# Pathogen screening in Semi Closed Containment Systems and open net pens with emphasis on the presence of *Tenacibaculum* spp. and development of Real Time RT-PCR assays for *Tenacibaculum* spp.

Thesis for the degree

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# ABSTRACT

Tenacibaculosis, caused by *Tenacibaculum* spp., has been an increasing problem in Norwegian Salmon farming causing reduced fish welfare and significant economic losses. Semi Closed Containment System (SCCS) is a rather new technology that have proved effective against sea lice, however its preventive effect on pathogens such as *Tenacibaculum* spp. has not yet been assessed. The aim of this study was to investigate whether a SCCS could reduce the presence of *Tenacibaculum* spp. associated with ulcerative disease compared to a traditional open net pen. A secondary aim was to establish Real Time RT-PCR assays to differentiate Clade I, II and III of *Tenacibaculum* spp. relevant to ulcerative disease in Norway.

Three Real Time RT-PCR assays were developed in this study: TffC3 targeting *Tenacibaculum finnmarkense* genomovar *finnmarkense* (Clade III), TfuC1 targeting *Tenacibaculum finnmarkense* genomovar *ulcerans* (Clade I) and TdC2 targeting *Tenacibaculum dicentrarchi* (Clade II). Testing of the assays confirmed that they were specific, sensitive and effective in detecting the target bacteria when using a reverse transcriptase and annealing temperature of 47 °C and 62 °C, respectively. The assays were used in pathogen screening of fish- and water samples from the SCCS and open net pen in this study. A previously developed assay targeting the bacterium *Moritella viscosa* ('typical' and 'variant') was also included in this screening as it is commonly found in skin lesions/ulcers in outbreaks of 'winter ulcers'.

Fish- (1-111 dps) and water samples (1-170 dps) were collected from the SCCS and open net pen at the study site with more frequent samplings during the first month post stocking as this is the period in which the risk of tenacibaculosis is at its highest. Fish tissue sampled from the skin and gills, and water samples were analyzed using the developed Real Time RT-PCR assays. The results showed that there was a significantly higher presence of *T. finnmarkense* fish from the open net pen compared to the SCCS when considering the gill tissue. It is concluded that *T. dicentrarchi* is likely not present in Northern Norway salmon farms and thus can be omitted from screening of outbreaks of ulcerative disease in the Northern Norway. *T. finnmarkense* is always present in the water 1-170 dps, while *M. viscosa* emerge in the water concurrent with the onset of ulcerative disease.

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# ABBREVIATIONS

| | |
|---|---|
| BAMA | Blod agar with sea salt |
| C | Clade |
| Ct | Cycle threshold |
| Dps | Days post stocking |
| FDRG | Fish Disease Research Group |
| gen. | Genomovar |
| h | Hour |
| HK | Housekeeping |
| L | Liter |
| MA | Marine agar |
| MB | Marine Broth (Difco 2216) |
| mL | Milliliter |
| MLSA | Multilocus Sequence Analysis |
| MPN | Most Probable Number |
| NE | Normalized Expression |
| nM | Nano Molar |
| NTC | Non-template Control |
| $R^2$ | Coefficient of determination |
| RK | RNA extraction control |
| rpm | Revolutions per minute |
| rRNA | ribosomal Ribonucleic acid |
| RT-PCR | Reverse Transcriptase Polymerase Reaction |
| SCCS | Semi Closed Containment System |
| µL | Microliter |

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# 1. INTRODUCTION

## 1.1 Norwegian Salmon Farming

Norwegian salmon production has increased enormously since the 1970s and has led to Norway being the largest salmon producing country globally, with a production share of 55,3% in 2015, exporting salmon for 52,2 billion NOK in 2019 (Iversen et al., 2020; Norwegian Seafood Council, 2020). Due to the large production of salmon, there has been issues with high mortality and disease, hence Norwegian salmon farming has had a history of high antibiotic consumption (Figure 1.1). In the 1980's and 1990's the consumption of antibiotics in Norwegian salmon farming increased as a result of large outbreaks of the bacterial diseases vibriosis (*Vibrio* spp.) and furunculosis (*Aeromonas salmonicida*) (Sommerset et al., 2005). In 1987, almost 50,000 kg active substance were used to treat diseased Norwegian salmon. However, the introduction of a fish immersion vaccine proved effective against *Vibrio* spp., and the antibiotic consumption used to treat vibriosis was reduced. With large outbreaks of furunculosis in the late 1980's and early 1990's the antibiotic consumption was once again raised. In 1990 nearly 40,000 kg antibiotics were prescribed to treat salmon suffering from furunculosis. The immersion vaccine did not provide sufficient protection against *A. salmonicida*, but after several years of testing different adjuvants and antigen combinations, the most effective vaccine proved to be one with all antigens in one oil-adjuvanted vaccine. This vaccine resulted in a significant reduction in the use of antibiotics which since then has been kept at a low and steady level (Sommerset et al., 2005).

## 1.2 Disease Situation in Norwegian Farmed Salmon

The notifiable diseases most prominent in terms of increase in the Norwegian salmon farming industry in 2020, were infectious salmon anemia (ISA) and furunculosis (*A. salmonicida*). Other notifiable viral diseases like pancreas disease (PD) and Cardiomyopathy syndrome (CMS) continue to be frequently diagnosed (Sommerset et al., 2021). These diseases, including other viral, bacterial and parasitic diseases, cause large economical losses as well as fish welfare issues in the Norwegian salmon farming industry. Due to the viral diseases being the most problematic issue in Norwegian aquaculture, antibiotic consumption remains at a low level although there have recently been prescribed antibiotics to threat salmon suffering of several severe bacterial diseases (e.g., Yersinosis). It was prescribed just over 600kg antibiotics to treat farmed salmon in 2017 and in 2018 the prescription increased to over 900kg. The increased use

of antibiotics was mostly due to outbreaks of yersinosis caused by the bacterium *Yersinia ruckeri* in larger salmon. However, in 2019 there was no reported treatments against yersinosis and the antibiotics consumption was reduced to only 223kg, which is similar to the consumption prescribed in 2015 and 2016. Since 2018, there have been an increase in reported cases of pasteurellosis in farmed Atlantic salmon caused by bacteria belonging to the genus *Pasteurella*. The disease induces high mortality in large salmon and due to the lack of a commercial vaccine, the disease could possibly give rise to a higher consumption of antibiotics in the near future (Sommerset et al., 2020). Two of the thirteen antibiotic treatments involving sea-farmed salmon were related to 'winter ulcers' and *Moritella viscosa* infections in 2019 (Sommerset et al., 2020).

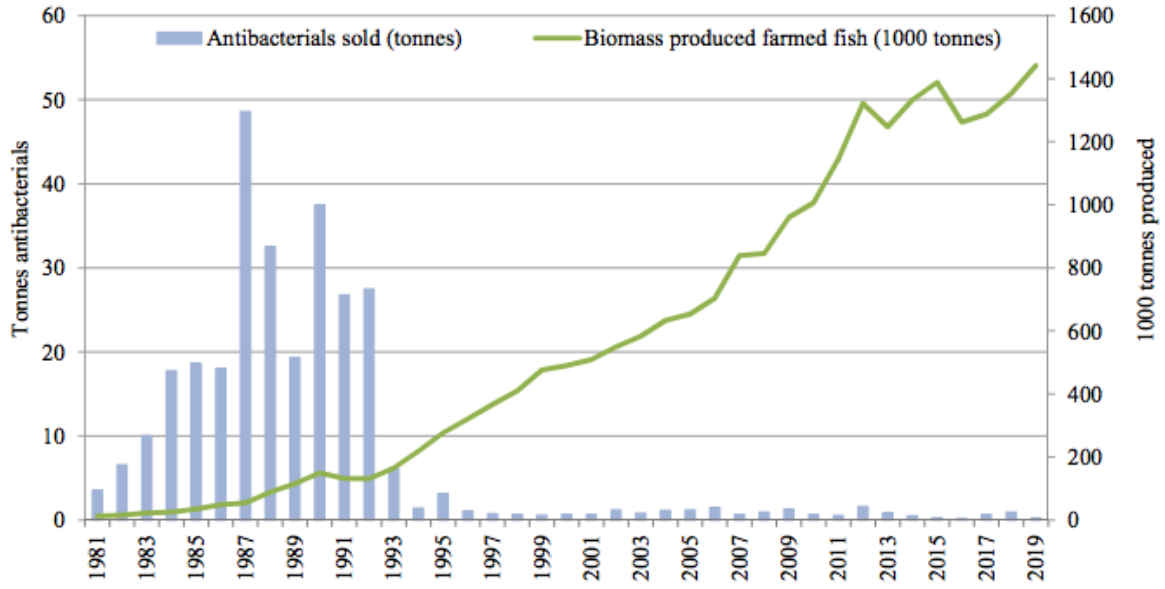


**Figure 1.1**. Sales, in tons of active substance, of antibacterial veterinary medicinal products for therapeutic use in farmed fish (including cleaner fish) in Norway in 1981-2019 vs. produced biomass (slaughtered) farmed fish (NORM/NORM-VET, 2019).

For several years, salmon lice (*Lepeophtheirus salmonis*) have been the most problematic issue in the Norwegian salmon farming industry. The large biomass and density of farmed Atlantic salmon have caused an increased salmon lice population. This has resulted in issues regarding elevated mortality in wild salmonids, as well as reduced fish welfare due to mechanical delousing treatments (Guarracino et al., 2018). There are also large economical losses in Norwegian salmon farming related to salmon lice due to costly treatments and that increased growth in the industry is put on hold until the lice problem is resolved.

Several control measures have been used to reduce the number of salmon lice but the effect has varied (Cerbule et al., 2020). Chemical treatments were initially a popular method used to combat salmon lice infestations, but recently it has been raised concerns about the potential negative impact the chemical treatment has upon copepod life underneath and surrounding the net pen and emergence of resistance in the lice population against the chemicals. Biological treatment using cleaner fish that eats salmon lice off the skin of the salmon has been a much used method in the last decade. This treatment raise concerns of cleaner fish welfare and the possible transmission of diseases between the salmon and cleaner fish (Imsland et al., 2014). Lately the number of non-chemical treatments against salmon lice has increased using e.g., heated water or freshwater baths (Powell et al., 2015), mechanical delousing using brushes or water jets, as well as laser technology. Even though the non-chemical treatments have shown good effect against sea-lice infestations, there has been some negative effects regarding fish welfare (Cerbule et al., 2020). New farming technologies using e.g., snorkel sea cages (Stien et al., 2016) or lice 'skirts' have been more frequently used in the last years. Closed Confinement Systems (CCS) have also shown promising results regarding protection against sea-lice, as well as reduced mortality (Balseiro et al., 2018; Nilsen, 2019; Øvrebø, 2020).

## 1.3 Bacterial Ulcerative Skin Diseases

Ulcerative bacterial skin diseases have been a growing issue in salmon farming related to economic losses due to downgrading at slaughter, as well as fish welfare issues. The 'winter ulcer disease' affecting Atlantic salmon has been reported since the early 1980's (Poppe, 1990). The pathological changes and bacteriological findings during the 'winter ulcer' pathogenesis in Atlantic salmon were first described by Lunder et al (1995). The acute stages of the disease are described as superficial lesions with scale loss and mild inflammation, while ulcers in the subacute stages extended down into the underlying muscle. The chronic stages of ulcers are seen histologically as severe inflammation of the dermis and the interstitial muscle tissue. A hyperplastic epidermis covers granulation tissue in the regenerative stage (Lunder et al., 1995).

Bacteria like *Aliivibrio wodanis* and *Tenacibaculum* spp. have been isolated from fish suffering from skin ulcers. Co-infections with *M. viscosa* and *Tenacibaculum* spp. have shown to cause severe skin ulcers in laboratory studies and are common in the field (Olsen et al., 2011).

Outbreaks of 'winter ulcer disease' continue to be reported although most farmed salmon in Norway are vaccinated with multi-component vaccines containing the *M.* viscosa-antigen.

However, field studies and experimental studies testing the *M. viscosa* vaccines reports a significant protection against mortalities and development of ulcerative disease (Karlsen et al., 2017). Most reported outbreaks of 'winter ulcer disease' are related to handling that could damage the skin barrier and create a breeding ground for skin ulcer bacteria (Sommerset et al., 2020).

Two major phenotypic and genetic clades ('typical' and 'variant') have been identified among *M. viscosa* isolates (Grove et al., 2010). The 'typical' *M. viscosa* induce high mortality in Atlantic salmon but not in rainbow trout, whilst the 'variant' *M. viscosa* induce some mortality in both species (Karlsen et al., 2014). It is believed that available vaccines protect against the 'typical' *M. viscosa*, but in recent years the causative agents of outbreaks of ulcerative disease have been reported to belong to the 'variant' *M. viscosa*. It has been speculated that the vaccination against 'typical' *M. viscosa* could lead to the spreading of 'variant' *M. viscosa* isolates (Takle et al., 2015). Bacteria in the genus *Tenacibaculum* have also been associated with outbreaks of 'winter ulcer disease', and due to the recent widespread use of marine agar (MA), these bacteria have been identified more often during routine diagnostics (Takle et al., 2015).

### 1.3.1 *Moritella viscosa*

*Moritella viscosa* has historically been considered to be the main etiological agent of 'winter ulcer disease' affecting salmonid fishes in several salmon producing countries, especially during the winter season concurrent with decreasing sea water temperatures. This bacterium is shown to cause skin ulcers as well as septicaemia and mortality in both field and laboratory experiments (Løvoll et al., 2009; Olsen et al., 2011). *M. viscosa* was previously known as *Vibrio viscosus* until a reclassification were proposed by Benediktsdóttir et al (2000). The basis of the reclassification was that *V. viscosus* revealed a 99±1 % sequence similarity to *Moritella marina* using 16S rRNA sequencing (Benediktsdóttir et al., 2000).

*Moritella viscosa* cells are gram-negative, short or elongated, non-spore forming rods that are motile by a single flagellum and can be cultivated in liquid or solid media. Colony morphology on bovine blood agar containing 2 % NaCl after 48 h incubation at 15 °C, are round, translucent, grey and hemolytic (Takle et al., 2015). The colonies are viscous and can form long threads when removed from the agar surface. The bacterium is catalase- and oxidase positive, acid

producing, grows at temperatures between 4-25 °C and requires NaCl for growth (Lunder et al., 2000).

## 1.4 Tenacibaculosis in Norway

Several outbreaks of severe skin and tissue damage in salmon all along the Norwegian west coast were reported in 2010. The lesions were mainly located to the head region but were also found on the fins and flanks. All outbreaks were related to low sea water temperature. Under microscopical examination of the skin lesions, long, rod-shaped bacteria were observed. With further cultivation on marine agar (MA), these bacteria were identified as close relatives to members of the genus *Tenacibaculum* (Bornø & Sviland, 2011).

The genus *Tenacibaculum* has become more known in the later years due to the high prevalence of ulcers in farmed salmonids. However, these bacteria have been observed in histological examinations of ulcers since the late 1980s in farmed salmonids in Norway (Olsen et al., 2011; Takle et al., 2015). Due to the difficulty of growing these bacteria on a supporting agar, *Tenacibaculum* spp. have not been identified during routinely diagnostics until recently, with the increased use of Marine agar (MA) that supports *Tenacibaculum* spp. growth (Olsen et al., 2011; Småge, 2018).

A challenge study was carried out by Olsen et al (2011) using two *Tenacibaculum* sp. isolates in order to test the role of these bacteria in the 'winter ulcer' pathogenesis in Atlantic salmon. The fish only developed ulcers when co-infected with *M. viscosa* or when scarified prior to bath challenge. Only one of the two isolates used in the challenge (Group 1 isolate F95B/98 = *Tenacibaculum finnmarkense* genomovar *ulcerans*, TNO010) (Olsen et al., 2017) was detected using immunohistochemistry, while the other isolate (F95C/98 = *Tenacibaculum piscium*) was not detected. These results argued that *Tenacibaculum* first and foremost act as secondary pathogens, although the degree of pathogenesis depends on the challenge isolate (Olsen et al., 2011; Småge et al., 2018).

Co-infections of *Tenacibaculum finnmarkense* and *M. viscosa* have been reported from studies of the microbiota of Atlantic salmon suffering from ulcerative disease, where analyses of skin/ulcers revealed a substantially higher presence of *Tenacibaculum* spp. compared to *M. viscosa* (Karlsen et al., 2017). In a challenge experiment conducted by Småge et al (2018), *T. finnmarkense* HFJ and *T. finnmarkense* Tsp.2 proved to be able to induce tenacibaculosis. The

findings from both of these studies, including the challenge experiment by Olsen et al (2011), suggests that *T. finnmarkense* plays an important role in the 'winter ulcer' pathogenesis as well as being able to induce ulcerative disease on its own, while *T. piscium* is not.

### 1.4.1 Clinical signs and gross pathology

Recently sea transferred smolt are usually most susceptible to tenacibaculosis caused by *T. finnmarkense* in Northern Norway. Smolts affected by the disease is typically characterized by abnormal swimming behavior. Tenacibaculosis commonly affects the unscaled parts of the skin, causing mouth erosion, frayed fins as well as tail rot and scale loss (Toranzo *et al.,* 2005; Småge, 2018). Although skin lesions/ulcer often appear in the head, tail and fin regions, ulcers may also appear in the bilateral and ventral side of the fish (Småge et al., 2017). The fish may show few or no sign of lesions in the scaled-covered part of the skin. However, when the ulcers/lesions appear in the scaled parts of the skin, the lesions are often seen as uneven with yellow margins surrounded by a large area of scale loss. These observations of ulcers are different from what is seen with 'winter ulcers' associated with *M. viscosa* infection, where the ulcers have a more defined, rounder form and a narrower zone of scale loss (Bruno et al., 1998; Småge, 2018). *M. viscosa* seems to affect the scaled parts of the skin, while *T. finnmarkense* affects the non-scaled skin (Salte et al., 1994). The cause of mortality in Northern Norwegian Atlantic salmon suffering from tenacibaculosis is unknown, however it is likely that destruction of the osmotic barriers of the skin plays an important role (Zydlewski et al., 2010). Smolts have a less favorable surface/volume ratio compared to that of larger salmon, which might explain why smolts are more affected by tenacibaculosis compared to larger fish. Mortality during outbreaks may also be associated with the release of potent exotoxins as experimentally shown for *T. maritimum* (Van Gelderen et al., 2009).

The gills of moribund Northern Norwegian Atlantic salmon smolts sampled from outbreaks of tenacibaculosis often presents little to no pathological signs, although *T. finnmarkense* can be detected in gill samples using Real Time RT-PCR (Småge, 2018). This indicate that gills may not be the preferred site of infection. There are typically no pathological findings internally associated with tenacibaculosis, which reflects that *T. finnmarkense* and *T. dicentrarchi* are rarely isolated from internal organs (Avendaño-Herrera et al., 2016; Olsen et al., 2011). This is opposed to what is commonly found with *T. maritimum*, which is associated with severe damage in the gills and can often be isolated from gills and internal organs (Frisch et al., 2018).

### 1.4.2 Diagnostics

To diagnose tenacibaculosis, microcopy of wet mount preparations collected from the margin of ulcers/lesions could be used to detect long rod-shaped bacteria (McBride, 2014). For further isolation, the bacteria should be grown on a suitable medium that supports *Tenacibaculum* spp. growth. Blood agar with 2 % NaCl (BAS) is routinely used in Norwegian aquaculture for bacteriological investigation, however, this medium does not support the growth of several *Tenacibaculum* spp. (Karlsen et al., 2017; Småge, 2018). Marine Agar (MA) should be used for bacteriological investigation of skin ulcers/lesions in Norway as this medium provides sea salt and nutrients which are absolute requirements for the growth of several *Tenacibaculum* spp. (Olsen et al., 2011; Suzuki et al., 2001).

When *Tenacibaculum* spp. are successfully isolated, the bacteria can be identified by sequencing the 16S rRNA gene by using universal primers for bacteria. However, the 16S rRNA gene provide little variation that can be used to identify *Tenacibaculum* species (Nowlan, 2020). It has therefore been recommended to use a multilocus sequence typing (MLST) targeting several housekeeping (HK) genes to separate between closely related strains of *Tenacibaculum* (Habib et al., 2014). The HK-gene *rlm*N has been proposed as a rapid, reliable and less demanding tool for phylogenetic analyses of *Tenacibaculum* spp., as an alternative to MLSA using several HK-genes (Olsen et al., 2017). Real-Time RT-PCR assays have been developed targeting the 16S rRNA gene of several *Tenacibaculum* spp. (Fringuelli et al., 2012; Nowlan, 2020), however the use of this gene has proved difficult due to the little variation among *Tenacibaculum* spp. As a result, HK-genes have been suggested for further Real Time RT-PCR assay development (Nowlan et al., 2021).

### 1.4.3 Genus *Tenacibaculum*

Bacteria of the genus *Tenacibaculum* belongs to the family Flavobacteriacea and have been isolated from several marine organisms as well as water samples (“www.bacterio.net/tenacibaculum,”). Members of the family Flavobacteriacea to which *Tenacibaculum* spp. belong have shown to be closely linked to phytoplankton blooms (Buchan et al., 2014). *T. maritimum* has also been associated with jellyfish (Ferguson et al., 2010; Fringuelli et al., 2012), suggesting that other *Tenacibaculum* spp. might also use these organisms as vectors. However, in Norway a *Tenacibaculum* sp. from the jellyfish *Dipleurosoma typicum* (Boeck) was shown to be unrelated to *T. finnmarkense* found during an

outbreak of tenacibaculosis at high sea water temperatures concurrent with jellyfish being present in great number prior to the outbreak (Småge et al., 2017).

Members of the genus *Tenacibaculum* was described by Suzuki et al in (200) as rod-shaped bacterial cell that adheres to the surfaces of marine organisms. Bacteria of this genus are gram-negative, 1,5-60 um long and 0,4-0,5 wide and produce a yellow pigment that is mainly zeaxanthin. Spores are not formed, flexirubin-type pigment is absent, and the cells are non-flagellated with a gliding motion. The bacteria are strictly aerobic heterotrophs and are catalase and oxidase positive. All strains are isolated from the marine environment and grows well on media containing sea water or sea salts. The type species in genus *Tenacibaculum* is *Tenacibaculum maritimum* (Suzuki et al., 2001).

### 1.4.4 Genetic diversity within the genus *Tenacibaculum* in Norway

A MLSA analysis conducted on 89 different Norwegian *Tenacibaculum* spp. strains isolated from both diseased and apparently healthy fish species in Norway revealed a considerable genetic diversity, and four major clades were identified within these *Tenacibaculum* spp. isolates (Olsen et al., 2017). Species belonging to three of the four clades had already been described as *T. dicentrarchi* (Clade II) and *T. finnmarkense* (Clade I, III) (Piñeiro-Vidal et al., 2012; Småge et al., 2016), whereas one of the clades encompassed bacteria that likely represented novel undescribed species (Olsen et al., 2017).

