-virulent strains of the virus are the origin of strains that cause Infectious salmon anaemia (ISA), a very serious disease in Norwegian aquaculture (Johansen *et al.*, 2011). Furthermore, a large number of hosts under stressful conditions may increase the risk of a disease developing into an epidemic (Snieszko, 1974; Krkošek, 2010).

The principles of marine production in modern aquaculture therefore greatly increases the risk of accumulating pathogen and virulence production, and since the semi-open salmon farms are always present in coastal waters, so does the risk of pathogen dispersion and transmission (Johansen *et al.*, 2011; Pettersen *et al.*, 2015). Previous studies with PRV-screening of farmed and wild salmonids suggest extensive spreading of the virus along the Norwegian coast and establishment in wild populations with evidence suggesting the virus to be farm-sourced (Garseth *et al.*, 2013a), and it is considered likely that PRV-1 is transmitted from farmed to wild salmon (Taranger *et al.*, 2015).

The proliferation of pathogens and virulence in modern aquaculture together with indications of extensive pathogen-spreading along the coastline of Norway and with local water currents, implies a high risk of farm-sourced transmission to sea trout, despite no indication of attraction to salmon farms. However, due to the newly discovered PRV-genotypes and their host-specificity (Garseth & Biering, 2018; Dahle & Olsen, 2019), one can assume that the risk of PRV-1 exchange between farmed Atlantic salmon and sea trout is low.

### 4.4 FRESHWATER RESERVOIRS

Investigating potential freshwater reservoirs of PRV-1 is important to establish transmission risk to resident brown trout that are interacting with sea migrants. PRV-1 screening of wild juvenile brown trout from the 15 rivers surveyed in this study provided no evidence of PRV-1 reservoirs in juveniles that hadn't been to sea yet. This suggests that possible PRV-1 infected sea trout or potential farmed escapees did not spread the virus to freshwater habitats.
Marine production sites typically have individuals from the same juvenile freshwater production site (Johansen et al., 2011), meaning that diseases can be introduced and spread over large distances (Lyngstad et al., 2008; Garseth et al., 2013a). One example of such an incident was the introduction of ISA to Chile with vertically (from mother to offspring) ISAV-infected Norwegian Atlantic salmon roe (Calbucura et al., 2008), causing devastating losses for the Chilean salmon industry as the virus spread through horizontal transmission (from fish to fish) during the seawater phase. A concern might therefore be that if a PRV-genotype is introduced to marine production sites with vertically infected farmed fish, the virus could horizontally transmit to surrounding wild sea trout. The infected sea trout which survives their spawning migration could then vertically transmit it to their offspring. Infected farmed escapees which spawn with brown trout or sea trout could also vertically transmit the virus. Consequently, farm-sourced PRV-genotypes could potentially be spread to freshwater habitats naïve to that virus.

Studying diseases in wild fish populations introduces a paradox as only the survivors are left to be studied. Fish weakened by disease are of elevated starvation and predation risk and will be removed from the system (McVicar, 1997) which might explain the absence of PRV-1 in the sampled specimen in this study. Furthermore, only tissues from the heart was extracted for PRV-1 analyses in this study, whilst that of Garseth et al. (2013a) and Garseth and Biering (2018) included tissues from both heart and kidney. Excluding tissue samples from the kidneys could therefore have affected the results. However, the low host-pathogen susceptibility-adaptability between PRV-1 and sea trout (Garseth & Biering, 2018) most likely explains the absence of positive results.

Given that recent studies have reported PRV-genotypes with various infection efficiencies and transmission routes on brown trout, there is a need for future studies involving Piscine orthoreovirus-3 (PRV-3) screening of juvenile brown trout that are interacting with sea migrants.
When assessing infection risk, one important factor to consider is whether the pathogen of focus is adapted to the host, and conversely, if the host is susceptible to that pathogen (Snieszko, 1974; Colquhoun et al., 2018b). This can be challenging, as hosts and pathogens are in a constant opposing co-evolution during which the pathogens are continuously adapting to the host population’s most common genotype (The Red Queen Hypothesis, e.g. Van Valen (1973)). The evolution of such pathogen specialization could be sped up due to farmed populations containing genetically homogenous groups of fish in very high densities (Mennerat et al., 2010), consequently, increase the risk of horizontal transmission (Snieszko, 1974).