Småge et al (2016) described *Tenacibaculum finnmarkense* strain HFJ and proposed this strain as a representative of a novel species in the genus *Tenacibaculum.* The phylogenetic placement of *T. finnmarkense* strain HFJ was not addressed in the MLSA conducted by Olsen et al (2017) due to the lack of a comparable gene sequence for the strain. However, using the *rlmN* gene and phylogenetically placing the HFJ strain and 20 strains representing the four clades, it was revealed that *T. finnmarkense* strain HFJ belongs to Clade III (Småge, 2018).

In a later phylogenetic study using full genomes, it was demonstrated that *T. dicentrarchi* strains form a cohesive group, whereas *T. finnmarkense* strains are split into two subclusters (Clade I and III) (Bridel *et al.,* 2018). This study showed that *T. finnmarksense* strain TNO010 belonged to Clade I, which was found to be pathogenic to Atlantic salmon smolts in the challenge study conducted by Olsen et al (2011). However, the ability of *T. finnmarkense* strain TNO010 to cause ulcerative disease in the study was only demonstrated when the skin was scarified prior

to infection or when the fish was co-infected with *M. viscosa*. *T. finnmarkense* strain HFJ that belongs to Clade III, is on the contrary able to induce tenacibaculosis as it presents in salmon farms in Northern Norway, without any co-infection or pre-stressors at low temperatures (4 °C) (Småge et al., 2018). In the same study it was found that there appears to be little or no fish-to-fish transfer of *T. finnmarkense* during outbreaks and that different *T. finnmarkense* strains can vary in virulence. *T. finnmarkense* strains belonging to Clade III seem to be more associated with severe outbreaks of tenacibaculosis, compared to members of Clade I (Lagadec et al., unpublished; Småge et al., 2018). In a recent taxonomic study, the name *T. finnmarkense* was validated with two genomovars: *T. finnmarkense* genomovar *finnmarkense* corresponding to Clade III and *T. finnmarkense* genomovar *ulcerans* corresponding to Clade I (Olsen et al., 2020). In the same study Clade IV isolates were described as a new species: *Tenacibaculum piscium*. However, this species has not been shown to be able to induce ulcerative disease on its own, unlike the three former clades (Klakegg et al,. 2019; Olsen et al., 2011; Småge et al., 2018).

Regarding geographical distribution, isolates from mid- and northern Norway seems to dominate Clade III, indicating that *T. finnmarkense* is more commonly found in northern Norway. Clade II, consisting of a cluster of *T. dicentrarchi* is dominated by isolates obtained from Western Norway (Figure 1.2) (Lagadec et al., unpublished; Olsen et al., 2017).

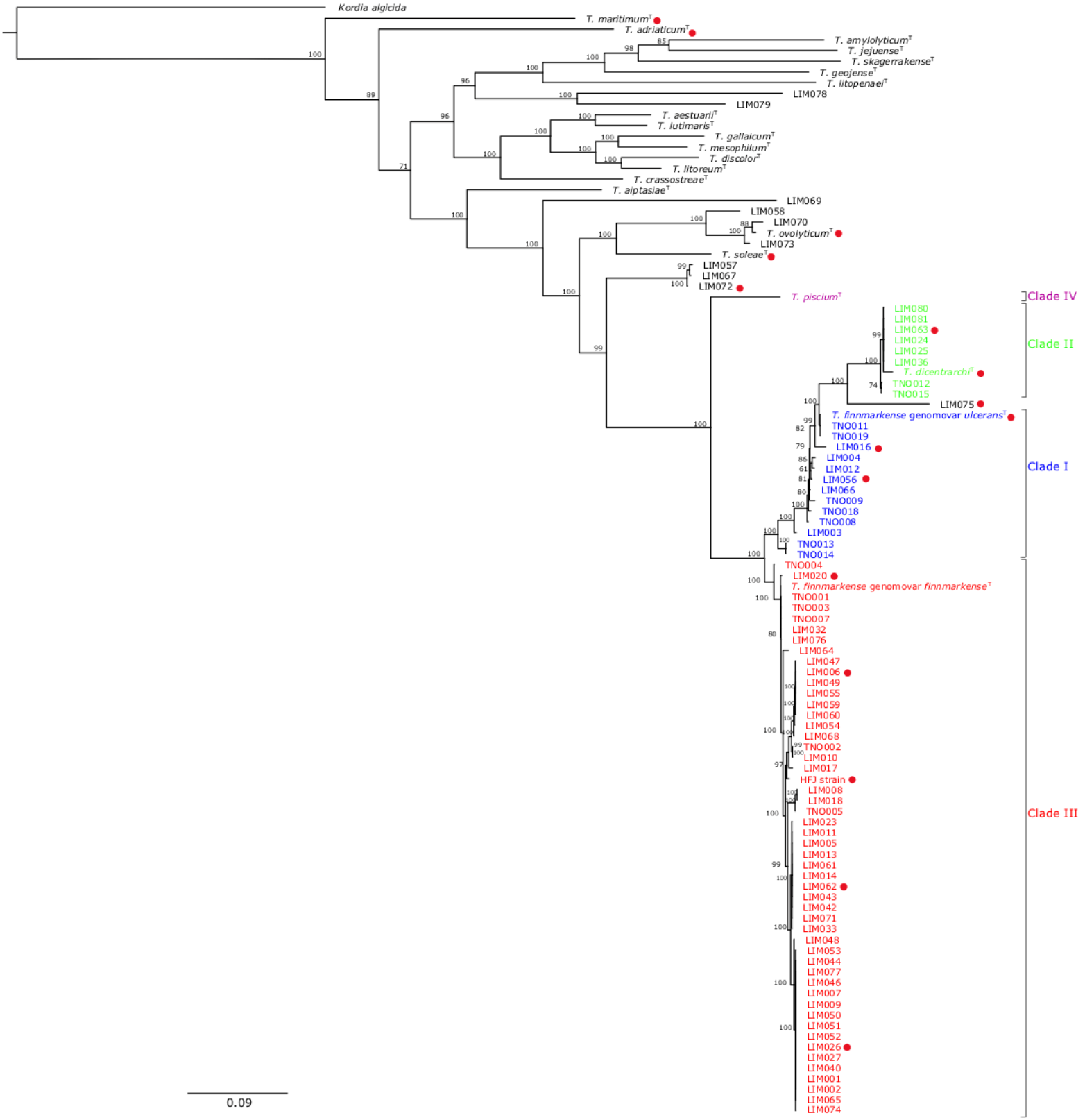


**Figure 1.2**. Phylogenetic three showing the phylogenetical placement of Norwegian *Tenacibaculum* spp. strains collected from outbreaks of ulcerative disease in Norway from 2017-2020. The isolates were isolated and included in the FHF funded study: 'Limit' and the MLST performed by Erwan Lagadec at the UiB. Alignments of single HK gene sequences for these isolates were used in the development of three Real Time RT-PCR assays. The red circles mark the isolates used in the specificity testing of the Real Time RT-PCR assays developed in this study (Lagadec et al., unpublished).

### 1.4.5 *Tenacibaculum finnmarkense*

*Tenacibaculum finnmarkense* was initially isolated from a skin lesion of a diseased Atlantic salmon in Finnmark, Norway and described by Småge et al (2016). *Tenacibaculum finnmarkense* strain HFJ is a gram-negative, aerobic, non-flagellated, long rod-shaped gliding bacteria. The cells are 5-25 x 0,5 um in size, the colony colour is yellow, growth is between 2-20 °C and only in the presence of sea salts. The bacterial cells are catalase-positive (weakly) and $H_2S$ negative (Småge et al., 2016).

Using 16S rRNA gene sequencing, Småge et al., (2016) found the strain to be closely related to *Tenacibaculum dicentrarchi* NCIMB $14598^T$ and *Tenacibaculum ovolyticum* NCIMB $13127^T$ and suggested that *Tenacibaculum* sp. strain HFJ should be classified as a representative of a novel species in the genus *Tenacibaculum* with the proposal name *Tenacibaculum finnmarkense* sp. nov. Olsen et al., (2020) proposed that the group of isolates commonly known as '*T. finnmarkense*' should be named *Tenacibaculum finnmarkense* sp. nov., with strain $TNO006^T$ as the type strain. Further proposing to subdivide *Tenacibaculum finnmarkense* sp. nov. into two genomovars; *T. finnmarkense* gen. *finnmarkense* with $TNO006^T$ as the type strain and *T. finnmarkense* gen. *ulcerans* with $TNO010^T$ as the type strain as seen in Figure 1.1. *T. finnmarkense* gen. *finnmarkense* isolates make up Clade III of Norwegian *Tenacibaculum* spp. isolates, while *T. finnmarkense* gen. *ulcerans* isolates groups into Clade I (Olsen et al., 2020).

### 1.4.6 *Tenacibaculum dicentrarchi*

*Tenacibaculum dicentrarchi* was first isolated and described from skin lesions in European sea bass in Spain (Piñeiro-Vidal et al., 2012). In Norway, *T. dicentrarchi* was isolated from Atlantic cod (*Gadus morhua*) in 2009-10 (Habib et al., 2014). The bacterial cells of *T. dicentrarchi* are strictly aerobic, gram-negative, gliding straight rods with a diameter of 0,3-0,5 µm and 2-40 µm in length (Piñeiro-Vidal et al., 2012). In aging cultures degenerative spherical cells are observed. On MA, colonies are flat, circular with uneven edges, pale-yellow in colour, with no adhesion to the agar. The growth occurs in media containing seawater with a temperature of 4-30 °C (optimum at 22-25 °C). The bacterial cells are catalase positive (weakly), $H_2S$ negative.

Based on phylogenetic analyses, *T. dicentrarchi* belongs to Clade II of Norwegian *Tenacibaculum* spp. strains recovered from outbreaks of ulcerative disease (Olsen et al., 2017, 2020). *T. dicentrarchi* seem to be more common in non-salmonid fish since three out of four salmon isolates belonging to Clade II were isolated from asymptomatic fish (Olsen et al. 2017).

The isolates from western Norway seems to dominate the *T. dicentrarchi* Clade II of Norwegian *Tenacibaculum* bacteria (Klakegg et al., 2019; Olsen et al., 2017).

## 1.5 New Farming Technologies

Due to the increasing problems with drug resistant salmon lice, pathogens causing high mortality, escapees and a big environmental impact, the Norwegian salmon farming industry has started to look into new farming technologies to combat these challenges (Nilsen, 2019; Øvrebø, 2020). These new technologies include a variety of new land-based facilities, development of offshore aquaculture, and a variation of Closed Containment Systems (CCS) including Semi Closed Containment Systems (SCCS).

Semi Closed Containment Systems is defined as a fish-producing facility that has an impenetrable barrier, or close to impenetrable, between the fish and the surrounding environment (Iversen et al., 2013). The use of SCCS reduces the time fish spend in open sea cages and could thus lower the negative impact the sea-phase has on the salmon in terms of e.g., sea lice infestations and pathogens. In a study by Øvrebø (2020), it was found that fish farmed in a SCCS had suffered lower basal stress compared to reference groups in open sea cages. Moreover, it was found that a SCCS significantly lowered salmon sea lice infestations, as well as causing higher weight gain and final weight of the fish compared to the reference fish in open sea cages. The conclusion of the study was that SCCS appear to have advantages compared to traditional production in open sea cages. However, the author emphasized that further research is needed to improve production in SCCS (Øvrebø, 2020). Several studies on fish stocked in Closed Containment Systems (CCS) have been conducted with the focus on e.g., mortality rate and growth performance showing promising results (Balseiro et al., 2018; Nilsen et al., 2020; Skaar & Bodvin, 1993). In a study by Nilsen et al (2020), different mortality causes were described in fish from CCS and one of the main causes of mortality were described as ‘Ulcers and fin rot’ accounting for 36,1 % of total mortality. This indicates that ulcerative disease can be a challenge when stocking fish in SCCS.

## 1.6 The Use of Real Time RT-PCR to Monitor the Presence of Fish Pathogens

Real Time RT-PCR uses fluorescent reporter molecules to monitor the production of amplification products during each cycle of the PCR reaction. This eliminates the need of gel electrophoresis to detect amplification products and DNA sequencing for amplicon identification. The simplicity, specificity and sensitivity of this method has made Real Time RT-PCR a benchmark technology for detection of RNA (Bustin et al., 2005).

In intensive fish farming it is important to monitor the presence of pathogens in a system (e.g., sea cage) in order stop the spread of harmful pathogens, either inside the given system or into the environment outside the system. Pathogen screening could potentially reveal infection diversity from which disease arises, patterns of infection and potential risks associated with agent transmission between wild and farmed hosts (Bateman et al., 2021). The monitoring of possible harmful pathogens is commonly performed using Real Time RT-PCR since the method is rapid and fairly inexpensive, but still efficient and sensitive.

The Norwegian salmon farming industry has been screening farmed salmon as risk assessment for several diseases. The surveillance of ISA-virus using e.g., Real Time RT-PCR in the monitoring has shown success reflected in the decrease of new ISA-virus cases in Norway the last two decades (Gjevre & Svendsen, 2018; Lyngstad et al., 2011; Nylund et al., 2019). When detecting ISA-virus in fish farms through screening, prior to outbreaks, the spread of disease may be reduced. There is also an economical aspect to pathogen screening. If the pathogen (e.g., ISAV) is detected prior to outbreak, the fish may still be slaughtered and sold, giving some economic gain.

Real Time RT-PCR assays have been developed to target Norwegian *Tenacibaculum* spp. isolates with success (Vold, 2014). Assays used for monitoring the presence of *T. maritimum* have also been developed (Downes et al., 2018; Fringuelli et al., 2012). Nowlan et al (2021) attempted to design Real Time RT-PCR assays targeting *T. finnmarkense* and *T. dicentrarchi* using the 16S rRNA gene as target. However, the authors were not successful and advised further research on HK-genes in the development of assays specific to *T. dicentrarchi* and the two genomovars of *T. finnmarkense*.

## 1.7 Aims of Study

The main aim of this study was to determine if a SCCS could reduce the presence of *Tenacibaculum* spp. compared to a traditional open net pen.

There has been an increased focus on screening fish and water the first month post sea transfer to provide a detailed picture of the emergence of pathogens that could lead to a disease situation. Hence, a secondary aim of this study was to develop three Real Time RT-PCR assays to differentiate between members belonging to Clades I, II and III in the genus *Tenacibaculum* spp., i.e., bacteria associated with ulcerative disease in Norwegian salmon production.

The 0-hypothesis for the current study is: A SCCS does not reduce the presence of *Tenacibaculum* spp. and the risk for ulcerative disease compared to an open net pen.

# 2. MATERIALS AND METHODS

## 2.1 Fish Sampling

### 2.1.1 Collecting fish at site

A total of 30 fish from the Semi Closed Containment System (SCCS) and 30 fish from a reference net-pen were collected and euthanized by an overdose of Benzoak vet. (ACD Pharmaceuticals AS) concurrent with scheduled welfare scoring and lice count conducted at both pens. This was performed weekly the first month after stocking and once a month after the first month (Table 2.1). Individual fish were placed in plastic bags marked with fish number before being frozen at -20 °C and shipped to the University of Bergen (UiB). The fish were stored at -20°C until being processed for tissue sampling. Information regarding fork length and weight for each fish was retrieved from the welfare scoring at the sites. Pre stocking fish from the smolt site were also collected on site, frozen and sent to UiB to be analyzed in order to check if the fish was infected with any known pathogens prior to stocking.

**Table 2.1.** Scheduled fish sampling plan with dates at study site from the SCCS and open net pen. *Fish from the SCCS were collected 09.11.2020, 55 days past stocking (dps), one day prior to fish from the open pen 10.11.2020 (56 dps).

| Sampling Number | Date of sampling | Days post stocking (Dps) | Number of fish collected | |
|---|---|---|---|---|
| | | | SCCS | Open net pen |
| **0** | 10.09.2020 | -5 | 30 | 30 |
| **1** | 16.09.2020 | 1 | 30 | 30 |
| **2** | 23.09.2020 | 8 | 30 | 30 |
| **3** | 30.09.2020 | 15 | 30 | 30 |
| **4** | 09.10.2020 | 24 | 30 | 30 |
| **5** | 14.10.2020 | 29 | 30 | 25 |
| **6** | 09.11.2020-10.11.2020* | 55-56* | 30 | 30 |
| **7** | 07.12.2020 | 83 | 30 | 30 |
| **8** | 04.01.2021 | 111 | 30 | 30 |

### 2.1.2 Sampling in lab Bergen

Frozen fish from the SCCS and the reference pen were thawed for 12 hours at 4 °C prior to tissue sampling. The fish were examined externally and internally before collecting the tissue samples to register signs of disease. Skin, gills and kidney tissues were sampled for subsequent RNA extraction and Real Time RT-PCR analyses. Skin tissues were sampled from under the jaw when no skin lesions/ulcers could be detected. If the fish was scored 1-3 in the Cermaq Welfare Scoring system with wounds or showed presence of skin lesions/ulcers, the skin tissue sample were collected from the affected areas. Gill samples were collected from the gill filaments (second gill arch) and kidney samples were collected from the head-kidney. All tissue samples were stored in 2 mL Eppendorf tubes and put on ice immediately after sampling. Scalpel and tweezers were sterilized between every tissue sample using 100 % ethanol and flame. Back-up samples from skin, gills and kidney were collected from every tissue and stored in a collection tube. All samples were stored at -20 °C until further processing.

## 2.2 Water Sampling

Water samples were collected at site every other day during the first month after stocking and once a week after the first month until 170 dps (Table 2.2). Specific water sampling bottles (VWR) were used to collect 0.5 L of water from the SCCS and the reference open net pen. A water sample was also sampled from the SCCS prior to fish stocking. The water samples were collected by lowering the 0.5 L water sample bottle with the cap unscrewed under the water surface, before opening in order to avoid retrieving surface film that may contain unwanted material. The water samples were immediately frozen at -20 °C after sampling, before being sent to Bergen for further analysis.

**Table 2.2.** Scheduled water sampling plan with dates at the study site. Sea water were sampled from both the SCCS and open net pen at each sampling. *At the first sampling 6 days prior to fish stocking, water was only sampled from the SCCS. The red line indicates the end of the first month after fish stocking. Samplings above this line were performed once every other day. Below the red line, water was collected once a week.

| Sampling Number | Date of sampling | Days post stocking |
|---|---|---|
| 1* | 09.09.2020 | -6 |
| 2 | 15.09.2020 | 0 |
| 3 | 17.09.2020 | 2 |
| 4 | 19.09.2020 | 4 |
| 5 | 21.09.2020 | 6 |

| | | |
|---|---|---|
| 6 | 23.09.2020 | 8 |
| 7 | 25.09.2020 | 10 |
| 8 | 27.09.2020 | 12 |
| 9 | 29.09.2020 | 14 |
| 10 | 01.10.2020 | 16 |
| 11 | 03.10.2020 | 18 |
| 12 | 05.10.2020 | 20 |
| 13 | 07.10.2020 | 22 |
| 14 | 09.10.2020 | 24 |
| 15 | 11.10.2020 | 26 |
| 16 | 13.10.2020 | 28 |
| 17 | 15.10.2020 | 30 |
| 18 | 22.10.2020 | 37 |
| 19 | 25.10.2020 | 40 |
| 20 | 29.10.2020 | 44 |
| 21 | 05.11.2020 | 51 |
| 22 | 12.11.2020 | 58 |
| 23 | 19.11.2020 | 65 |
| 24 | 20.11.2020 | 66 |
| 25 | 26.11.2020 | 72 |
| 26 | 03.12.2020 | 79 |
| 27 | 10.12.2020 | 86 |
| 28 | 17.12.2020 | 93 |
| 29 | 24.12.2020 | 100 |
| 30 | 31.12.2020 | 107 |
| 31 | 07.01.2021 | 114 |
| 32 | 14.01.2021 | 121 |
| 33 | 21.01.2021 | 128 |
| 34 | 28.01.2021 | 135 |
| 35 | 04.02.2021 | 142 |
| 36 | 11.02.2021 | 149 |
| 37 | 18.02.2021 | 156 |
| 38 | 25.02.2021 | 163 |
| 39 | 04.03.2021 | 170 |

### 2.2.1 Water filtration

Two water filtration methods were evaluated for use in this study. The first method following an in-house protocol (FDRG) described below were compared to a second method following a protocol provided by Hu (Hu, 2017) using glass microfiber filters (Whatman®) with pore size 0.7 μm . The methods were compared by collecting two flasks each containing 1.0 L sea water and adding 20 μL *Halobacterium salinarum* to each flask. A Real Time RT-PCR assay (Hsal) targeting *H. salinarum* (Andersen et al., 2010a) were further used to compare the obtained Ct-values after filtration. The first method following the in-house protocol presented the lowest Ct-value and this method was thus used for the subsequent water filtration of the water samples from the SCCS and open net pen at the study site.

Water filtration was performed following the manufacturer's instructions (VWR). A volume of 0.5 L of sea water was vacuum filtered through one-layer of Cellulose Nitrate Filters (Sartorius Stedim Biotech GmbH) with pore size 1.2 μm with a stainless steele vacuum filtration system (Microsart®) for 47 mm diameter membranes with a water flow of 0.2-0.5 liters per min. A volume of 10 μl of *H. salinarum* were added to each flask prior to filtration. The filtration system was washed using soap and distilled water between the filtration of each water sample. The filters were placed upside down in 1.4 mL of lysis buffer (E.Z.N.A total RNA kit OmegaBioTek) in 50 mm diameter petri dishes and sealed with parafilm and shaken for 10 minutes (150 rpm) at room temperature on a Mini-Shaker (Biosan). Two portions consisting of 350 μL of lysis buffer were removed from the petri dish and transferred into two 2.0 mL Eppendorf tubes. The samples were mixed with 350 μL 70 % ethanol, vortexed and frozen at -80 °C prior to RNA extraction following the cultured cell protocol from the E.Z.N.A total kit.

## 2.3 RNA Extraction using TRIzol® Reagent

RNA from tissue samples were extracted following the manufacturer`s protocol (Sigma-Aldrich TRIzol® Reagent). The tissue samples contained in 2 mL Eppendorf tubes were kept on ice while sterile steel beads (5 mm) were added to the lid of the tubes containing skin. The beads were sterilized using 100 % ethanol and flame before being added to the tube lids. A volume of 1.0 mL TRIzol® was added to the tissue samples and homogenized in a TissueLyser (QIAGEN) at 30/s for 3 minutes for kidney and gills, while tubes containing skin samples were homogenized for 6 minutes. The samples were then incubated for 5 minutes at room temperature before adding 200 μl chloroform (Sigma-Aldrich) to the tubes. The tubes were shaken by hand for 20 seconds and incubated for 5 more minutes. The samples were centrifuged

at 12 000 x g for 15 minutes at 4 °C to separate the mixture into three phases, with the upper aqueous phase containing the RNA. A volume of 500 µL of the aqueous phase was added to an Eppendorf tube containing 500 µL isopropanol. The tubes were subsequently turned upside down to mix and further incubation of the solution at room temperature for 10 minutes. The samples were centrifuged at 12 000 x g for 15 minutes at 4 °C to form a pellet of RNA. The supernatant was removed, and the pellet washed using 75 % ethanol and centrifuged at 12 000 x g for 5 minutes at 4 °C. The last washing step was repeated using 100 % ethanol. The supernatant was removed, and the pellet left to dry for 10 minutes to remove any trace of alcohol before the pellet was eluted in 150 µL 60 °C nuclease-free water, vortexed and stored at -80 °C. Controls followed the same procedures as the samples, only they did not contain any tissues.

## 2.4 RNA Extraction using the E.Z.N.A total RNA kit

RNA extraction from water samples was performed by Cermaq Staff using the E.Z.N.A Total RNA Kit I (Omega BioTek), following the Cultured cells protocol provided by the manufacturer.

## 2.5 Real Time RT-PCR

The AqPATH-ID™ One-Step RT-PCR Kit (Applied Biosystems) was used and the manufacturer's instructions followed when analyzing the RNA by Real Time RT-PCR.

**Table 2.3.** Primers, probes and their target used in this study.

| Assay | Primer/ Probe | Sequence | Reference |
|---|---|---|---|
| **TffC3 (*Tenacibaculum finnmarkense* genomovar *finnmarkense*)** | Forward<br>Reverse<br>Probe | ACATGACGCATCGTTGGTTGAA<br>ATGCCCGTATTTGAAAGGTACTTT<br>CGTTGTGTAAATCACTT | Present study |
| **TfuC1 (*Tenacibaculum finnmarkense* genomovar *ulcerans*)** | Forward<br>Reverse<br>Probe | ACATGACGCATCGTTGGTTAAC<br>ATGCCCGTATTTGAAAGGTACTTT<br>CGTTGCGTAAATCACTT | Present study |

| | | | |
|---|---|---|---|
| **TdC2 (*Tenacibaculum dicentrarchi*)** | Forward<br>Reverse<br>Probe | ACATGACACACCGTTGGTTAAC<br>ATACCCGTATTTAAAAGGTAGTTT<br>CGTTGTGTAAATCATCT | Present study |
| **EF1A (Elongationfactor salmon)** | Forward<br>Reverse<br>Probe | CCCCTCCAGGACGTTTACAAA<br>CACACGGCCCACAGGTACA<br>ATCGGTGGTATTGGAAC | (Olsvik, Lie, Jordal, Nilsen, & Hordvik, 2005) |
| **MvOmpA (*Moritella viscosa*)** | Forward<br>Reverse<br>Probe | GATGATAACGCAACAGCAG<br>CGGAAACTTACACCAGATAATG<br>TCTTGGAGCAGGTCTAGAATATACACCAG | (Vold, 2014) |
| **Tb_rpoB (*Tenacibaculum* spp.)** | Forward<br>Reverse<br>Probe | GGAGCAAACATTGACCAAATT<br>GGTATGTCCGTAACGTGGAA<br>TCCTGCTTGATCAGTTAAAGCGT | (Vold, 2014) |
| **Tb_tuf (*Tenacibaculum* spp.)** | Forward<br>Reverse<br>Probe | AGTGTGACGTCCACCTT<br>CTGTAAGCCAGGTTCTGT<br>TTTCAATACATACACCTCAGC | (Vold, 2014) |
| **Hsal (*Halobacterium salinarum*)** | Forward<br>Reverse<br>Probe | AGGCGTCCAGCGGA<br>GGGAAATCTGTCCGCTTAACG<br>CCGGTCCCAAGCTGAACA | (Andersen, Hodneland, & Nylund, 2010b) |

The TffC3 assay was used to target *Tenacibaculum finnmarkense* genomovar *finnmarkense* in the samples. TfuC1 to target *Tenacibaculum finnmarkense* genomovar *ulcerans*, TdC2 to target *Tenacibaculum dicentrarchi*, the MVOmpA to target *Moritella viscosa*, and EF1A targeting the elongation factor alpha of *Salmo salar*.

The MicroAmp Optical 96-well Reaction Plates (Applied Biosystems) were used in the Real-Time RT-PCR reaction. The plate was put on ice while adding the mastermix and template; 10.5 uL mastermix and 2 uL template (total volume 12.5 µl) was added to each well. The mastermix consisted of 6.25 µl 2X R-PCR buffer, 1.0 µl forward primer, 1.0 µl reverse primer, 0.22 µl probe, 0.25 µl Enzyme mix and 1.78 µl nuclease-free water to adjust the volume of the mastermix to 10.5 µl. An RNA-extraction control (RK) was added to every run in order to detect possible contamination during the RNA extraction procedure. A non-template control (NTC) consisting of nuclease free water was added to each run to detect possible contamination during the adding of template to the plate. After adding the template, the plate was sealed with MicroAmp™ Optical Adhesive Film (Applied biosystems (Thermo Fisher Scientific), centrifuged for 20 seconds and analyzed using an Applied Biosystems Quantstudio 3 Real-

Time PCR system. The reactions were run according to the Standard AgPath setup with optimal reverse transcriptase- and annealing temperatures as determined in section 2.6.5; Reverse transcription for 10 minutes at 47 °C, polymerase activation for 10 minutes at 95 °C, 45 cycles of DNA-dissociation for 15 seconds at 95 °C and annealing and elongation for 45 seconds at 62 °C for the TffC3, TfuC1 and TdC2 Real Time RT-PCR assays. Other Real Time RT-PCR assays used in the current study were run with a reverse transcriptase and annealing according to the standard AgPath Setup with temperatures of 45 °C and 60 °C, respectively. The amplification curves were given a fixed threshold at 0.1.

## 2.6 Real-Time RT-PCR Assay Development

Results from a MLSA based on genotyping of 89 Norwegian *Tenacibaculum* isolates from tenacibaculosis outbreaks in several fish species revealed a considerable genetic diversity of *Tenacibaculum* isolates that could be separated into four major clades (Olsen et al 2017). Isolates belonging to the species *T. dicentrarchi* made up Clade II, *T. piscium* represent Clade IV, and *T. finnmarkense* makes up Clade I and Clade III (divided into two genomovars; *T. finnmarkense* genomovar *finnmarkense* (CIII) and *T. finnmarkense* genomovar *ulcerans* (CI) (Olsen et al., 2020). Bacteria from these different clades cannot be differentiated using the existing *Tenacibaculum* spp. Real-Time RT-PCR assays. The Tb_tuf Real-Time RT-PCR assay seems to be very sensitive to *Tenacibaculum* spp., but the assay is not specific enough to differentiate between CI, CII and CIII of *Tenacibaculum* spp. that are relevant to ulcerative disease in Norway (Vold, 2014). Tb_rpoB seems to be specific to *T. finnmarkense* genomovar *finnmarkense* (Vold, 2014), but still there is no available assays targeting *T. finnmarkense* genomovar *ulcerans* and *T. dicentrarchi.* After several specificity tests of the available *Tenacibaculum* spp. assays (Tb_tuf and Tb_rpoB) it was deemed necessary to design new assays due to the lack of specificity of the current assays. New assays to target *T. finnmarkense* gen. *finnmarkense, T. finnmarkense* gen. *ulcerans* and *T. dicentrarchi* using the same housekeeping gene (*tgt*) were developed.

### 2.6.1 Target genes and Real-Time RT-PCR primer and probe selection

77 strains were used to create three *Tenacibaculum* spp. Real Time RT-PCR assays to discriminate Clades I, II or III. Primer and probe design were performed by Professor Are Nylund and alignments were provided by Erwan Lagadec (FDRG) at the University of Bergen. The 77 *Tenacibaculum* spp. strains consisted of: 15 *Tenacibaculum* spp. strains isolated in

Norway (Habib et al., 2014), the HFJ strain isolated and described in 2016 (Småge et al., 2016), type strains from GenBank (including the newly described *T. piscium*, *T. finnmarkense* gen. *finnmarkense* and *T. finnmarkense* gen. *ulcerans* (Olsen et al., 2020) and 57 *Tenacibaculum* spp. strains isolated in the frame of an ongoing project funded by FHF ('Limit').

All these *Tenacibaculum* spp. were included in a MLST analysis using primers targeting seven housekeeping genes *atpA* (567 bp), *dnaK* (573 bp), *glyA* (558 bp), *gyrB* (597 bp), *infB* (564 bp), *rlmN* (549 bp) and *tgt* (486 bp) as previously described by Habib et al (2014). Sequence alignments were constructed for all seven loci separately using AlignX in the VectorNTI 9.0.0 software package (Invitrogen). The sequences were trimmed and adjusted to correct reading frames in GeneDoc (Nicholas, 1997). Concatenation of the seven housekeeping genes was performed using Kakusan4 (Tanabe, 2011). The substitution rate, codon position and best fit model for the individual loci were calculated with Kakusan4. The Bayesian phylogenetic analysis was performed in MrBayes 3.2 (Ronquist & Huelsenbeck, 2003) with a proportional codonproportional data bloc and a Markov Chain Monte Carlo (MCMC) analysis. The run included 20,000,000 generations and trees were sampled every 1000 generations. The initial 10,000 trees were discarded as a conservative "burn-in" in TreeAnnotator and the final tree was visualized in FigTree v1.4.3 (Figure 1.2). Based on the phylogenetic analysis (Prof. Are Nylund), the gene *tgt* was found to be most suitable for development of the three wanted assays. All three assay are located in the same region of *tgt* with the probes covering nucleotides 373 – 389 (Accession no: MK414132)

The following steps were taken in the assay development process:

1. Testing the specificity and sensitivity of each assay using pure-culture *Tenacibaculum* spp. field isolates.
2. Optimalization of the primers and probes concentration.
3. Efficacy testing of the assays.
4. Testing the assays on tissues.

### 2.6.2 Culturing of bacterial isolates

All *Tenacibaculum* spp. field isolates used in the assay development are listed in Table 2.4. The isolates were cultured and prepared for RNA extraction using two different methods; bacterial growth in Marine Broth (Difco 2216) for 48-72 hours, 160 rpm at 16 °C and direct plating onto Blood agar with sea salt (BAMA) plates for 48-72 hours at 16 °C.

**Reviving of freeze-dried NCIMB type strain cultures**

*T. finnmarkense* gen. *ulcerans* type strain TNO010$^T$ (NCIMB 15239) and *T. finnmarkense* gen. *finnmarkense* type strain TNO006$^T$ (NCIMB 15238) were ordered freeze-dried from NCIMB and had to be revived. The strains were supplied as cultures freeze-dried on filter paper within glass ampoules which were sealed under vacuum. A glass slide was carefully used to score around the circumference of the ampoule around the middle where a cotton wool plug had been placed at freezing. Force were applied until the ampoule broke and the cotton wool plug were removed. Flame were applied to the end of the ampoule and 0.5 mL of MB were transferred to the ampoule containing the freeze-dried culture and gently mixed using a pipette. The bacterial suspension was cultured on BAMA for 48-72 hours at 16 °C and in 20 mL Marine Broth (Difco 2216) for 48-72 hours and 160 rpm at 16 °C.

**Bacterial growth in Marine Broth (MB)**

200 µL of frozen inoculum (bacterial stock solution of *Tenacibaculum* spp.) was added to Marine Broth (Difco 2216) and then cultured at 16 °C and 140 rpm for 48 hours. 1.0 mL of the suspension was added to 2.0 mL Eppendorf tubes and frozen at -20 °C prior to RNA extraction.

**Bacterial growth on Marine Blood Agar (BAMA)**

100 µL of frozen inoculum (bacterial stock solution of *Tenacibaculum* spp. isolates) was added to BAMA plates and grown for 48 hours at 16 °C. A volume of 10 µL of bacterial colonies was collected from the plates using a bacterial loop and added to 1.0 mL MB in a 2.0 mL Eppendorf tube. The samples were homogenized into a homogenous suspension. Most of the suspension was added to a 15 mL Falcon tube with approximately 7.0 mL MB and further homogenized. A volume of 1.0 mL of the suspension was added to a 2.0 mL Eppendorf tube and frozen at -20 °C prior to RNA extraction.

**RNA Extraction of bacterial isolates**

RNA from *Tenacibaculum* spp. field isolates was extracted following the manufacturer's protocol (TRIzol® Reagent) and the same steps were performed as described in section 2.3, except for the following adjustments: RNA extraction from all *Tenacibaculum* spp isolates was performed by making a suspension of 1.0 mL MB and pure-culture bacterial isolates. To separate the bacterial cells from the MB the tubes were centrifuged for 4 minutes at 12.000 x g. The supernatant was removed, and 1.0 mL nuclease free water added to the tubes and vortexed. The tubes were centrifuged for 4 minutes at 12.000 x g before removing the

supernatant. 1.0 mL of TRIzol® was added to the tubes containing the bacterial cells, and vortexed for 5 minutes to homogenize the suspension before adding 200 µL Chloroform to the mix.

**Table 2.4.** List of *Tenacibaculum* spp. field isolates used in the specificity testing of the Real-Time RT-PCR assays. *Strain LIM075 (INTER) is phylogenetically placed between Clade I and Clade II. **Strain LIM072 do not belong to CIV but is phylogenetically closely related. Type species strain names are obtained from Park et al (2017) (Park et al., 2017).

| Isolate name | Species | Clade |
|---|---|---|
| *T. finnmarkense* gen. *finnmarkense* HFJ | *T. finnmarkense* genomovar *finnmarkense* | III |
| *T. dicentrarchi* 35/09$^T$ (NCIMB 14598) | *T. dicentrarchi* | II |
| *T. finnmarkense* gen. *ulcerans* TNO010$^T$ (NCIMB 15239) | *T. finnmarkense* genomovar *ulcerans* | I |
| LIM056 | *T. finnmarkense* genomovar *ulcerans* | I |
| LIM016 | *T. finnmarkense* genomovar *ulcerans* | I |
| LIM063 | *T. dicentrarchi* | II |
| LIM075 | *Tenacibaculum* sp. | INTER* |
| LIM006 | *T. finnmarkense* genomovar *finnmarkense* | III |
| LIM020 | *T. finnmarkense* genomovar *finnmarkense* | III |
| LIM026 | *T. finnmarkense* genomovar *finnmarkense* | III |
| LIM062 | *T. finnmarkense* genomovar *finnmarkense* | III |
| LIM072 | *T. piscium** | IV** |
| *T. soleae* LL04 12.1.7$^T$ (NCIMB 14368) | *T. soleae* | - |
| *T. ovolyticum* IFO 15947$^T$ (NCIMB 13127) | *T. ovolyticum* | - |
| *T. maritimum* IFO 15946$^T$ (NCIMB 2154) | *T. maritimum* | - |
| *T. adriaticum* B390$^T$ (DSM 18961) | *T. adriaticum* | - |

### 2.6.4 Most Probable Number (MPN)

MPN is a dilution method for estimating the number of viable bacterial cells in a sample. In this method, tubes of broth are inoculated with progressively more diluted samples of a bacterial cell suspension, incubated and examined for growth. The number of bacteria in a sample is estimated based on the statistical probability of each sample containing viable cells (Blodgett,

2010; Hogg, 2013). In this study, the MPN method was performed in order to estimate the number of *Tenacibaculum* spp. bacterial cells in 1.0 mL of MB. All estimations using the MPN method were performed when the bacteria were in the exponential growth phase.

The MPN method was performed using 10-fold dilutions in duplicate with 8 replicates per dilution. To make the 10-fold, 900 µL of MB were transferred into 11 x 2 2.0 mL Eppendorf tubes using a multi pipette. 1.0 mL of the bacterial suspension was transferred to a 2.0 mL Eppendorf tube. 100 µL of the bacterial suspension were transferred to the first tube containing 900 µL MB and mixed by pipetting up and down 10 times. Next, 100 µL of the content in the first Eppendorf tube were transferred to the second tube and mixed. This was repeated for all 11 Eppendorf tubes (Figure 2.1). The chosen dilutions were selected and added to a 96-well plate with 8 replicates per dilution 8 (Figure 2.2). $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$, $10^{-10}$ and $10^{-11}$ were the chosen dilutions for every MPN performed in the present study.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| X | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ | $10^{-11}$ |
| X | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ | $10^{-11}$ |

**Figure 2.1**. Illustration of the two parallels in the MPN dilution series of bacteria cultivated in MB. The X represents a non-diluted sample of the bacterial culture.

| | **Parallel 1** | | | | | | **Parallel 2** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ | $10^{-11}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ | $10^{-11}$ |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

**Figure 2.2.** Illustration of the 96-well plate with 8 (A-H) replicates per dilution. The samples are gradually more diluted with column 1 and 7 being the least diluted and column 6 and 12 being most diluted. The degree of dilution is illustrated with a gradually lighter shade in the wells.

The 96-well plates were incubated for at least 48 hours at 16 °C to observe growth but were analyzed after 168 hours (one week) of incubation. The plate was held against a light source and observed underneath to analyze the bacterial growth. A positive well (growth) appears as precipitation in the well. No growth indicates that the samples have been diluted as much as possible for growth. The positive wells were counted for each dilution, which could have a maximum of 8 positive wells and 8 negatives. Figure 2.3 illustrates an example of how to read a MPN plate incubated at 16°C for 48h. The X represents growth in the wells. Parallel 1 reads 8 8 5 1 0 0 and parallel 2 reads 8 8 4 1 0 0 (Figure 2.3). Three numbers from each parallel are chosen, preferably ending with a zero, this means parallel 1 is 8 5 1 and parallel 2 is 8 4 1.

| | **Parallel 1** | | | | | | **Parallel 2** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ | $10^{-11}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ | $10^{-9}$ | $10^{-10}$ | $10^{-11}$ |
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| **A** | X | X | X | | | | X | X | X | | | |
| **B** | X | X | X | | | | X | X | X | | | |
| **C** | X | X | | | | | X | X | X | | | |
| **D** | X | X | X | | | | X | X | | X | | |
| **E** | X | X | | X | | | X | X | | | | |
| **F** | X | X | X | | | | X | X | | | | |
| **G** | X | X | | | | | X | X | X | | | |
| **H** | X | X | X | | | | X | X | | | | |

**Figure 2.3.** An example on how to read the 96-well plate incubated for 48 hours at 16 °C. In this example parallel 1 should be read as 8 5 1 and parallel 2 as 8 4 1. Growth of parallel 1 stops at the $10^{-10}$ dilution. Growth of parallel 2 stops at the $10^{-10}$ dilution.

To estimate the MPN of the three numbers from the 96-well plate, an MPN-reference table for 8 replicates was used (Blodgett, 2010). The MPN value for the three numbers taken from the MPN-reference table is multiplied by the $10^{X}$ of the middle number and multiplied by 10 because the bacterial suspension had been diluted with 100 µL into 900 µL of MB. The MPN values of parallel 1 plus parallel 2 divided by two gives the average MPN value and the value is given in cells/mL.

### 2.6.5 Specificity testing of Real-Time RT-PCR assays

The specificity of the assays was tested using RNA extracted from field isolates of *Tenacibaculum* spp., strain HFJ of *T. finnmarkense* gen. *finnmarkense* and the type species of *T. finnmarkense* gen. *ulcerans* and *T. dicentrarchi* listed in Table 2.4. This was performed to ensure the specificity of the assays and that the assays only amplified target RNA. All reactions were performed in triplicates and run according to the Standard AgPath setup with the optimal primer and probe concentrations listed in Table 3.2. The assay specificity was tested for all assays using four different reverse transcriptase and annealing temperatures as listed in Table 2.5. These analyses were performed to identify the temperature that made the assays most specific and only amplify the target RNA. The adjustments of the temperature for the reverse transcriptase and annealing steps were performed based on guidelines in the AgPath-ID™ One-Step Rt-PCR Reagents User Guide (ThermoFisher).

**Table 2.5.** The different reverse transcriptase and annealing temperatures tested in the assay specificity testing. * the standard temperatures according to the Standard AgPath setup.

| Specificity test number | Reverse Transcriptase Temperature (°C) | Annealing Temperature (°C) |
|---|---|---|
| ***1** | 45 | 60 |
| **2** | 47 | 62 |
| **3** | 48 | 63 |
| **4** | 49 | 64 |

### 2.6.6 Optimalization of the Real-Time RT-PCR assays

Optimalization of the Real-Time RT-PCR assays was performed in order to determine the optimal primer and probe molarity combination. RNA from *T. finnmarkense* gen. *finnmarkense* strain HFJ were used as target for the TffC3 assay because the *T. finnmarkense* gen. *finnmarkense* type strain TNO006 (NCIMB 15238) showed no sign of growth after being cultured as described in section 2.6.2. RNA from *T. finnmarkense* gen. *ulcerans* type strain TNO010 (NCIMB 15239) was used as target for the TfuC1 assay and RNA from *T. dicentrarchi* type strain 35/09 (NCIMB 14598) was used as target for the TdC2 assay. All reactions were performed in triplicates. The assays were optimized at a 47 °C reverse transcriptase temperature and an annealing temperature of 62 °C.

The assays were initially tested against different concentrations of forward and reverse primer using a standard probe concentration of 120 nM. The following forward/reverse primer concentrations in nM were tested for all assays: 400/400, 400/600, 400/800, 600/400, 600/600, 600/800, 800/400, 800/600 and 800/800. After determining the optimal forward/reverse primer concentration for each assay, the probe was optimized using these probe concentrations: 50, 75, 100, 125, 150, 175, 200, 225 nM. Optimal forward/reverse primer and probe concentrations are listed in Table 3.2.

### 2.6.7 Sensitivity and Efficacy testing of the Real-Time RT-PCR assays

An efficacy test was conducted for the three developed assays to test the assay's efficacy to detect target template. A bacterial tenfold dilution series from $10^0$ to $10^{10}$ of *T. finnmarkense* genomovar *finnmarkense* strain HFJ (assay: TffC3), *T. finnmarkense* gen. *ulcerans* TNO010$^T$ (NCIMB 15239) (assay: TfuC1), and *T. dicentrarchi* 35/09$^T$ (NCIMB 14598) (assay: TdC2) were used to test the efficacy of the assays. The *Tenacibaculum* spp isolates used in the analyses were grown in separate MB cultures and 1.0 mL was collected from the cultures and used to make the tenfold dilution series. RNA was extracted from each of the bacterial tenfold dilutions according to the description given in section 2.6.2 and a Real-Time RT-PCR reaction was performed using the Standard AgPath setup with reverse transcriptase and annealing temperatures of 47 °C and 62 °C, respectively. All reactions were run in triplicates. The bacterial tenfold dilution was performed in order to quantify the number of bacterial cells that corresponds to a given Ct-value. The number of bacterial cells was determined in the $10^0$-dilution using the MPN method as described in 2.6.4 (Table 2.6).