During this study the original concepts of PRV-genotypes and host-susceptibility were re-evaluated, and discoveries were made leading to new knowledge in this field of research. The Norwegian veterinary institute (Colquhoun et al., 2018a; Dahle & Olsen, 2019) reported strong indications that genetically different PRV-strains cause disease in different salmonid species, but the genotypes can, to some extent, also infect between the species. A very recent survey for PRV in salmonids in Norway conducted by Garseth et al. (2019) found that sea trout are less susceptible to PRV-1, or PRV-1 is less adapted to sea trout, than Atlantic salmon. However, the Garseth et al. (2019) survey provides the first evidence of genotype PRV-3 in wild sea trout. The survey suggested that in Norway, PRV-3 is more prevalent in the marine environment and concludes that PRV-3 is a common virus in sea trout in Norway. PRV-3 is a new genotype of PRV and was first described in Norway in 2015 in association to HSML-like lesions in farmed rainbow trout (Olsen et al., 2015). Olsen et al. (2015) conducted the study in response to HSML-like disease outbreaks in farmed rainbow trout all originating from the same brood stock. PRV-3 was detected in all affected sites, including two marine sites where apparently clinically healthy fish from infected hatcheries developed the disease after sea transfer. Despite similar pathology to HSML, Olsen et al. (2015) detected a different course of the disease, where the disease...
outbreak occurred primarily in young fish during the fresh water phase (in contrast to HSMI in Atlantic salmon which primarily occurs 5-9 months after sea transfer). Since all affected fish originated from the same brood stock, Olsen et al. (2015) speculated that PRV-3 could also transmit vertically.

This study did not test for PRV-3 in juvenile brown trout which might explain the absence of positive results in the pathogen screening. However, none of the brown trout in Garseth et al. (2019) survey tested positive for PRV-3, but the study species all originated from non-anadromous lakes. The Norwegian veterinary institute (Dahle & Olsen, 2019) reported that PRV-3 is less common in Norwegian farming of rainbow trout. The virus might therefore be enzootic to Norway and subsequently been introduced to the industry from wild salmonids, potentially through horizontal transmission from wild sea trout. However, the origin of PRV-3 is unknown (Garseth et al., 2019) and one should therefore express caution in speculating too much.

Due to documented low PRV-1 prevalence in wild sea trout (Garseth et al., 2013b) and newly discovered PRV-genotypes and their host-specificity (Dahle & Olsen, 2019; Garseth et al., 2019), one can assume that the risk of PRV-1 exchange between farmed salmonids and sea trout is low. However, the documented high prevalence of PRV-3 in wild sea trout (Garseth et al., 2019) raises concerns regarding exchange of this virus with farmed rainbow trout.

5. Conclusive Remarks

The principles of modern aquaculture allow pathogen and virulence production that greatly exceeds levels found in natural populations, and permits spreading of pathogens both with local water currents and over extensive distances along the Norwegian coastline. The complex life-history of sea trout with its prolonged persistence in coastal areas elevates the risk of pathogen exchange with farmed salmonids, despite no indications of attraction to salmon farms.
Based on brand-new knowledge about PRV-genotypes and host-susceptibility, the genotype PRV-1 is unlikely to infect sea trout. However, it has also been newly discovered that some genotypes could infect across salmonid species, where the genotype PRV-3 may infect between sea trout and farmed rainbow trout. Regardless of PRV-genotype, the risk of cross-infestation is probably elevated during seasons with unstable environmental conditions, but likely only up to an intermediate risk level regarding PRV-1. The frequency of disease-outbreaks in Norwegian aquaculture is likely to increase with the annual increase in farmed salmonid production, further elevated by ongoing climate change, and the risk of transmission to sea trout is therefore likely to increase with it. Infectious diseases in Norwegian aquaculture can therefore be assumed to decrease the benefits of marine migration for sea trout, leading to a decline in number of anadromous individuals of a population.

In this study, there were no indications of sea trout being attracted to the salmon farms, nor any evidence to suggest freshwater reservoir of PRV-1. However, in Tosenfjorden, the sea trout resided within the fjord system during the main season of HSMI-disease outbreak in salmon farms, and the hydrodynamic dispersal model indicated that local water currents could transport pathogens up to 90 km from the source at surface and 450 km at ~30 m depth.

6. References


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