**Table 2.6.** List of isolates used in the assay sensitivity and efficacy testing and the number of bacterial cells/mL for each isolate. The numbers were calculated using the MPN method.

| Isolate Name | Bacterial cells/mL |
|---|---|
| *T. finnmarkense* gen. *finnmarkense* HFJ | 5,95 x $10^7$ |
| *T. dicentrarchi* type strain 35/09 (NCIMB 14598) | 9,7 x $10^8$ |
| *T. finnmarkense* gen. *ulcerans* type strain TNO010 (NCIMB 15239) | 3,8 x $10^8$ |
| *M. viscosa* BF21 | 6,2 x $10^8$ |

A traditional tenfold RNA dilution series ranging from $10^0$ to $10^{10}$ using the same target RNA mentioned above was also performed using the Standard AgPath setup with reverse transcriptase and annealing temperatures of 47 °C and 62 °C, respectively, as with the bacterial tenfold dilution series. The reactions were run in triplicates. The average Ct-values in the $10^0$ to $10^{-5}$ dilutions for each triplicate of the bacterial dilution from and RNA dilution were plotted in Microsoft Excel to create a standard curve and to further calculate the coefficient of determination ($R^2$) and slope of the graph. The Ct-values from the bacterial dilution series and RNA dilution series were also compared, proving the RNA dilution series to be the most accurate. The efficacy of the assays was calculated using Formula 1.

Formula 1: $$\mathbf{E} = \mathbf{10}^{\left(-\frac{\mathbf{1}}{\mathbf{-slope}}\right)}$$

A sensitivity test was performed on all assays using the same bacterial tenfold dilution series and RNA tenfold dilution series from $10^0$ to $10^{10}$ as used for the efficacy testing. This analysis was performed in order to test the ability of the assays to detect small amount of template and its ability to amplify target RNA when diluted to minute concentrations. The Real-Time RT-PCR was performed using the Standard AgPath setup with reverse transcriptase and annealing temperatures of 47 °C and 62 °C, respectively. The optimal primer and probe concentrations listed in Table 3.2 were used in the sensitivity test.

### 2.6.8 Assay testing using field case samples

The Real Time RT-PCR assays developed in this study were further tested using Real Time RT-PCR and extracted RNA from fish samples from field outbreaks of suspected tenacibaculosis. This was conducted in order to test the assays specificity when each RNA sample likely consisted of several *Tenacibaculum* spp. species. All the fish that were tissue sampled from field cases of suspected tenacibaculosis were sampled and put on RNAlater by Cermaq Fish Health personnel. The fish was further euthanized, frozen and sent to Bergen where it was thawed prior to tissue sampling. The gills and skin around ulcers/lesions were tissue sampled. Tissue sampling and Real Time RT-PCR analyses were performed by Cermaq staff.

Microsoft Excel was used to make bar charts to illustrate the prevalence of *Tenacibaculum* spp. and *M. viscosa*. In all tables presenting Ct-values obtained from Real Time RT-PCR a separate

colour is used for the different ranges of the Ct-values from low to high. Ct-values marked in bright pink ranges from 10.0-19.9, light pink ranges from 20.0-29.9 and orange ranges from 30.0-39.9. The Ct-values marked with light yellow colour have a value above 40.0 and were deemed negative (Table 1, Appendix).

## 2.7 Density

Density is a measure of the number of bacteria (in this case bacteria RNA related to volume of tissue) in the fish. Density is given as an estimate of the amount of target RNA in the samples. Normalized expression (NE) and reverse Ct-value are used to express density. An important part of this study was to investigate the presence of *T. finnmarkense* and *M. viscosa* in skin ulcers and water samples and further compare the density of these pathogens. In the current study, Real Time RT-PCR assays targeting two different housekeeping-genes were used in the analyses; TffC3 and TfuC1 with the *tgt*-gene as target and MvOmpA targeting the OmpA-gene. This means there could be a different expression of the HK-genes in the bacterial cells which in turn could affect the Ct-value. Due to this, the obtained Ct-values are not directly comparable between the TffC3, TfuC1 and MvOmpA assays. In order to compensate for this issue, the Ct-value with the corresponding number of bacteria were investigated for the Real Time RT-PCR assays used in the comparison of pathogen density. From the results, it is evident that the assays have a comparable sensitivity and that can be used in the evaluation of the density of pathogens (Table 3.4). Nevertheless, this issue needs to be considered when comparing the density of *Tenacibaculum* spp. and *M. viscosa* using the assays developed in this study.

### 2.7.1 Calculation of normalized expression

The Ct-values obtained from Real Time RT-PCR analyses were normalized coherent with the reference gene in order to correct possible differences in the amount of RNA in the sample. EF1A was used for Atlantic salmon and Hsal for *H. salinarum*. Normalized expression was calculated using Formula 2. $E_{ref}$ is the assay efficacy and $Ct_{ref}$ is the Ct-value of the reference gene. $E_{target}$ and $Ct_{target}$ is the efficacy of the current pathogen to be normalized using the coherent Ct-value. Negative samples were excluded from the calculation of normalized expression.

Formula 2: $$\mathbf{NE_{Tissue}} = \frac{(\mathbf{E_{ref}})^{\mathbf{Ct_{ref}}}}{(\mathbf{E_{target}})^{\mathbf{Ct_{target}}}}$$

The normalized expression values were further recalculated to NE-fold to demonstrate the variation between the samples. To calculate the NE-fold values the NE-values were divided by the lowest $NE_{min}$ value for the given group of fish (Formula 3).

Formula 3: $$\mathbf{NE_{fold}} = \frac{\mathbf{NE}}{\mathbf{NE_{min}}}$$

All NE-fold values were log-transformed due to the large variation between the values, which better illustrates the number of pathogens in the fish groups.

### 2.7.2 Reverse Ct-values

All Ct-values were reversed using Formula 4 in order to better present the Ct-values. By reversing the Ct-value, high values (low Ct-values) indicate high density. This was only performed when the Ct-values of the reference gene were close to stabile. Negative samples were presented as 0.

Formula 4: $$\mathbf{Density = 40 - Ct\ value}$$

## 2.8 Statistical Analyses

GraphPad Prism (v. 9.0.0) was used to perform all statistical analyses in order to examine the difference in presence of *Tenacibaculum* spp. in the SCCS and open net pen at the study site. The positive *Tenacibaculum* spp. fish were aggregated, and not normally distributed, hence nonparametric tests were performed to compare different groups. A Mann-Whitney test was performed to determine the difference in two groups (e.g., TffC3 vs. TfuC1 in gill tissue from the SCCS). The Kruskal-Wallis test was performed followed by a Dunn's multiple comparisons test in order to compare the difference in three or more groups (e.g., different pathogens in skin ulcers). Log NE-fold values and reverse Ct-values were used to perform statistical analyses to examine the variation in density of a given pathogen in different fish groups and water sampling.

P-values < 0.05 are considered significant for all statistical analyses. Results are presented as *= $P \leq 0.05$, **= $P \leq 0.01$, ***= $P \leq 0.001$ and ****= $P \leq 0.0001$. GraphPad Prism (v. 9.0.0) and Microsoft® Excel (v. 16.43) were used to construct bar and column graphs.

# 3. RESULTS

## 3.1 Real Time RT-PCR Assay Testing

### 3.1.1 Assay Specificity

Three assays developed in this study and the specificity of the assays were tested using 16 different *Tenacibaculum* spp. isolates (Table 2.4). The assays were tested against RNA extracted from pure bacterial cultures.

**TffC2**

The TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense* tested positive for isolates of *T. finnmarkense* gen. *finnmarkense* but were also "weakly positive" (fluorescence tail) for isolates of *T. finnmarkense* gen. *ulcerans* using the standard AgPath Setup with a reverse transcriptase temperature of 45 °C and annealing temperature of 60 °C (high Ct-values, range 32.5-32.9) (Figure 3.1A). TffC3 did not give positive results for *T. dicentrarchi* nor other *Tenacibaculum* type species-isolates. When testing the assay specificity of TffC3, TfuC1, TdC2 using reverse transcriptase and annealing temperatures of 47 °C and 62 °C, the TffC3 assay gave "weak signals" for *T. finnmarkense* gen. *ulcerans* and high Ct-values (Figure 3.1B) (Table 22, Appendix).

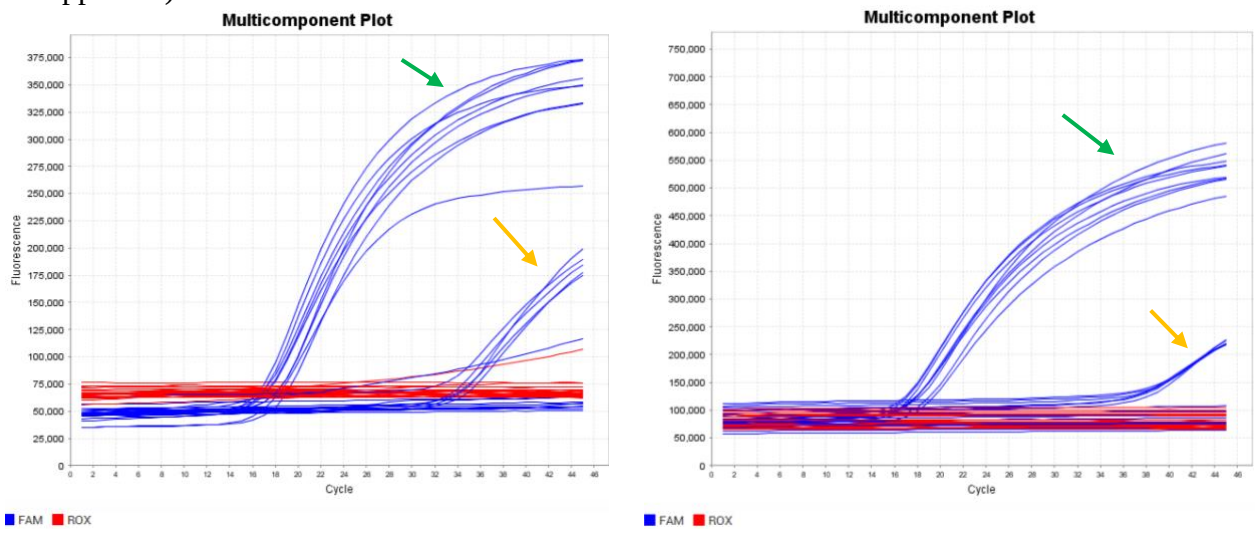


**Figure 3.1.** Multicomponent plot from the specificity testing of the TffC3 Real Time RT-PCR assay (targeting *T. finnmarkense* gen. *finnmarkense*. **A:** The specificity testing performed using the standard AgPath Setup with reverse transcriptase and annealing temperature of 45 °C and 60 °C. **B:** The specificity testing run at the standard AgPath Setup with reverse transcriptase and annealing temperature of 47 °C and 62 °C. Green arrow: TffC3 positive for isolates of *T. finnmarkense* gen. *finnmarkense*. Yellow arrow: TffC3 "weakly positive (fluorescent tail) for isolates of *T. finnmarkense* gen. *ulcerans.*

**TfuC1**

When using the standard AgPath setup with a reverse transcriptase temperature of 45 °C and annealing temperature of 60 °C, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans* tested positive for isolates of *T. finnmarkense* gen. *ulcerans.* The assay did also test positive for *T. finnmarkense* gen. *finnmarkense* (high Ct-values, range 27.1-30.9) (Figure 3.2A). TfuC1 did not test positive for isolates of *T. dicentrarchi* nor other *Tenacibaculum* type species-isolates. TfuC1 tested negative for *T. finnmarkense* gen. *finnmarkense* with increased reverse transcriptase- and annealing temperatures (47 °C and 62 °C, respectively) (Figure 3.2B) (Table 21, Appendix).

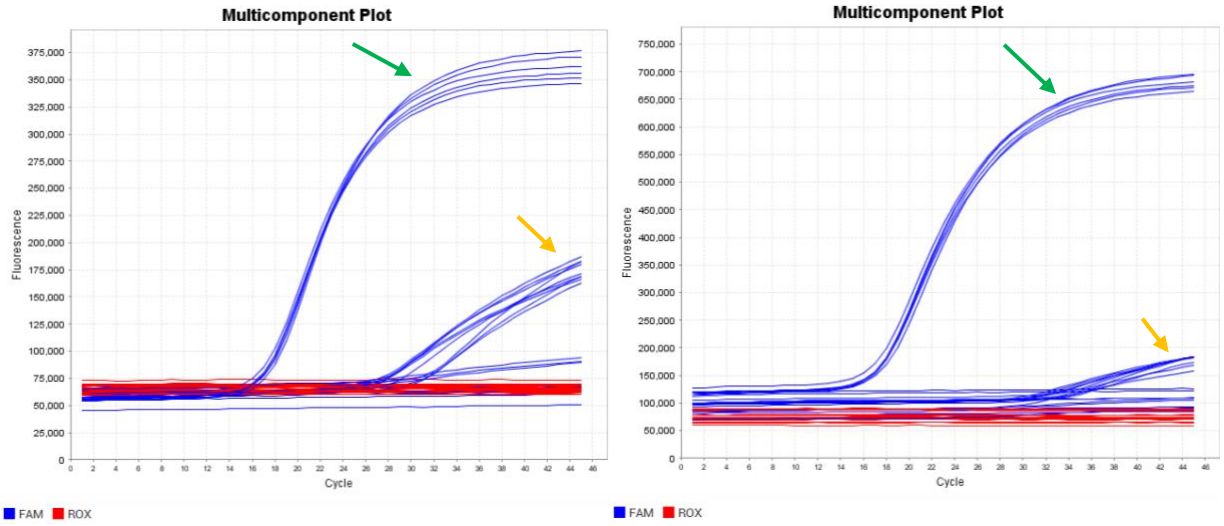


**Figure 3.2.** Multicomponent plot from the specificity testing of the TfuC1 Real Time RT-PCR assay (targeting *T. finnmarkense* gen. *ulcerans.* **A:** The specificity testing performed using the standard AgPath Setup with reverse transcriptase and annealing temperature of 45 °C and 60 °C. **B:** The specificity testing performed using the standard AgPath Setup with reverse transcriptase and annealing temperature of 47 °C and 62 °C. Green arrow: TfuC1 positive for isolates of *T. finnmarkense* gen. *ulcerans*. Yellow arrow: TfuC1 "weakly positive" (fluorescent tail) for isolates of *T. finnmarkense* gen. *finnmarkense*.

**TdC2**

The TdC2 assay targeting *T. dicentrarchi* tested positive for isolates of *T. dicentrarchi* (*T. dicentrarchi* 35/09$^{T}$, NCIMB 14598) and negative for isolates of *T. finnmarkense* gen. *finnmarkense*, *T. finnmarkense* gen. *ulcerans* and other *Tenacibaculum* type species (Figure 3.3) (Table 22, Appendix).

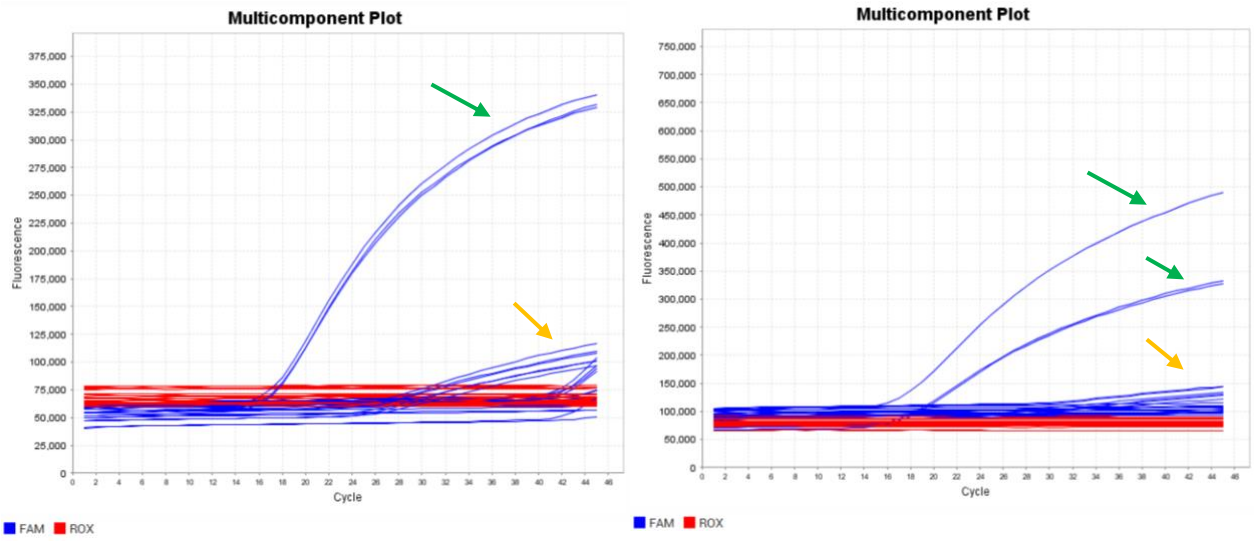


**Figure 3.3.** Multicomponent plot of specificity testing of the TdC2 Real Time RT-PCR assay (targeting *T. dicentrarchi.* **A:** The specificity testing performed using the standard AgPath Setup with reverse transcriptase and annealing temperature of 45 °C and 60 °C. **B:** The specificity testing performed using the standard AgPath Setup with reverse transcriptase and annealing temperature of 47 °C and 62 °C. Green arrows: TdC positive for isolates of *T.dicentrarchi*. Yellow arrow: TdC2 giving a fluorescent tail for isolates of *T. finnmarkense* gen. *finnmarkense* (deemed negative).

**Table 3.1.** The three Real Time RT-PCR assays developed in this study and the bacterial isolates the assays tested positive for using the standard AgPath Setup with a reverse transcriptase temperature of 47 °C and annealing temperature of 62 °C. All *Tenacibaculum* spp. isolates used in the specificity testing of the assays are listed in Table 2.4. *"Weak positives" that could be considered negative.

| TffC3 | TfuC1 | TdC2 |
|---|---|---|
| *T. finnmarksense* gen. *finnmarkense* - LIM026 | *T. finnmarkense* gen. *ulcerans* - LIM056 | *T. dicentrarchi* - LIM063 |
| *T. finnmarksense* gen. *finnmarkense* - LIM020 | *T. finnmarkense* gen. *ulcerans* - LIM016 | *T. dicentrarchi* type strain 35/09 (NCIMB 14598) |
| *T. finnmarksense* gen. *finnmarkense* - LIM006 | *T. finnmarkense* gen. *ulcerans* type strain TNO010 (NCIMB 15239) | **T. finnmarkense* gen. *ulcerans* type strain TNO010 (NCIMB 15239) |
| *T. finnmarkense* gen. *finnmarkense* HFJ | **T. finnmarkense* gen. *finnmarkense* HFJ | |
| **T. finnmarksense* gen. *ulcerans* - LIM056 | | |
| **T. finnmarksense* gen. *ulcerans* - LIM016 | | |
| **T. finnmarkense* gen. *ulcerans* type strain TNO010 (NCIMB 15239) | | |

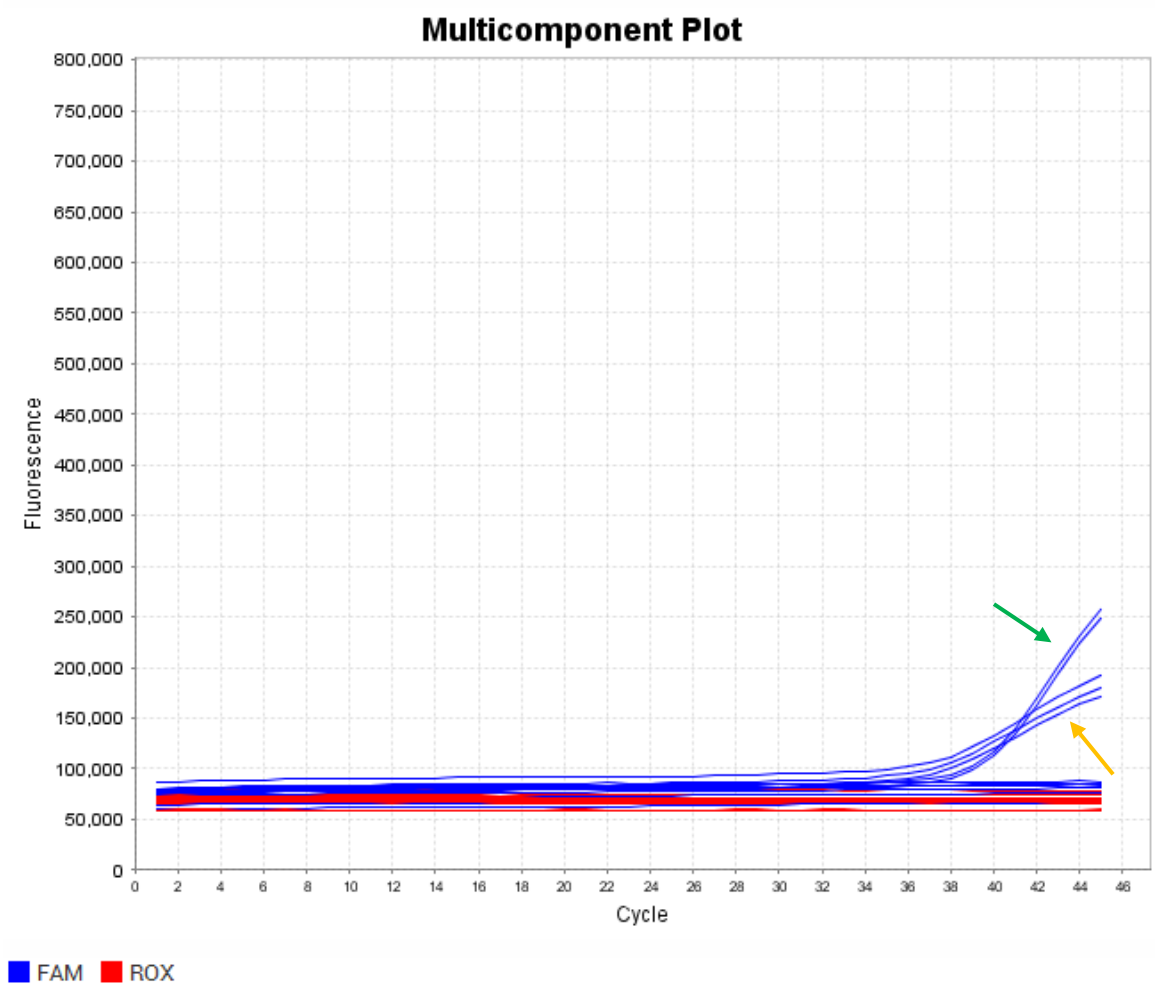


**Figure 3.4.** The multicomponent plot curves of the TffC3 assay performed using reverse transcriptase and annealing temperatures of 47 °C and 62 °C. positive for *T. finnmarkense* genomovar *finnmarkense* HFJ diluted to $10^{-6}$ (green arrow). Yellow arrow: TffC3 "weakly positive" (or fluorescent tail) for *T. finnmarkense* gen. *ulcerans*$^{T}$. The obtained Ct-values illustrated with these curves were close to equal (green arrow: 37.8, yellow arrow: 36.4). There was a considerable difference in the degree of exponentiality of the curves.

The reverse transcriptase temperature was further increased to 48 °C and 49 °C and the annealing temperature was increased to 63 °C and 64 °C which made the TffC3 assay specific to *T. finnmarkense* gen. *finnmarkense* but causing the TdC2 assay targeting *T. dicentrarchi* to be less sensitive (Table 22, Appendix).

Due to these results, a reverse transcriptase temperature of 47 °C and annealing temperature of 62 °C were further used. These temperatures were subsequently used in the assay testing and when using the assays for Real Time RT-PCR for field cases of suspected tenacibaculosis and in the screening of *Tenacibaculum* spp. of the fish in the SCCS and open net pen.

### 3.1.3 Assay optimalization

The Real Time RT-PCR assays developed in this study were optimized using different combinations of forward/reverse primer and probe concentrations as described in section 2.6.6. Optimized primer and probe concentrations (nM) for each assay are listed in Table 3.2.

**Table 3.2.** The different assays developed in this study and their optimized forward and reverse primer and probe concentration. Concentrations are given in nanomolar (nM)

| Assay | Forward primer (nM) | Reverse primer (nM) | Probe (nM) |
|---|---|---|---|
| **TffC3** | 800 | 800 | 175 |
| **TfuC1** | 800 | 800 | 175 |
| **TdC2** | 800 | 800 | 175 |

### 3.1.3 Assay efficacy

The assay efficacy testing was carried out as described in section 2.6.7. Figure 3.5 displays a line diagram obtained from the efficacy testing of the three Real Time RT-PCR assays developed in this study. The slope, coefficient of determination ($R^2$) and efficacy are presented in Table 3.3. All three assays were found to be most efficient between $10^0$ and $10^{-6}$ dilution and efficiently detect target RNA up to a Ct-value of 33.

**Table 3.3.** The slope, coefficient of determination ($R^2$) and efficacy for the assays developed and used in this study including MvOmpA targeting *M. viscosa* (Vold, 2014).

| Assay | Slope | Coefficient of determination – $R^2$ | Efficacy | Efficacy percent (%) |
|---|---|---|---|---|
| **TffC3** | 3.2493 | 0.9981 | 2.0312 | 103.12 |
| **TfuC1** | 3.2878 | 0.9997 | 2.0144 | 101.44 |
| **TdC2** | 3.3121 | 0.9994 | 2.0041 | 100.41 |
| **MvOmpA** | 3.6394 | 0.9935 | 1.8826 | 88.27 |

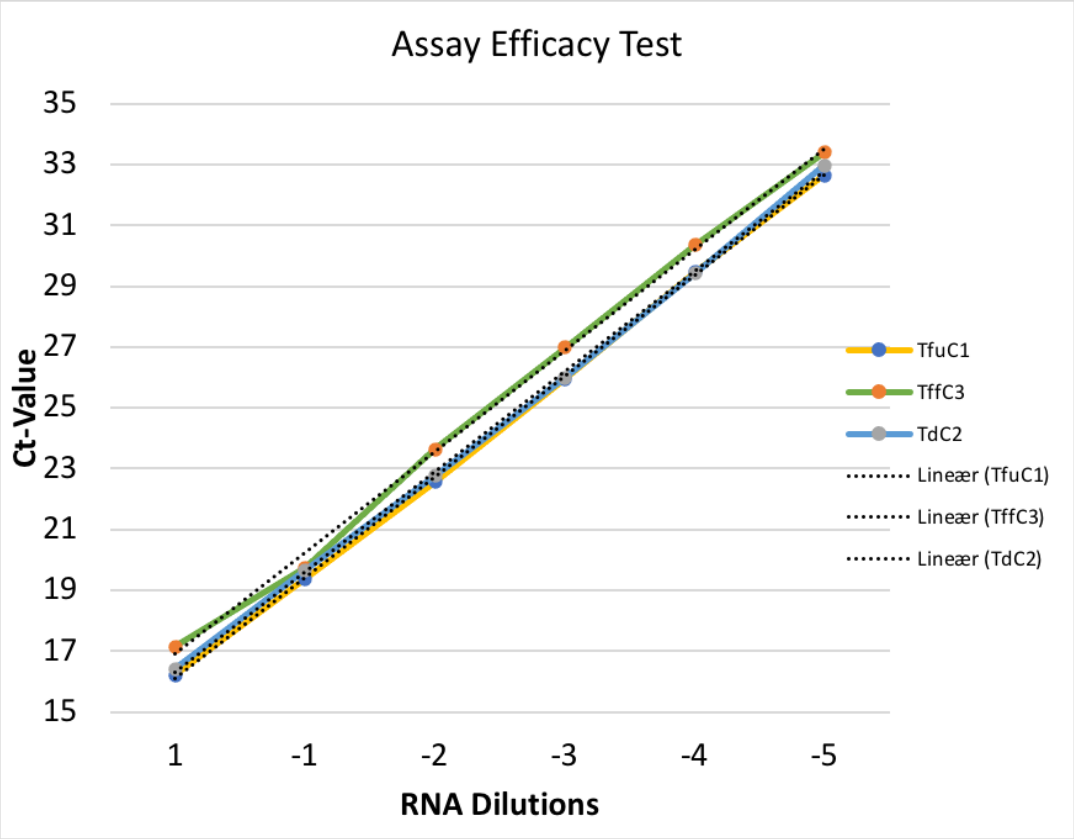


**Figure 3.5.** The standard curve of the three Real Time RT-PCR assays developed in this study: TffC3, TfuC1 and TdC2. Each with a linear trendline. Y-axis is the Ct-value and x-axis is the tenfold RNA-dilutions ranging from 1 to $10^{-5}$.

### 3.1.4 Assay sensitivity

The sensitivity testing of the Real Time RT-PCR assays developed in this study was carried out as explained in section 2.6.7. The assays proved to be able to detect pure culture of *Tenacibaculum* spp. diluted $10^{-6}$. The MPN method explained in section 2.6.4 were used to calculate number of bacterial cells/mL in the non-diluted sample. By dividing the number of cells by 10 for each dilution, the number of bacterial cells is estimated down to a $10^{-6}$ dilution. Although this method may not be accurate enough to absolute quantify the number of bacterial cells in the diluted samples, it provides an estimated number of bacterial cells per mL. Estimated number of bacterial cells/mL of each dilution and the coherent Ct-value obtained by using Real Time RT-PCR are presented in Table 3.4. The TffC3 assay was positive for two of three replicates (triplicates) in the $10^{-6}$ dilution while the TfuC1 and TdC2 assays were positive for one of three replicates in the $10^{-6}$ dilution. This indicate that TffC3 is the most sensitive assay.

**Table 3.4.** Estimated number of bacterial cells/mL with the corresponding dilution for each of the Real Time RT-PCR assays developed in this study including the MvOmpA assay targeting *M. viscosa*. Ct-values are obtained from Real Time RT-PCR. *2/3 replicates tested positive. **1/3 of the replicates tested positive.

| RNA Tenfold Dilution | TffC3 | | TfuC1 | | TdC2 | | MvOmpA | |
|---|---|---|---|---|---|---|---|---|
| | Bacterial cells/mL | Ct-value | Bacterial cells/mL | Ct-value | Bacterial cells/mL | Ct-value | Bacterial cells/mL | Ct-value |
| 1 | 59 500 000 | 17.1 | 380 000 000 | 16.2 | 970 000 000 | 16.4 | 620 000 000 | 14.2 |
| $10^{-1}$ | 5 950 000 | 19.7 | 38 000 000 | 19.4 | 97 000 000 | 19.7 | 62 000 000 | 16.8 |
| $10^{-2}$ | 595 000 | 23.6 | 3 800 000 | 22.6 | 9 700 000 | 22.8 | 6 200 000 | 19.3 |
| $10^{-3}$ | 59 500 | 27.0 | 380 000 | 26.0 | 970 000 | 26.0 | 620 000 | 22.7 |
| $10^{-4}$ | 5950 | 30.4 | 38 000 | 29.5 | 97 000 | 29.4 | 62 000 | 26.5 |
| $10^{-5}$ | 595 | 33.4 | 3800 | 32.6 | 9700 | 33.0 | 6200 | 29.9 |
| $10^{-6}$ | 59.5 | 37.8* | 380 | 35.3** | 970 | 36.7** | 620 | 33.6 |
| $10^{-7}$ | 5.95 | Neg | 38.0 | Neg | 97.0 | Neg | 62,0 | 36.2** |

### 3.1.5 Field testing of the developed *Tenacibaculum* spp. Real Time RT-PCR assays

The Real Time RT-PCR assays developed in this study were tested using samples from field outbreaks of ulcerative skin disease where *Tenacibaculum* spp. were suspected to be associated with the disease (Figure 3.6). Fish from several of these outbreaks were screened for *Tenacibaculum* spp. using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, TfuC1 targeting *T. finnmarkense* gen. *ulcerans* and TdC2 targeting *T. dicentrarchi.* The diseased fish was also screened for the presence of *M. viscosa* using the MvOmpA assay as well as the E1FA assay targeting the salmon elongation factor as a control. The screening included four sites in Troms and Finnmark (1TF, 2TF, 3TF and 4TF), one study site with the SCCS located in Nordland, and fish from a challenge experiment (CE) (Solheim, 2020) that had been challenged with *T. finnmarkense* gen. *finnmarkense* strain HFJ.

The Real Time RT-PCR analyses conducted on skin samples from outbreaks at site 1TF, 2TF, 3TF and 4TF confirmed that the three assays developed in this current study could differentiate *Tenacibaculum* spp. from a single sample where several *Tenacibaculum* spp. bacteria likely to be present. Only the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense* tested positive using extracted RNA from the challenge experiment that used *T. finnmarkense* gen.

*finnmarkense* HFJ. Ct-values obtained from Real Time RT-PCR from the field cases and from the challenge experiment are presented in Table 23, Appendix.

**Figure 3.6.** Fish from the field case sites suffering from ulcerative disease. The two left pictures are fish from the 1TF site and the four pictures on the right are fish collected from the 3TF site. All fish suffers from large, uneven bilateral skin ulcers. Mouth erosion and fin- and tail rot are also present in the fish.

## 3.2 Fish- and Water Sampling from the SCCS and open net pen at the Study Site

### 3.2.1 Screening of the fish samples collected from the SCCS and open net pen at the study site 1 – 111 dps

A total of 30 fish (475 fish in total) from sampling 1-8 from the SCCS and open net pen were screened for *Tenacibaculum* spp. and *M. viscosa* using Real Time RT-PCR and the assays developed in this study including MvOmpA. All fish samples tested negative for *M. viscosa.* Fish from the first sampling (1 dps) and outbreak of ulcerative disease in the SCCS (152 dps) were screened for *T. dicentrarchi* and tested negative. TdC2 were therefore not included in the further *Tenacibaclum* spp. screening in the following samplings. Number of *T. finnmarkense*, *T. dicentrarchi* and *M. viscosa* positive samples are listed in Table 3.5. Bar graphs (Figure 3.7) illustrates the prevalence of *T. finnmarkense* in the SCCS and open net pen at the study site, while Fig. 3.8 shows the presence of *T. finnmarkense* gen. *finnmarkense* and *T. finnmarkense* gen. *ulcerans* in the SCCS and open net pen at the study site. A detailed overview of all obtained Ct-values from Real Time RT-PCR are listed in the Appendix (Table 2-19).

**Table 3.5.** Overview of the *Tenacibaculum* spp. and *M. viscosa* positive fish samples from the SCCS and open net pen at the study site. Tissue samples were collected from the skin under the jaw when no lesions/ulcer could be detected and the gills. A total of 30 fish were tissue sampled from each sampling. The number 0 indicates no positive fish samples from the given sampling. The light-yellow coloured numbers show samplings with 1-10 positive fish. Dark yellow-coloured numbers illustrate samplings with 10-20 positive fish. *At the 5th sampling, 25 fish from the open net pen were collected. **At the 6th sampling fish from the SCCS were collected 55 dps and fish from the open net pen were collected 56 dps. NT = not tested

| Sampling | Days post stocking | Tissue | Real Time RT-PCR Assay | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SCCS | | | | Open net pen | | | |
| | | | TffC3 | TfuC1 | TdC2 | MvOmpA | TffC3 | TfuC1 | TdC2 | MvOmpA |
| **1** | 1 | Skin | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 |
| **2** | 8 | Skin | 0 | 0 | NT | 0 | 6 | 0 | NT | 0 |
| **3** | 15 | Skin | 3 | 0 | NT | 0 | 0 | 0 | NT | 0 |
| **4** | 24 | Skin | 4 | 0 | NT | 0 | 0 | 2 | NT | 0 |
| **5** | 29* | Skin | 1 | 0 | NT | 0 | 1 | 0 | NT | 0 |
| **6** | 55 - 56** | Skin | 0 | 0 | NT | 0 | 0 | 0 | NT | 0 |
| **7** | 83 | Skin | 3 | 0 | NT | 0 | 4 | 3 | NT | 0 |
| **8** | 111 | Skin | 1 | 0 | NT | 0 | 6 | 2 | NT | 0 |
| **1** | 1 | Gill | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| **2** | 8 | Gill | 2 | 0 | NT | 0 | 7 | 6 | NT | 0 |
| **3** | 15 | Gill | 2 | 3 | NT | 0 | 2 | 0 | NT | 0 |
| **4** | 24 | Gill | 2 | 0 | NT | 0 | 1 | 11 | NT | 0 |
| **5** | 29* | Gill | 1 | 1 | NT | 0 | 2 | 2 | NT | 0 |
| **6** | 55 - 56** | Gill | 1 | 0 | NT | 0 | 3 | 4 | NT | 0 |
| **7** | 83 | Gill | 4 | 9 | NT | 0 | 3 | 2 | NT | 0 |
| **8** | 111 | Gill | 1 | 0 | NT | 0 | 5 | 11 | NT | 0 |
| **Total positive samples** | | | **25** | **13** | **0** | **0** | **41** | **46** | **0** | **0** |

Figure 3.7 illustrates the presence of *T. finnmarkense* in skin and gill tissue from the SCCS and open net pen at the study site 1-111 dps. There was no significant difference in the presence of *T. finnmarkense* in skin tissue from the SCCS and open net pen (P = 0.3542). There was a significantly higher presence of *T. finnmarkense* in gill tissue in the open net pen compared to the SCCS (*P = 0.0451). No significant difference was found when comparing the presence of *T. finnmarkense* in the open net pens and SCCS regardless of tissue.

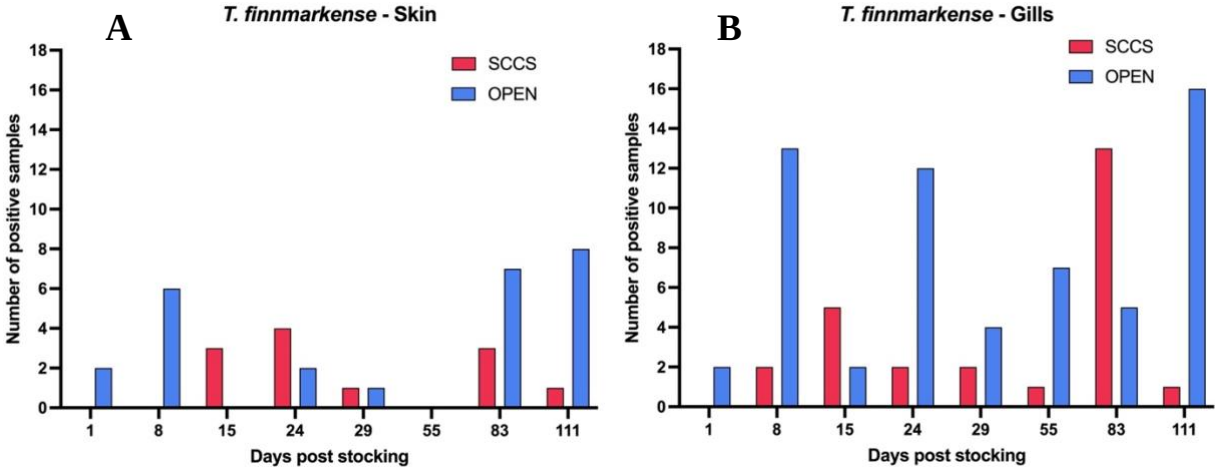


**Figure 3.7.** Presence of *T. finnmarkense* in skin **(A)** and gill **(B)** tissue from the SCCS and open net pen (OPEN) at the study site 1-111 dps. Days post stocking (dps) of the sampling are illustrated on the x-axis. Y-axis shows number of *T. finnmarkense* positive samples obtained by Real Time RT-PCR analyses. A total of 30 fish were collected at each sampling except for 29 days post stocking when there were 25 fish collected from the open net pen. At the 6$^{th}$ sampling, fish from the SCCS were collected 55 dps and fish from the open net pen were collected 56 dps.

Figure 3.8 illustrates the presence of *T. finnmarkense* gen. *finnmarkense* and *T. finnmarkense* gen. *ulcerans* in skin- and gill tissue from the SCCS and open net pen at the study site 1-111 dps. Statistical analyses were performed on all categories (TffC3 vs. TfuC1 in skin and gills in SCCS and open net pen) to disclose any significant difference. There was a significantly higher presence of *T. finnmarkense* gen. *finnmarkense* compared to *T. finnmarkense* gen. *ulcerans* in skin tissue from the SCCS (*P = 0.0256). There was not a significant difference in the presence of *T. finnmarkense* gen. *finnmarkense* compared to *T. finnmarkense* gen. *ulcerans* in gill tissue from the SCCS (P = 0.2005), skin tissue from the open net pen (P = 0.6454) and in gill tissue from the open net pen (P = 0.6900).

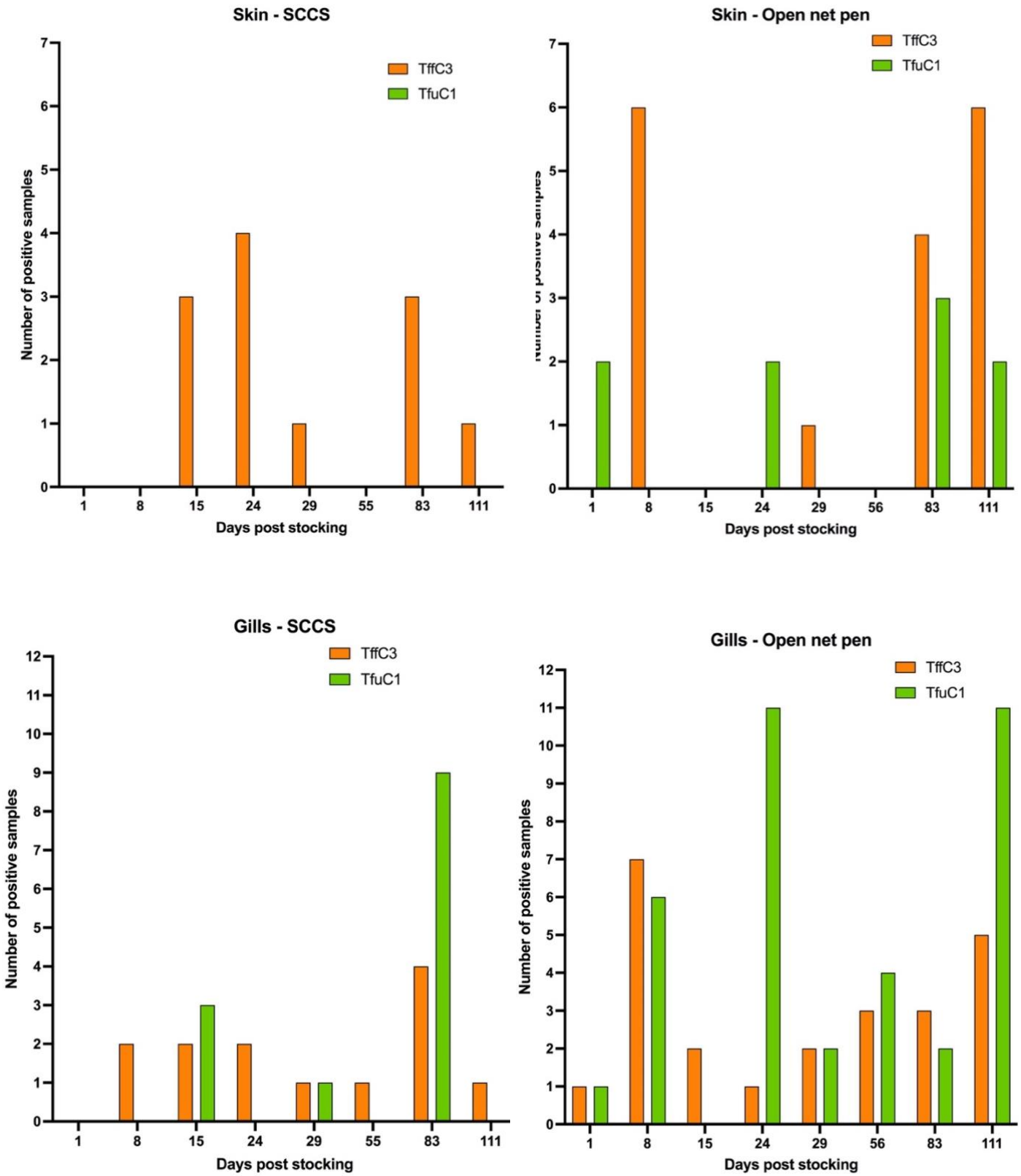


**Figure 3.8.** Bar charts illustrating the prevalence of *T. finnmarkense* gen. *finnmarkense* (TffC3) and *T. finnmarkense* gen. *ulcerans* (TfuC1) in the SCCS (right) and open net pen (left) from skin (upper figures) and gill (lower figures) tissue. Days post stocking (dps) of the sampling are illustrated on the x-axis. Y-axis shows number of *T. finnmarkense* positive samples obtained by Real Time RT-PCR analyses. A total of 30 fish were collected at each sampling except for 29 days post stocking when there was 25 fish collected from the open net pen. At the 6$^{th}$ sampling, fish from the SCCS were collected 55 dps and fish from the open net pen were collected 56 dps.

**Figure 3.9.** Fish (F8) from the SCCS at the study site 140 dps (9th fish sampling) that could be considered a representation of the 'healthy fish' in sampling 1-8. Fish from samplings 1-9 were visually healthy and showed no sign of disease. The fish had been frozen and thawed prior to sampling. Only fish from sampling 1-8 have been analyzed in the current study.

### 3.2.2 Screening of the water samples collected from SCCS and open net pen at the study site 0 – 170 dps

One water sample from the SCCS and open net pen was collected at each sampling (Table 2.2) and analyzed by Real Time RT-PCR using assays: TffC3 (targets *T. finnmarkense* gen. *finnmarkense*), TfuC1 (targets *T. finnmarkense* gen. *ulcerans*), MvOmpA (targets *M. viscosa*) and Hsal (targets *H. salinarum*). All water samples (N=38) collected from the SCCS tested positive for *T. finnmarkense* gen. *finnmarkense.* The first *M. viscosa* positive water sample was collected 66 dps in both the SCCS and open net pen, while the water samples were *T. finnmarkense* positive sporadically from 1-170 dps. Overview of Ct-values obtained from Real Time RT-PCR analyses are listed in the Appendix (Table 21). Figure 3.10 illustrates the density of *T. finnmarkense* and *M. viscosa* in the water samples (0.5 L) using normalized expression (Log NE-fold). Water samples from the SCCS did not have any significant difference in presence of *T. finnmarkense* gen. *finnmarkense, T. finnmarkense* gen. *ulcerans* and *M. viscosa* (TffC3 vs. TfuC1 P>0.9999, TffC3 vs. MvOmpA P = 0.4513 and TfuC1 vs. MvOmpA P = 0.4711) (Figure 3.11A). Water samples from the open net pen showed a higher presence of *T. finnmarkense* gen. *finnmarkense* compared to *M. viscosa* *P = 0.0358 (Figure 3.11B). There was no significant difference in density in the following groups from the open net pen: TffC3 vs. TfuC1 P = 0.1851 and TfuC1 vs. MvOmpA P = 0.5700.

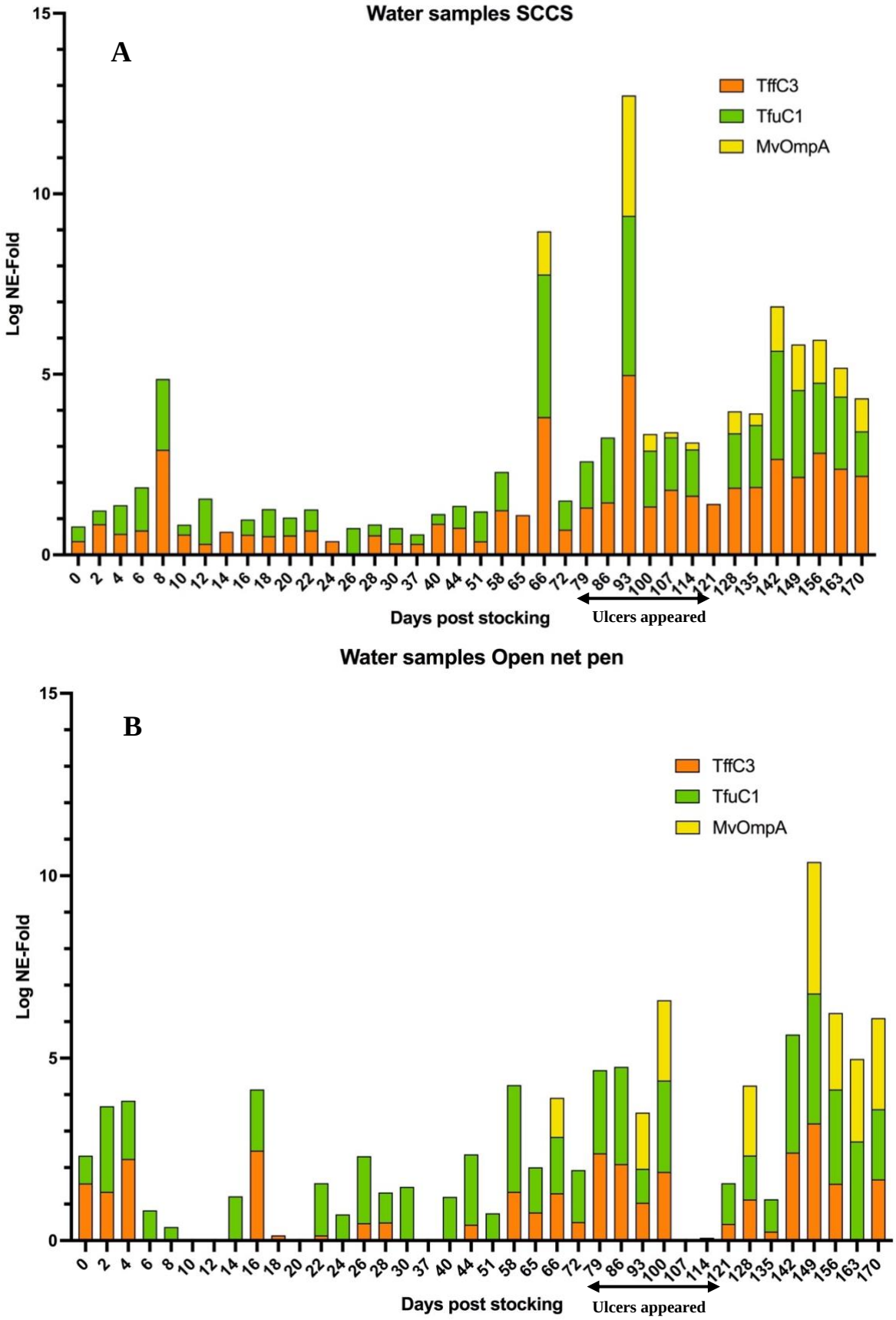


**Figure 3.10.** Density of TffC3 (target *T. finnmarkense* gen. *finnmarkense*), TfuC1 (target *T. finnmarkense* gen. *ulcerans*) and MvOmpA (target *M. viscosa*) in water samples 0-170 dps (38 samplings) in SCCS **(A)** and open net pen **(B)** at the study site. The data is presented as Log NE-fold. The black arrow illustrates the timepoint of which ulcers started emerging in both pens at the study site.

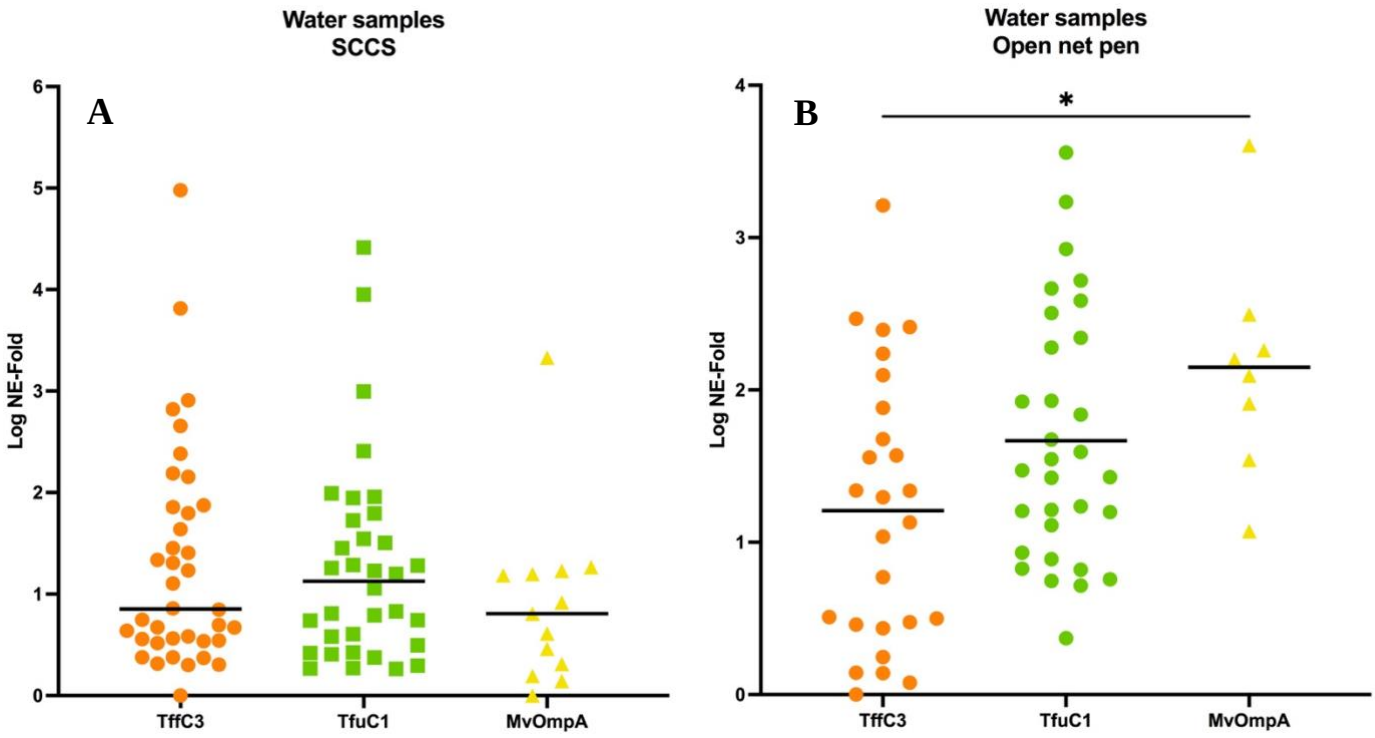


**Figure 3.11**. Density of TffC3 (target *T. finnmarkense* gen. *finnmarkense*), TfuC1 (target *T. finnmarkense* gen. *ulcerans*) and MvOmpA (target *M. viscosa*) in water samples 0-170 dps (38 samplings) in SCCS **(A)** and open net pen **(B)** at the study site. The data is presented as Log NE-fold. *P = 0.0358.

**Table 3.6.** Sea water temperatures (°C) obtained from the SCCS and open net pen at the study site from September to April. SCCS intake water is collected from 15 m deep.

| Month | Temperature (°C) | |
|---|---|---|
| | SCCS | Open net pen |
| September 2020 | 11.70 | 11.80 |
| October 2020 | 10.30 | 10.30 |
| November 2020 | 8.20 | 8.20 |
| December 2020 | 6.70 | 6.70 |
| January 2021 | 5.60 | 5.60 |
| February 2021 | 4.70 | 4.70 |
| March 2021 | 4.10 | 4.10 |
| April 2021 | 4.40 | 4.40 |

### 3.2.3 Outbreak of ulcerative disease in the SCCS at the study site

Fish in the SCCS started developing clinical signs of ulcerative disease and suspected tenacibaculosis around 4 months (ca. 130 dps) post stocking (Figure 3.12-3.14). A total of 20 fish were collected 152 dps and skin samples from the area around the mouth, ulcers/lesions in the muscle of the fish and gills were tissue samples. The tissues were screened using Real Time RT-PCR assays TffC3, TfuC1, TdC1, MvOmpA and EF1A (reference gene) targeting *T. finnmarkense* gen. *finnmarkense*, *T. finnmarkense* gen. *ulcerans*, *T. dicentrarchi*, *M. viscosa* and the salmon elongation factor, respectively. Ct-values obtained from the Real Time RT-PCR analyses are listed in the Appendix (Table 19). The fish suffered from severe mouth erosion, fin rot and scale loss (Figure 3.12A). Most of the fish had large ulcers on the bilateral side or abdomen penetrating the skin into the muscle (Figure 3.13C). Many of the large margins of the ulcers in the skin were uneven (Figure 3.14A).


**Figure 3.12. A:** Fish (F3) collected from the SCCS (152 dps) with clinical signs often found in fish suffering from tenacibaculosis. The fish suffers mouth erosion, severe scale loss and tail rot. **B, C:** A closer look at F3 from the SCCS (152 dps). **B** - severe mouth erosion. The skin epithelia has eroded leaving the bone and cartilage of the mouth exposed. **C** - severe fin rot affecting the caudal fin. All fish had been euthanized, frozen and thawed prior to tissue sampling.

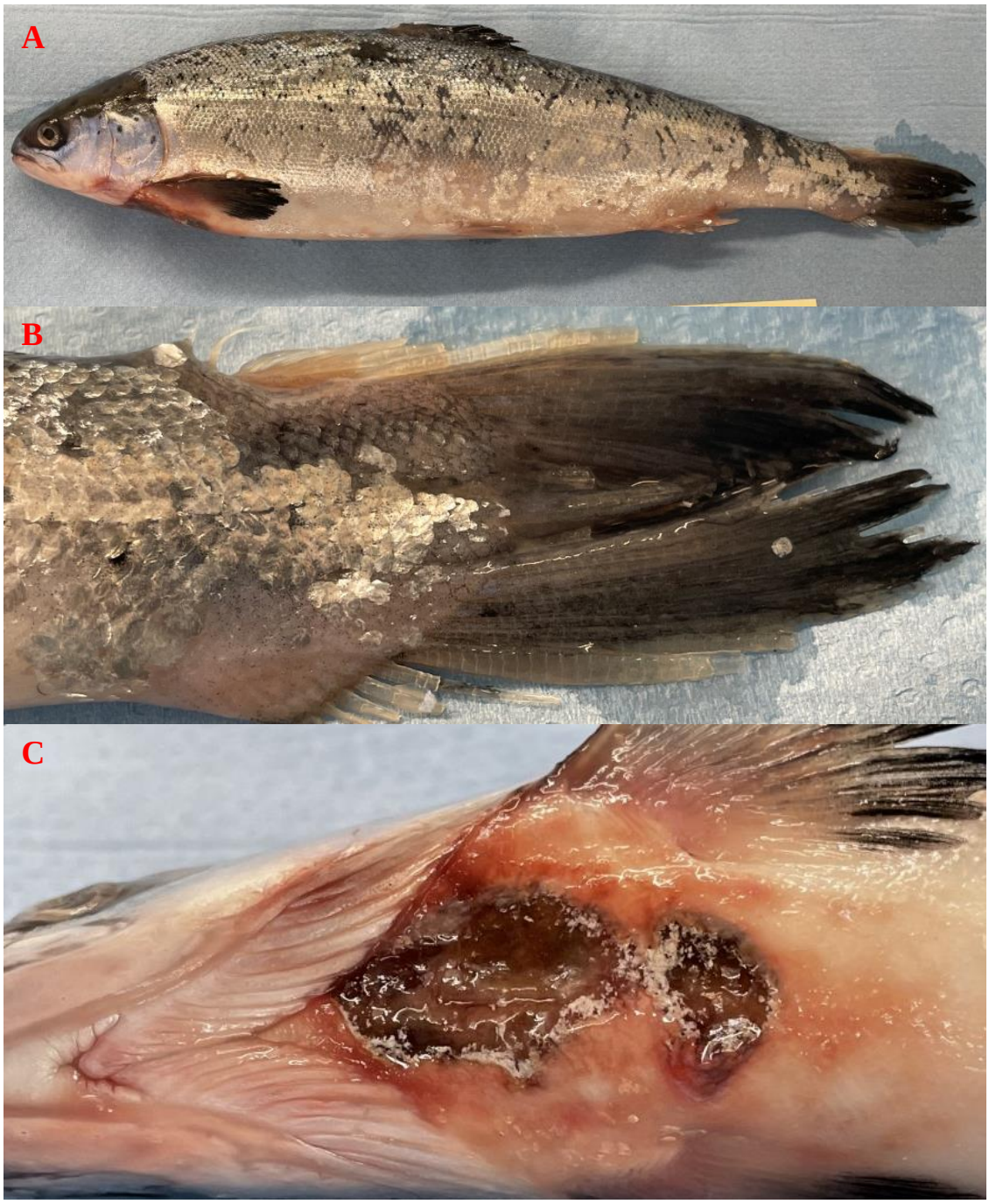


**Figure 3.13. A:** Fish (F2) collected from the SCCS (152 dps) with clinical signs often associated with tenacibaculosis. The fish has severe scale loss, tail and fin rot and a large ulcer on the abdomen between the pectoral fins. **B:** The same fish as seen in A (F2) collected 152 dps from the SCCS suffering from severe fin rot affecting the caudal fin. **C:** Fish (F2) from the SCCS 152 dps, suffering from a large uneven abdominal ulcer between the pectoral fins. All fish had been euthanized, frozen and thawed prior to tissue sampling.


**Figure 3.14.** Fish from the SCCS (152 dps) with large uneven bilateral ulcers penetrating the muscle (**A**). Fish from the SCCS at the study site (152 dps) with severe jaw erosion (**B**).

RNA from tissue samples from a total of 20 fish was tested for the presence of *Tenacibaculum* spp. using the Real Time RT-PCR assays developed in the current study. Figure 3.15 shows the density of *T. finnmarkense* gen. *finnmarkense* (TffC3), *T. finnmarkense* gen. *ulcerans* (TfuC1) and *M. viscosa* (MvOmpA) in skin tissue from mouth, margin of bilateral and ventral ulcers and gill tissue. The statistical analyses were performed on Log NE-fold and significance are presented in Figure 3.15 with coherent P-values in the figure text. There was no significant difference in the density of *T. finnmarkense* gen. *finnmarkense*, *T. finnmarkense* gen. *ulcerans* and *M. viscosa* in the following groups (based on Log NE-fold values): skin from margin of ulcers (TffC3 vs. TfuC1 P = 0.1912, TfuC1 vs. MvOmpA P>0.9999 and TffC3 vs. MvOmpA P = 0.6573) and skin from mouth area (TffC3 vs. TfuC1 P = 0.0795). All fish tested negative for *T. dicentrarchi* and the TdC2 assay targeting this species was thus discarded from the figures illustrating the density of *Tenacibaculum* spp. and *M. viscosa.*

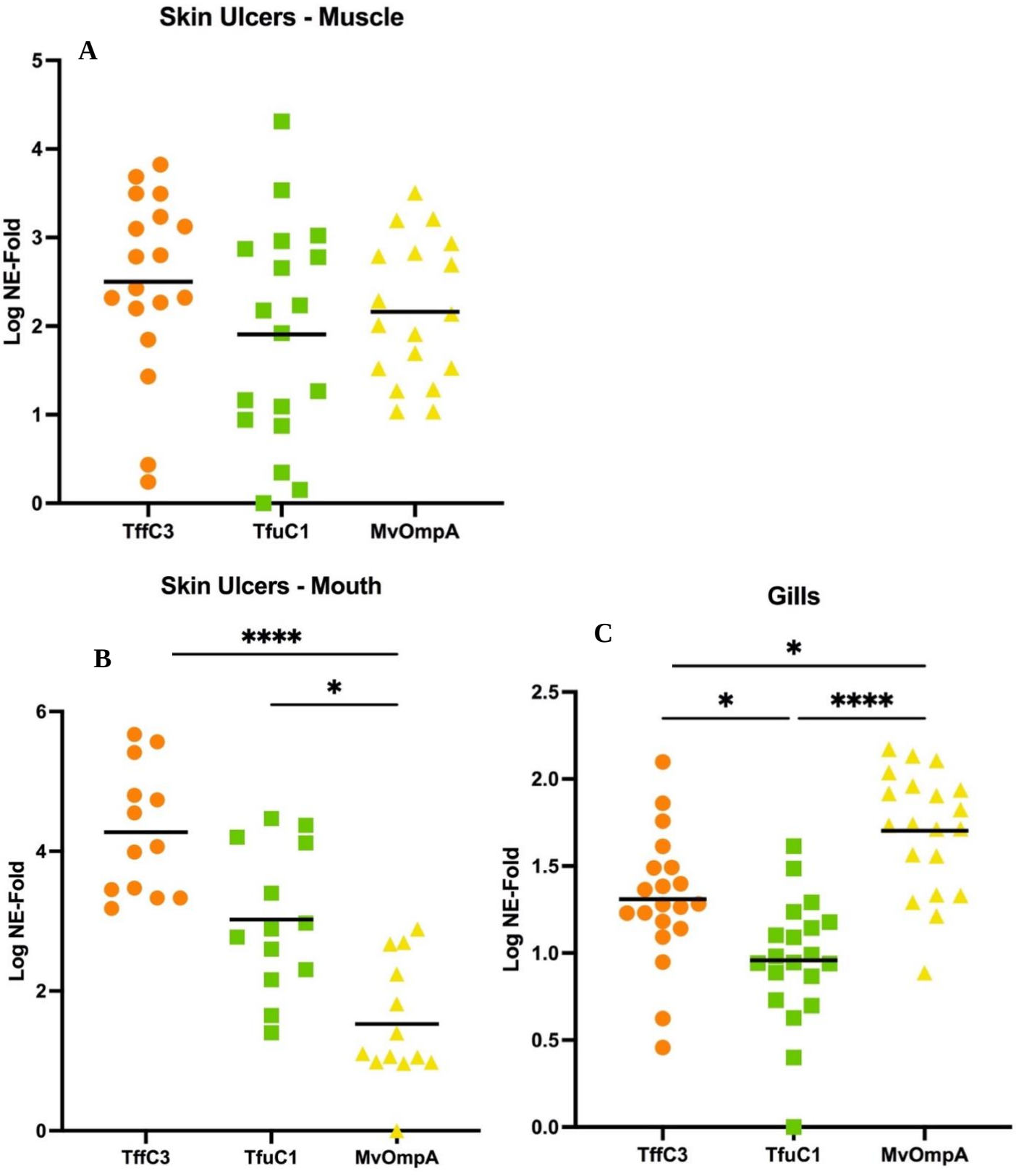


**Figure 3.15**. Density of TffC3 (target *T. finnmarkense* gen. *finnmarkense*), TfuC1 (target *T. finnmarkense* gen. *ulcerans*) and MvOmpA (target *M. viscosa*) in skin tissue from the margin of ulcers (**A**), mouth area (**B**) and gill tissue (**C**) 152 dps from the SCCS at the study site. The data is presented as Log NE-fold. **A**: No significant difference in the presence of TffC3, TfuC1 and MvOmpA. **B**: *P = 0.0219, ****P<0.0001. **C**: *P = 0.0320 (TffC3 vs. TfuC1), *P = 0.0253 (TffC3 vs. MvOmpA) and ****P<0.0001.

### 3.2.4 Outbreak of ulcerative disease in the open net pen at the study site

Fish in the SCCS continued developing ulcers, and after approximately 6 months post stocking, fish from the open reference net pen started developing ulcers similar to the ones in the SCCS. A total of 6 fish from both the SCCS and open net pen were tissue sampled from ulcers and gills and tested for *Tenacibaculum* spp. and *M. viscosa* using Real Time RT-PCR and the assays developed in this study including MvOmpA targeting *M. viscosa.* The Ct-values obtained the Real Time RT-PCR analyses are listed in Table 20, Appendix. Figure 3.16 illustrates the ratio between *T. finnmarkense* gen. *finnmarkense*, *T. finnmarkense* gen. *ulcerans* and *M. viscosa* from skin tissue and gill tissue from the SCCS and the open net pen based on reversed Ct-values. There was a significantly higher presence of *T. finnmarkense* gen. *finnmarkense* than *T. finnmarkense* gen. *ulcerans* (**P = 0.0087) and *M. viscosa* (*P = 0.0124) in skin ulcers. In samples from the gill tissue there was a significantly higher presence of *T. finnmarkense* gen. *finnmarkense* compared to *T. finnmarkense* gen. *ulcerans* (**P = 0.0013).

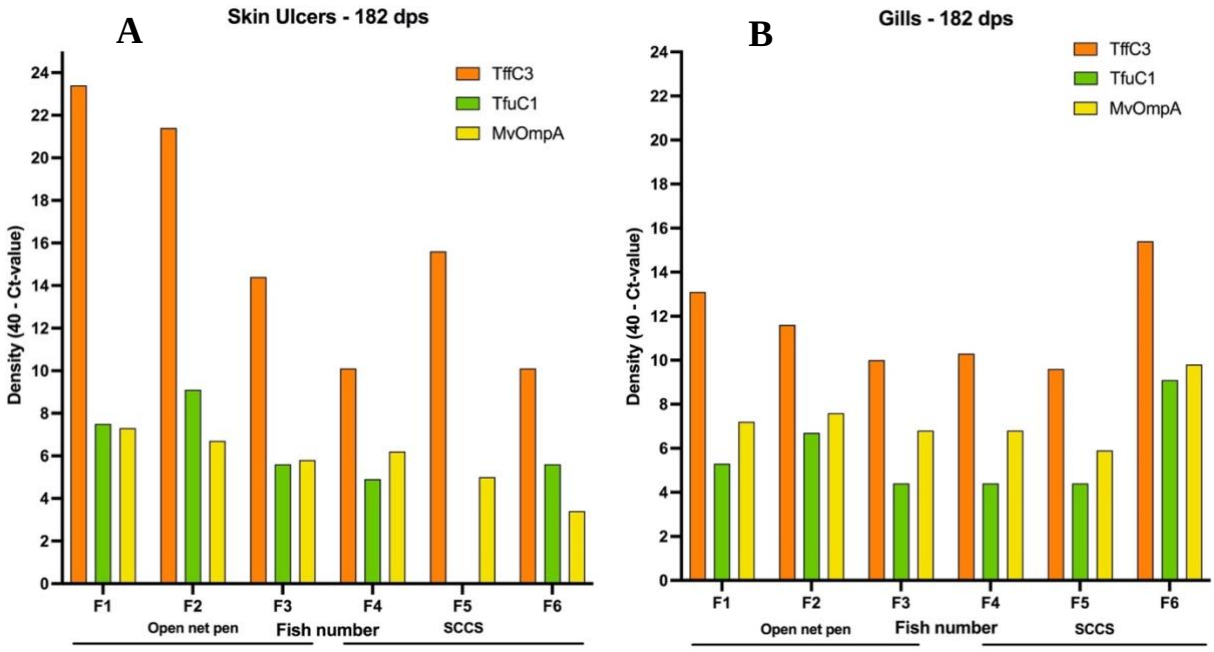


**Figure 3.16.** Bar chart illustrating the prevalence of *Tenacibaculum* spp. and *M. visocosa* in the skin-ulcers **(A)** and gill tissue **(B)** of the fish collected from the SCCS (F4-6) and open net pen (F1-3) at the study site 182 dps. The TffC3 assay target *T. finnmarkense* gen. *finnmarkense*, TfuC1 target *T. finnmarkense* gen. *ulcerans* and MvOmpA target *M. viscosa*. Y-axis represent reverse Ct-values and x-axis is fish number. TfuC1 vs. MvOmpA = not significant (P>0.9999).

Skin tissue from ulcers showed lower Ct-values for TffC3 compared to TfuC1 and MvOmpA. All fish tested negative for TdC2. Tissue from the skin around ulcers and gills of the fish from the SCCS and open net pen at the study site were analyzed histologically by PHARMAQ Analytic. The histology from skin showed filamentous bacteria accordant to morphology associated with *Tenacibaculum* spp. (Figure 3.17). The gills presented no histopathological signs of disease. The skeletal muscle showed signs of some filamentous bacteria.

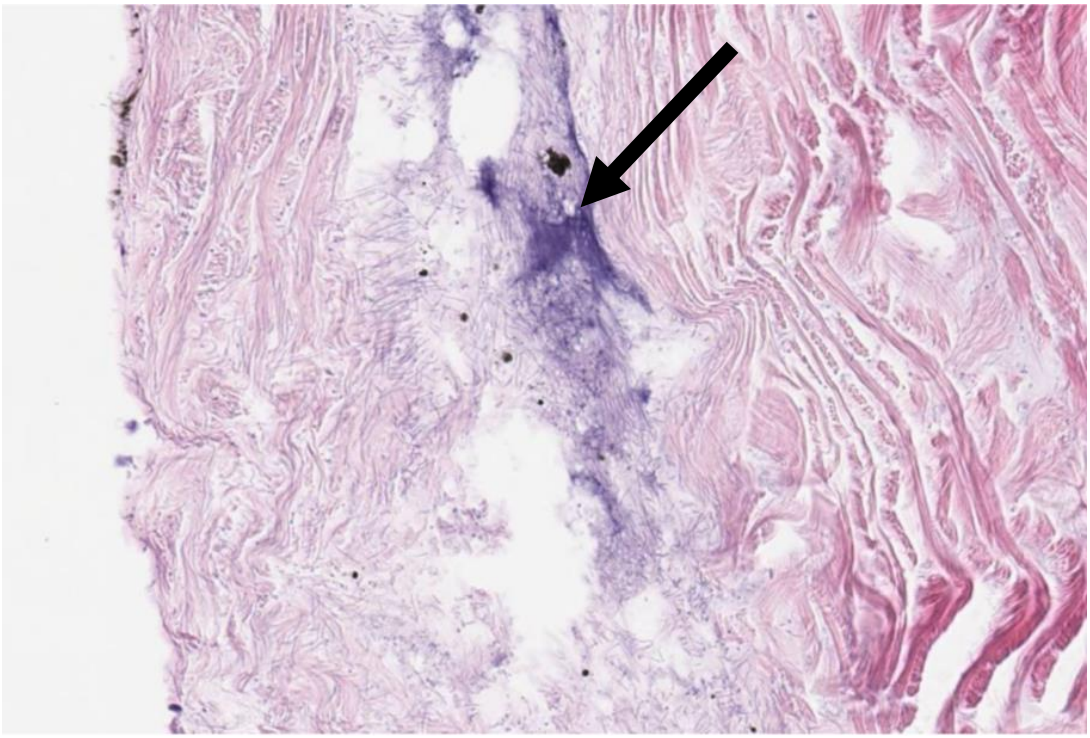

**Figure 3.17.** Skin from Fish 1 (F2) from the open net pen at the study site 182 dps. The arrow points at a thick layer of long rod-shaped bacteria in the epidermis.

# 4. DISCUSSION

## 4.1 Real Time RT-PCR Assay Development for *Tenacibaculum* spp. and Evaluation of its Use for Field Diagnostics

During the last decade, there has been an increased focus on *Tenacibaculum* spp. as a causative agent of ulcerative disease in Norwegian salmon farming. *T. finnmarkense* and *T. dicentrarchi* are commonly isolated during outbreaks of ulcerative disease in Norway. These bacteria have been shown to be primary pathogens in laboratory studies reproducing the clinical signs as described for tenacibaculosis (Klakegg et al., 2019; Småge et al., 2018). Although *T. finnmarkense* is able to induce tenacibaculosis on its own, *M. viscosa* is also commonly found in outbreaks of ulcerative disease, particularly in cases of 'winter ulcers' in larger fish at low sea water temperatures (below 8 °C). The bacterium *Aliivibrio wodanis* is also commonly identified in these cases but has not been shown to cause disease in laboratory studies (Bårdsgjære, 2002; Takle et al., 2015). The common perception today is that *M. viscosa* is not the sole agent of ulcerative disease in Norway, but that such diseases involve several bacteria. Diagnostic tools are important in the detection of pathogens responsible for disease and to further establish solutions to the problem related to the pathogens. Real Time RT-PCR assays are rapid and inexpensive diagnostic tools to use in the detection of specific pathogens. The development of specific Real Time RT-PCR assays for the detection of the *Tenacibaculum* spp. associated with field cases of ulcerative disease (i.e., tenacibaculosis and 'winter ulcers') were therefore attempted in this study.

From a MLSA analysis conducted on 89 Norwegian *Tenacibaculum* spp. isolates (mostly isolated from farmed Atlantic salmon) it was identified that the isolates related to ulcerative bacterial skin disease grouped into four major clades (Olsen et al., 2017). Clade I consisted of *T. finnmarkense* gen. *ulcerans* isolates and Clade III consisted of *T. finnmarkense* gen. *finnmarkense* isolates. Clade II consisted of *T. dicentrarchi* isolates, whilst Clade IV consisted of *T. piscium* isolates (Olsen et al., 2017, 2020). Results from several challenge studies have shown that it is only isolates belonging to Clades I-III that are relevant to the development of ulcerative skin disease in Atlantic salmon (Klakegg et al., 2019; Olsen et al., 2011; Olsen et al., 2020; Småge et al., 2018). However, only *T. finnmarkense* gen. *finnmark*ense (Clade III) and *T. dicentrarch* (Clade II) have been shown to cause disease on its own (Klakegg et al., 2019;

Småge et al., 2018). Yet, it has been shown that *T. finnmarkense* gen. *ulcerans* (Clade I) could be an important pathogen in connection with development of the 'winter ulcer disease' (Olsen et al., 2011). In order to investigate the presence and abundance of *Tenacibaculum* spp. of Clade I-III in water samples and in healthy and diseased salmon in this study, Real Time RT-PCR assays targeting *Tenacibaculum* spp. Clades I-III were needed.

Several TaqMan Real Time RT-PCR assays targeting the 16S rRNA gene have been developed in order to differentiate *T. finnmarkense* (Clade I and III) and *T. dicentrarchi* (Clade II), however the specificity of the assays were deemed moderate (Nowlan et al., 2021). These findings are consistent with another 16S rRNA Real Time RT-PCR assay targeting *T. maritimum* which, although being highly sensitive and efficient, obtained positive results for *T. soleae* (Fringuelli et al., 2012). Based on variable stretches in the 16S rRNA genes, the genes have been successfully used for specific PCR primer design, however, the 16S rRNA gene from *Tenacibaculum* species are conserved and provide little genetic variation that can be used for separation between species (Nowlan, 2020). Nowlan et al (2021) thus recommends that housekeeping genes (e.g., *gyrB, ygfO*) should be investigated as better target genes for developing Real Time RT-PCR assays that can differentiate between the *Tenacibaculum* spp. Clades I-III. Habib et al (2014) also proposed in their study that future MLSA surveys of *Tenacibaculum* strains should use the 6 loci *atpA, dnaK, glyA, infB, rlmN* and *tgt* (HK-genes). Unfortunately, there are currently few available housekeeping gene sequences in the Gene Bank for Norwegian *Tenacibaculum* spp. isolates. Thus, *Tenacibaculum* spp. HK gene sequences used in this study to develop Real Time RT-PCR assays were collected through a comprehensive MLST analysis in connection to the 'Limit'-project, funded by the Norwegian Seafood Research Fund (FHF). From these HK gene sequences, an alignment of a total of seven loci (*atpA, dnaK, glyA, gyrB, infB, rlmn*, and *tgt*) from 77 Norwegian *Tenacibaculum* spp. isolates were investigated for use in the development of the Real Time RT-PCR assays targeting Clade I-III. All three assays in this study (TffC3, TfuC1, TdC2) were designed targeting the *tgt*-gene since this gene was found to have the most suitable variation between the *Tenacibaculum* spp. clades.

During the specificity testing of the developed Real Time RT-PCR assays, it was evident that the reverse transcriptase and annealing temperature needed to be raised from the standard 45 °C/60 °C, to 47°C/62 °C in order to make the assays more specific, whilst still being sensitive (Table 22, Appendix). This issue occurred with all three assays, but it was most prominent with

the TffC3 and TfuC1 assays as they both seemed to amplify RNA from isolates belonging to both target clades. From the results it appeared that bacterial RNA from the incorrect *Tenacibaculum* spp. clade was amplified when using a lower temperature (i.e., the standard AgPath Setup). In this case it resulted in Ct-values of approximately 30.0, which thus appeared to be weak positive results when analyzing the results from the Real Time RT-PCR amplification plot. However, when analyzing the same results in the multicomponent plot, the curves did not have the correct incline compared to other positive samples that gave the same Ct-value (Figure 3.4) and were therefore considered as negative. All weak positive samples were analyzed in the multicomponent plot to decide whether they were positive or negative. The assays were also tested using RNA from field cases of tenacibaculosis resulting in low Ct-values and a large amount of target RNA present (Table 23, Appendix). In these cases, the assays did not amplify bacterial RNA from the incorrect *Tenacibaculum* spp. clade even though the Ct-values were as low as in the specificity testing where the RNA was extracted from pure bacterial cultures. It seems the assays are more suitable for field case of tenacibaculosis, which was the intended use of the assays, compared to use on pure cultures of bacteria. It should be added that cloning of *Tenacibaculum* spp. from a mixed culture with representatives of all clades may constitute a problem with respect to obtaining a pure clone of a specific *Tenacibaculum* species. However, we have not found any other evidence suggesting that the clonal cultures of representatives from the different clades of *Tenacibaculum* spp. are contaminated.

Six points of dilution were used when calculating the assay efficacy (TffC3, TfuC1, TdC2), but since the sixth dilution did not produce positive replicates, it was decided to perform the efficacy testing using five points of dilution. Using five points of dilution still fulfills the recommendation by ThermoFisher. All three assays developed in this study had an assay efficacy between 90-110% which is acceptable according to ThermoFisher (Table 3.3). The sensitivity testing of the assays proved that all three assays were able to amplify target RNA down to a $10^{-6}$ dilution of the RNA. For the TffC3 assay, 2/3 replicates tested positive in $10^{-6}$ dilution, while 1/3 replicates tested positive in the $10^{-6}$ dilution for TfuC1 and TdC2. This make the assays less efficient after Ct-values of approximately 33.0, which is why the $10^{-6}$ dilution was excluded from the assay efficacy calculation as previously discussed.

In this study it was attempted to absolute quantify the number of bacterial cells to a corresponding Ct-value. Knowing this, gives information about how many bacteria are present

in the skin of both healthy and diseased fish, and in water samples. This was carried out by estimating the number of bacterial cells in 1.0 mL MB for the type strains (or a representative strain) representing Clades I-III using the MPN method (see section 2.6.4). An optimal assay efficiency coincides with a change of 3.3 in Ct-value between each tenfold dilution (ThermoFisher). The bacterial tenfold dilution series was therefore made at the exponential growth phase of the target strains that were tested. However, the 3.3 drop in Ct-value for each dilution in the assay efficiency testing was not found to be completely accurate. This may have been a result of inaccurate pipetting when creating the bacterial tenfold series or during the RNA extraction for each dilution. *T. maritimum* has been reported to be able to produce a substantial amount of extracellular polymers or 'slime' (Avendaño-Herrera et al., 2006; Burchard et al.,1990). This may also be the case for *T. finnmarkense* and *T. dicentrarchi* which may have agglutinated and stuck to the tube walls, resulting in a non-homogenous bacterial solution that could have affected the accuracy in the dilution series. Due to the inaccurate results from the bacterial tenfold dilution series, a traditional RNA tenfold dilution series was used to quantify the bacteria. Based on the results listed in Table 3.4, the TffC3 assay seems to be the most sensitive assay. A similar issue, as seen for the bacterial tenfold dilution series, appeared with the corresponding RNA dilution series. Although the drop in Ct-value were closer to 3.3 for each dilution for the RNA compared to the bacterial dilution, it was not exactly 3.3 for each dilution. For the TffC3 assay the $10^0$ to $10^{-1}$ dilution the Ct-value dropped from 17.1 to 19.7 which means there could be an overestimation of the assay sensitivity because there might be more RNA in the dilutions than if the dilution series were accurate. This means the TffC3 assay might be closer to TfuC1 and TdC2 when considering the sensitivity of this assay.

The Real Time RT-PCR assays developed in this study were tested using both field samples of fish suspected to be suffering from tenacibaculosis as well as RNA obtained from a challenge experiment using *T. finnmarkense* gen. *finnmarkense* strain HFJ as the challenge isolate. In conclusion, the Real Time RT-PCR assay development resulted in three efficient, specific and sensitive assays that are able to differentiate *T. finnmarkense* gen. *ulcerans* (CI), *T. finnmarkense* gen. *finnmarkense* (CIII) and *T. dicentrarchi* (CII). The assays can be recommended for use in screening of *T. finnmarkense* and *T. dicentrarchi* in connection with outbreaks of ulcerative disease. Although the assays are specific to their clade, the reverse transcriptase and annealing temperatures should be set to 47 °C and 62 °C, respectively, to increase the specificity and the multicomponent plot should be analyzed in order to discard any false positives.

## 4.2 Fish and Water Screening in SCCS and open net pen with Emphasis on the Presence of *Tenacibaculum* spp. and *M. viscosa*

### 4.2.1 Real Time RT-PCR screening of fish samples 1-111 days post stocking (dps) at the study site

All fish examined in the period from 1-111 dps appeared healthy and did not show any clinical signs of ulcerative disease, although the Real Time RT-PCR analyses of skin and gill tissue confirms the presence of *T. finnmarkense* in both skin and gill tissues in fish from both the SCCS and open net pen (Table 3.5). There was a significantly higher presence of *T. finnmarkense* in gill tissue from the open net pen compared to the SCCS (*P = 0.0451). As gills are in direct contact with the environment, they are thought to be especially susceptible to waterborne pathogens, environmental changes and infections. Analyses of gill tissue have therefore been suggested to reflect the water quality with emphasis on the presence of pathogens (Downes et al., 2018). Taking this into consideration, the gill analyses could indicate that *T. finnmarkense* should be less abundant in water samples from the SCCS. However, there was no significant difference in the presence of *T. finnmarkense* in the SCCS and the open net pen based on the water samples (P >0.9999). In fact, there was no *T. finnmarkense* negative water samples from the SCCS, and only 4/38 (N=38) water samples tested negative from the open net pen (Table 21, Appendix). These findings indicate that water samples may poorly reflect what pathogens may be present on the gills. However, the Ct-values obtained from the *T. finnmarkense* positive water samples are in most of the samplings above the level that the TffC3 and TfuC1 assays were deemed most efficient. This could in turn affect the reliability of the obtained Ct-values.

Pre stocking fish and the first fish sampling from the SCCS and open net pen at the study site were screened for the presence of *T. dicentrarchi.* All fish tested negative (Table 3.5), which was expected since most of the known Clade II isolates have been obtained from western Norway at higher temperatures (above 8 °C) (Klakegg et al., 2019; Olsen et al., 2017). Diseased salmon from the study site and other sites in Troms and Finnmark (northern Norway) suffering from ulcerative skin disease were also screened for the presence of *T. dicentrarchi* and tested negative. Due to these results, fish from samplings 2-8 (Table 2.1) were not tested for the presence of *T. dicentrarchi.* Members of this species does not seem to be present in healthy nor diseased fish suffering from ulcerative disease in Northern Norwegian salmon farms. It remains

to be seen if increased water temperature (e.g., due to climate change) could lead to outbreaks of *T. dicentrarchi* in Northern Norway. Based on the analyses conducted in the present study, it is suggested that *T. dicentrarchi* can be omitted when screening farmed salmon for the presence of *Tenacibaculum* spp. in Northern Norwegian fish farms as of now.

### 4.2.2 Real Time RT-PCR screening water samples at the study site 1-170 dps

The Ct-values obtained during analyses of water samples (TffC3 and TfuC1 assays) indicated a relatively low presence of *T. finnmarkense* (Ct >30.0) for most sampling dates (Table 21, Appendix), although the presence of *T. finnmarkense* increased with days post stocking. This indicates that *T. finnmarkense* is generally present in the environment, both in the SCCS and in the open pen, throughout the water sampling period. The number of *T. finnmarkense* in the water samples increases with number of days from when the fish were stocked, which is especially apparent in the SCCS (Figure 3.10A). From these results it appears that *T. finnmarkense* accumulates inside the SCCS, unlike in the open net pen where the water flows directly through the pen. The sporadic presence of *T. finnmarkense* in the water may be as a result of seasonal changes in sea water temperature, as described for *T. maritimum* (Downes et al., 2018). In the current study it seems that *T. finnmarkense* has an increase in presence in the SCCS regardless of seasonal changes in sea water temperature. However, the water samples in this study were analyzed over a period of approximately six months, while Downes et al (2018) studied the change in *T. maritimum* presence as a part of a longitudinal study over 16 months. Hence, six months may not be enough time to investigate seasonal change in the abundance of *T. finnmarkense.*

Another interesting aspect in the SCCS is that no *T. finnmarkense* nor *M. viscosa* were detected in the water sample prior to fish stocking. This indicate introduction of *T. finnmarkense* in connection with the delivery of the fish (well boat) or that the bacteria enter during intake of water, and that the presence of salmon provide a substrate for growth and accumulation of these bacteria. This notion should be further investigated.

*M. viscosa* was initially detected in water samples 66 dps in both the SCCS and open net pen (Table 21, Appendix). The next water sampling positive for *M. viscosa* was 93 dps and the fish at the study site started to develop ulcers 80-110 dps and it was in this period the sea water temperature dropped. This indicate that the presence of *M. viscosa* in the water can be an important factor for the development of ulcerative disease (Lunder et al., 1995). It has been

discussed that *Tenacibaculum* spp. are only able to induce ulcerative disease when the fish has preexisting skin injury, and may only act as a cofactor aggravating the pathogenesis of 'winter ulcer disease' associated with *M. viscosa* (Olsen et al., 2011). In the current study it may seem that *T. finnmarkense* could be present in the water at all time without causing any disease on its own. Challenge experiments using *Tenacibaculum* spp. strains inducing high mortality have only been conducted on small fish (>200 g) (Klakegg et al., 2019; Olsen et al., 2011; Småge et al., 2018). The effect *Tenacibaculum* spp. has on larger fish in challenge studies, like the ones screened in this study, has not been investigated to the authors knowledge. It could be that both *T. finnmarkense* and *M. viscosa* needs to be present in order to induce ulcerative disease and mortality in larger fish. The presence of both *T. finnmarkense* and *M. viscosa* in the water might lead to enough pathogen pressure in the environment to induce ulcerative disease in salmon. This was described in the study by Olsen et al (2011) where salmon were co-infected with *Tenacibaculum* spp. and *M. viscosa* which led to the development of ulcers.

### 4.2.3 Real Time RT-PCR screening of fish samples obtained during an outbreak of ulcerative disease at the study site

Fish in the SCCS started to develop ulcers approximately 80-110 dps (section 3.2.3). The results of the screening revealed high levels of *T. finnmarkense* gen. *finnmarkense, T. finnmarkense* gen. *ulcerans* and *M. viscosa* in all tissues. *Tenacibaculum* spp. is reported to cause erosion of the mouth and head region as well as the caudal fin (Olsen et al., 2011; Småge, 2018; Toranzo et al., 2005). This is in accordance with the findings in the screening of diseased fish in the SCCS. The statistical analyses based on Log NE-fold values from Ct-values obtained from skin tissue from ulcers in the mouth region of the fish resulted in a significantly higher presence of *T. finnmarkense* gen. *finnmarkens* (****P<0.0001) and *T. finnmarkense* gen. *ulcerans* (*P = 0.0219), compared to *M. viscosa* (Figure 3.15B) (keeping in mind that the assays are targeting two different HK genes, *tgt* in *Tenacibaculum* spp and OmpA in *M. viscosa*). This supports the notion that *T. finnmarkense* has an affinity for collagen rich tissue such as the tissues in the mouth and fins (Olsen et al., 2011; Småge, 2018). The analyses of gill tissue resulted in relatively high Ct-values, with *M. viscosa* being significantly more present compared to *T. finnmarkense* gen. *finnmarkense* (*P = 0.0253) and *T. finnmarkense* gen. *ulcerans* (****P<0.0001) (Figure 3.15C). Although the statistical analyses indicate a higher presence of *M. viscosa* compared to *T. finnmarkense* in the gills and a lower presence in the tissue sampled from the mouth, it should be mentioned that the *T. finnmarkense* Real Time RT-PCR assays (TffC3 and TfuC1) and *M. viscosa* assay (MvOmpA) targets different HK-genes. Due to this,

the Ct-values cannot be directly compared without taking the possible difference in expression of the HK-genes in the cells into consideration. However, the bacterial cell count using the MPN method (section 2.6.4) estimates the bacterial cell number with the corresponding Ct-value quite accurately (Table 3.4.). This estimation was used as a reference when comparing Ct-values obtained from Real Time RT-PCR assays targeting different HK-genes.

Interestingly, no gill tissue sampled from healthy fish at the study site (1-111 dps) tested positive for *M. viscosa.* This indicates that *M. viscosa* emerges in the gills concurrent with the development of ulcers. In challenge experiments with *M. viscosa* conducted on Atlantic salmon, the bacterium was first detected by Real Time RT-PCR in the gill tissue (Løvoll et al., 2009). Therefore, the authors suggested that the gills might be a port of entry for *M. viscosa*. However, in the same study, *M. viscosa* was not detected in the gills using immunohistochemistry. Gills from fish suffering from ulcerative disease at the study site (open net pen) were examined histologically, but no histopathological signs nor the presence of bacteria with morphology associated with *Tenacibaculum* spp. or *M. viscosa* were observed. These findings correlate with the results presented by Løvoll et al (2009) and supports the hypothesis that *M. viscosa* might be using the gills as a port of entry. However, the notion that this bacterium somehow is able to penetrate intact gill tissue without causing damage, then enter the blood stream before causing the skin ulcers seem to be unlikely, yet not impossible. Perhaps a more likely scenario is the bacterium entering the blood stream through damaged skin (e.g., due to mechanical handling) before reaching the gill tissue. Another possibility is that *T. finnmarkense* has already caused infection in the skin providing a site of entry for *M. viscosa* as a secondary pathogen. This could further lead to the accumulation of *M. viscosa* in the ulcer and surrounding water. A high presence of *M. viscosa* in the water could lead to the detection of the bacterium in the gill tissue. Analysis of kidney samples from both healthy and diseased fish could indicate the route of infection for *M. viscosa,* and in hindsight this should have been performed in the current study. If the gill- and kidney tissue were to test positive for *M. viscosa,* but no ulcers are present, it would suggest that the gills are a port of entry for the bacterium. Nonetheless, further studies are needed to investigate the route of infection for *M. viscosa.*

### 4.2.4 The causative agents of the outbreak of ulcerative disease at the study site

Skin ulcers started to appear at the study site around 80-110 dps, and fish in the SCCS were suffering from more ulcers compared to the reference open net pen (Cermaq fish health personnel observations). The *M. viscosa* Ct-values obtained from water samples comparing the

two pens reflect this observation (*P = 0.0164). This is also reflected in the gills of diseased fish from the SCCS, where there was a significantly higher presence of *M. viscosa* compared to *T. finnmarkense* gen. *finnmarkense* (*P = 0.0253) and *T. finnmarkense* gen. *ulcerans* (****P<0.0001). Subsequently this indicates that *M. viscosa* could be the main causative agent or at least an important factor in the outbreak of ulcerative disease in the SCCS at the study site. As carried out in section 4.2.3, the presence of *T. finnmarkense* and *M. viscosa* have been compared using Ct-values obtained from Real Time RT-PCR assays based on different HK-genes. However, Table 3.4 suggests a somewhat similar sensitivity (and cell count with a corresponding Ct-value) of the assays and the Ct-values obtained from the different assays were thus compared in this study.

The fish at the study site were stocked in the SCCS and open net pen in the month of September and the ulcers emerged approximately 80-110 dps (Desember). This was concurrent with the onset of decreasing seawater temperatures (Table 3.6). The water temperatures from 1-170 dps were almost identical in the SCCS and open net pen, however the temperature in both pens clearly decreased below 8 °C. 'Winter ulcer disease' is most frequently observed during the winter months when temperatures are low (less than 8 °C) (Løvoll et al., 2009; Poppe, 1990), which correlate with the timepoint of which the ulcerative disease started appearing. The main causative agent or agents of 'winter ulcer disease' is not fully known, but *M. viscosa* have been the bacterium that has most often been associated with the disease (Benediktsdóttir et al., 2000; Bruno et al., 1998; Lunder et al., 1995). However, *T. finnmarkense* have been reported to play an important role in the 'winter ulcer' pathogenesis (Olsen et al., 2011). Bacteria of the genus *Tenacibaculum* have been shown to inhabit the surface of skin of both healthy salmon and salmon suffering from skin ulcers (Karlsen et al., 2017). The study site suffered a power failure approximately 130 dps which caused lack of light in both the SCCS and reference net pen. From the literature it is clear that any damage to the skin that negatively affect the protective barrier or environmental stressors that negatively affects the immune system function, makes fish more susceptible to disease (Fast et al., 2008; Kristensen et al., 2012; Sundh et al., 2010). Considering that the power failure happened shortly prior to the outbreak at site, it may have stressed the fish causing damage to the skin due to panicked behavior. From the analyses performed in this study it is evident that *T. finnmarkense* is present in the skin and water from the time of stocking and onwards. A potential damage to the skin may therefore have given *T. finnmarkense* an opportunity to establish the infection as it would have facilitated excess to the collagen rich dermis layer in which the bacteria seems to have a preference (Olsen et al., 2011;

Småge et al., 2018). From the histological analysis of skin tissue obtained from ulcers at the study site, a large amount of long rod-shaped bacteria resembling *Tenacibaculum* spp. morphology were identified (Figure 3.17). Although *M. viscosa* historically have been associated with being the main causative agent of 'winter ulcer disease' (Lunder et al., 1995), these findings indicate that *T. finnmarkense* has a role in the 'winter ulcer disease' alongside factors such as time or year and water temperature.

### 4.2.5 Can keeping fish in a SCCS reduce the risk for ulcerative disease?

Several studies have been conducted on mortality rates of fish stocked in CCS and more frequently the last decades due to fast progress in new farming technologies (Balseiro et al., 2018; Nilsen et al., 2020; Skaar & Bodvin, 1993; Øvrebø, 2020). Nilsen et al (2020) reported raised mortality in a CCS due to 'ulcers and fin rot' which accounted for 36.1 % of the total mortality. Fish stocked in the SCCS, as well as the open net pen, at the study site also suffered from ulcerative disease which have caused raised mortality. Although there was a significantly higher presence of *T. finnmarkense* in gills of fish in the open net pen compared to the SCCS, fish in both pens developed ulcerative disease. This suggests that a SCCS does not reduce the risk of bacterial skin ulcer disease.

Increased growth, reduced mortality, lower sea lice numbers and reduced stress are some of many reported benefits of using SCCS (Nilsen, 2019; Øvrebø, 2020). However, the findings in this study, supported by Nilsen et al (2020), further suggests that a SCCS does not reduce the risk of ulcerative disease due to *Tenacibaculum* spp. and *M. viscosa*, and that fish stocked in a SCCS is at the same risk of suffering from ulcerative disease as fish stocked in an open net pen. Although the main goal of using SCCS is to reduce the pressure of sea lice, some modifications might make the fish stocked in SCCS at less risk of developing ulcerative disease. First and foremost, the inlet water intake may play an important role. As mentioned in section 4.2.4, 'Winter ulcer disease' often emerges at low and decreasing sea water temperatures. The temperature in the SCCS will differ with the depth of the intake water and at the study site the SCCS has a water intake at 15 m. By collecting water from depths with stable temperatures, one might avoid the fluctuating temperatures in the SCCS. As a result, there will be less risk of decreasing temperatures that seems to be one of the triggers of the development of 'winter ulcer disease'. However, the water level at which sea lice is predominantly found should first and foremost be taken into consideration when choosing the water depth of intake water. The optimal temperature for salmon is in a range between 8 °C and 14 °C which should be reflected

(Mowi, 2020). Approximately 25 m depth would provide effective protection against sea lice (Nilsen, 2019), as well a stable environment regarding sea water temperature. The pre stocking water sample from the SCCS tested negative for *T. finnmarkense*, which indicates that the amount of *T. finnmarkense* present in the water at 15 m depth is low. This further means that the SCCS could have been contaminated with *T. finnmarkense* from the sea water in the well boat at fish delivery and the bacterium could further have accumulated inside the SCCS. The source of water intake in the well boats when delivering fish to SCCS should be assessed and the distribution of *T. finnmarkense* in the water column should be further investigated.

Filtration of intake water is another possible solution to avoid sea lice, however the filter technology (2011) is not able to filtrate small particles like bacteria and virus (e.g., IPN-virus) (Rosten, 2011). Filtration equipment used for efficient removal of small pathogens in CCS (e.g., *Tenacibaculum* spp.) should be further investigated. Nonetheless, the most relevant treatment of intake water in the reduction of *Tenacibaculum* spp. is disinfection of the intake water using UV. Rud et al (2017) detected *Tenacibaculum* and *M. viscosa* in SCCS trough a deep sequencing of the bacterial microbiota from Atlantic salmon post-smolt production, even though the fish appeared non-symptomatic. The authors suggested that future development of SCCS should consider to include intake water treatment, such as mechanical and UV-filtration to improve biosecurity (Rud et al., 2017). However, UV-resistant pathogens exist (e.g., IPN-virus), and the removal of these pathogens cannot be guaranteed using UV disinfection. Other disinfections like chlorine and ozone could be used, although these methods would be more complicated to use in sea water compared to UV (Rosten, 2011).

The findings in this study also suggests that regular screening of the water in the net pen can be used in risk assessments for predicting possible outbreaks of 'winter ulcer disease' associated with *M. viscosa*, as it seems the bacteria emerges some time prior to outbreaks of ulcerative disease. However, the screening of water samples is not recommended for prediction of ulcerative outbreaks associated with *T. finnmarkense* since these bacteria are present in a quite high number at all time in the sea water as observed in this study. Screening of water samples would be a less demanding and cost-efficient tool to detect ulcer related pathogens compared to fish sampling, although one likely achieves a more reliable prediction by screening fish samples.

# 5. CONCLUSION AND FUTURE RESEARCH

The effect of a Semi Closed Containment System (SCCS) on the presence of *Tenacibaculum finnmarkense* was investigated in this study. It is concluded that the SCCS does not reduce the presence of *T. finnmarkense* compared to the open net, yet there was detected more *T. finnmarkense* in the gills of fish from the open net pen compared to the SCCS. The water samples showed a constant presence of *T. finnmarkense* in both the SCCS and open net pen and at both high (11.8 °C) and low (4.1 °C) seawater temperatures. However, the presence of *T. finnmarkense* was shown to steadily increase inside the SCCS. This indicates that there is a slow accumulation of *T. finnmarkense* inside the SCCS compared to the open net pen, in which the presence of *T. finnmarkense* varied between 1-170 dps. Another interesting aspect is that *T. finnmarkense* is not detected in the SCCS water samples prior to fish stocking. This suggests that *T. finnmarkense* is either introduced into the system during stocking or via the intake water. *M. viscosa* was detected in the water samples from both pens 66 dps, concurrent with the onset of ulcerative disease at the study site. This indicates that *M. viscosa* is introduced into the system through the intake water at low seawater temperature (6.7-4.1 °C). The ulcers started occurring around the month of December (approximately 80 dps) at decreasing sea water temperatures. This suggest that the outbreak of ulcerative disease at the study site was a classic case of 'Winter ulcer disease' with both *M. viscosa* and *T. finnmarkense* as the causative agents of the disease. This study also concludes that *T. dicentrarchi* is likely not present in Northern Norway salmon farms as of now and thus can be omitted from screening of outbreaks of ulcerative disease in the Northern Norway. The three Real Time RT-PCR assays developed in this study can differentiate Clade I, II and III of *Tenacibaculum* spp. relevant to ulcerative disease in Norway. The Real Time RT-PCR assays are specific, sensitive and efficient at targeting their intended bacterium when using a reverse transcriptase and annealing temperature of 47 °C and 62 °C, respectively. Therefore, the assays can be used as an adequate tool for monitoring the presence of both *T. finnmarkense* and *T. dicentrarchi* in water samples and as diagnostic tools when investigating outbreaks of ulcerative disease in Norwegian salmon farms.

For future research, potential disinfection methods of intake water in SCCS should be investigated in order to avoid unwanted pathogens such as *Tenacibaculum* spp. and *M. viscosa* gaining access to the system, as this likely will reduce the risk of ulcerative disease. As only the screening of pathogens associated with ulcerative disease were included in this study, the effect of SCCS on the presence of other pathogens (e.g., *Piscine orthoreovirus*) should also be

carried out. From this study it is evident that screening of water samples can be used as a tool for monitoring the presence of *T. finnmarkens*e and *M. viscosa*. This suggest that water samples also can be used as a tool for monitoring other pathogens and should therefore be further investigated. The possible introduction of unwanted pathogens via well-boats when delivering fish to SCCS should also be further investigated. Moreover, the infection route of *M. viscosa* and the interaction between *M. viscosa* and *T. finnmarkense* in causing 'winter ulcers' should be further researched.

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# APPENDIX

## Recipes

BAMA (Blood agar with sea salt):

- Peptone from animal tissue 5.0 g
- Yeast extract 1.0 g
- Coral pro salt (Red sea) 37.2 g
- Bacteriological agar 15.0 g
- Distilled water 950 mL
- Sterile defibrinated sheep blood 50 mL

MB (Marine Broth)

- Peptone from animal tissue 5.0 g
- Yeast extract 1.0 g
- Coral pro salt (Red sea) 37.2 g
- Distilled water 1000 mL

## Colour scheme for Ct-values

**Table 1.** The colour scheme used in the presentation of Ct-values obtained from Real Time RT-PCR analyses. Ct-values above 40.0 were deemed negative.

| Colour | Ct-value |
|---|---|
| Bright pink | 10.0-19.9 |
| Light pink | 20.0-29.9 |
| Orange | 30.0-39.9 |
| Light yellow | >40.0 (Negative) |

# Real Time RT-PCR: Ct-values

## Pre stocking samples

**Table 2.** Overview of the Ct-values from the pre stocking samples obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi*, the MvOmpA assay targeting *Moritella viscosa* and the EF1A assay targeting the salmon elongation factor. Samples were collected from the skin under the jaw when no lesions could be detected and the gills. Days post stocking (dps) are listed in the table.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| 0-F1 | Skin | -5 | neg | neg | neg | neg | 21,7 |
| 0-F2 | Skin | -5 | neg | neg | neg | neg | 19,3 |
| 0-F3 | Skin | -5 | neg | neg | neg | neg | 19,8 |
| 0-F4 | Skin | -5 | neg | neg | neg | neg | 19,7 |
| 0-F5 | Skin | -5 | neg | neg | neg | neg | 18,7 |
| 0-F6 | Skin | -5 | neg | neg | neg | neg | 18,8 |
| 0-F7 | Skin | -5 | neg | neg | neg | neg | 20,4 |
| 0-F8 | Skin | -5 | neg | neg | neg | neg | 19,4 |
| 0-F9 | Skin | -5 | neg | neg | neg | neg | 16,4 |
| 0-F10 | Skin | -5 | neg | neg | neg | neg | 20,6 |
| 0-F11 | Skin | -5 | neg | neg | neg | neg | 18,2 |
| 0-F12 | Skin | -5 | neg | neg | neg | neg | 17,6 |
| 0-F13 | Skin | -5 | neg | neg | neg | neg | 18,1 |
| 0-F14 | Skin | -5 | neg | neg | neg | neg | 17,1 |
| 0-F15 | Skin | -5 | neg | neg | neg | neg | 20,1 |
| 0-F16 | Skin | -5 | neg | neg | neg | neg | neg |
| 0-F17 | Skin | -5 | neg | neg | neg | neg | 18,1 |
| 0-F18 | Skin | -5 | neg | neg | neg | neg | 17,6 |
| 0-F19 | Skin | -5 | neg | neg | neg | neg | 19,9 |
| 0-F20 | Skin | -5 | neg | neg | neg | neg | 19,3 |
| 0-F21 | Skin | -5 | neg | neg | neg | neg | 20,5 |
| 0-F22 | Skin | -5 | neg | neg | neg | neg | 20,3 |
| 0-F23 | Skin | -5 | neg | neg | neg | neg | 18,1 |
| 0-F24 | Skin | -5 | neg | neg | neg | neg | 18,0 |
| 0-F25 | Skin | -5 | neg | neg | neg | neg | neg |
| 0-F26 | Skin | -5 | neg | neg | neg | neg | 17,9 |
| 0-F27 | Skin | -5 | neg | neg | neg | neg | 20,4 |
| 0-F28 | Skin | -5 | neg | neg | neg | neg | 18,9 |
| 0-F29 | Skin | -5 | neg | neg | neg | neg | 37,1 |
| 0-F30 | Skin | -5 | neg | neg | neg | neg | 20,4 |
| 0-F1 | Gill | -5 | neg | neg | neg | neg | 16,8 |
| 0-F2 | Gill | -5 | neg | neg | neg | neg | 16,2 |
| 0-F3 | Gill | -5 | neg | neg | neg | neg | 15,2 |
| 0-F4 | Gill | -5 | neg | neg | neg | neg | 15,3 |
| 0-F5 | Gill | -5 | neg | neg | neg | neg | 15,2 |
| 0-F6 | Gill | -5 | neg | neg | neg | neg | 14,3 |
| 0-F7 | Gill | -5 | neg | neg | neg | neg | 15,4 |
| 0-F8 | Gill | -5 | neg | neg | neg | neg | 15,6 |
| 0-F9 | Gill | -5 | neg | neg | neg | neg | 14,8 |
| 0-F10 | Gill | -5 | neg | neg | neg | neg | 16,6 |
| 0-F11 | Gill | -5 | neg | neg | neg | neg | 14,8 |
| 0-F12 | Gill | -5 | neg | neg | neg | neg | 14,8 |
| 0-F13 | Gill | -5 | neg | neg | neg | neg | 14,9 |
| 0-F14 | Gill | -5 | neg | neg | neg | neg | 14,6 |
| 0-F15 | Gill | -5 | neg | neg | neg | neg | 15,3 |
| 0-F16 | Gill | -5 | neg | neg | neg | neg | 14,9 |
| 0-F17 | Gill | -5 | neg | neg | neg | neg | 14,3 |
| 0-F18 | Gill | -5 | neg | neg | neg | neg | 15,2 |
| 0-F19 | Gill | -5 | neg | neg | neg | neg | 15,4 |
| 0-F20 | Gill | -5 | neg | neg | neg | neg | 14,5 |
| 0-F21 | Gill | -5 | neg | neg | neg | neg | 15,6 |
| 0-F22 | Gill | -5 | neg | neg | neg | neg | neg |
| 0-F23 | Gill | -5 | neg | neg | neg | neg | 14,9 |
| 0-F24 | Gill | -5 | neg | neg | neg | neg | 15,2 |
| 0-F25 | Gill | -5 | neg | neg | neg | neg | 15,7 |
| 0-F26 | Gill | -5 | neg | neg | neg | neg | 15,4 |
| 0-F27 | Gill | -5 | neg | neg | neg | neg | 16,0 |
| 0-F28 | Gill | -5 | neg | neg | neg | neg | 14,8 |
| 0-F29 | Gill | -5 | neg | neg | neg | neg | 15,3 |
| 0-F30 | Gill | -5 | neg | neg | neg | neg | 16,4 |

## Fish samples 1-111 dps in the SCCS and open net pen at the study site

**Table 3**. Fish samples 1 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi*, the MvOmpA assay targeting *Moritella viscosa* and the EF1A assay targeting the salmon elongation factor. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C1 - F1 | Skin | 1 | neg | neg | neg | neg | 19,3 |
| C1 - F2 | Skin | 1 | neg | neg | neg | neg | 19,0 |
| C1 - F3 | Skin | 1 | neg | neg | neg | neg | 20,2 |
| C1 - F4 | Skin | 1 | neg | neg | neg | neg | 20,1 |
| C1 - F5 | Skin | 1 | neg | neg | neg | neg | 19,4 |
| C1 - F6 | Skin | 1 | neg | neg | neg | neg | 20,2 |
| C1 - F7 | Skin | 1 | neg | neg | neg | neg | 20,7 |
| C1 - F8 | Skin | 1 | neg | neg | neg | neg | 19,4 |
| C1 - F9 | Skin | 1 | neg | neg | neg | neg | 19,4 |
| C1 - F10 | Skin | 1 | neg | neg | neg | neg | 20,3 |
| C1 - F11 | Skin | 1 | neg | neg | neg | neg | 21,3 |
| C1 - F12 | Skin | 1 | neg | neg | neg | neg | 20,8 |
| C1 - F13 | Skin | 1 | neg | neg | neg | neg | 19,3 |
| C1 - F14 | Skin | 1 | neg | neg | neg | neg | 22,2 |
| C1 - F15 | Skin | 1 | neg | neg | neg | neg | 19,9 |
| C1 - F16 | Skin | 1 | neg | neg | neg | neg | 19,1 |
| C1 - F17 | Skin | 1 | neg | neg | neg | neg | 20,0 |
| C1 - F18 | Skin | 1 | neg | neg | neg | neg | 21,2 |
| C1 - F19 | Skin | 1 | neg | neg | neg | neg | 20,7 |
| C1 - F20 | Skin | 1 | neg | neg | neg | neg | 22,9 |
| C1 - F21 | Skin | 1 | neg | neg | neg | neg | 18,7 |
| C1 - F22 | Skin | 1 | neg | neg | neg | neg | 20,6 |
| C1 - F23 | Skin | 1 | neg | neg | neg | neg | 18,7 |
| C1 - F24 | Skin | 1 | neg | neg | neg | neg | 19,0 |
| C1 - F25 | Skin | 1 | neg | neg | neg | neg | 18,8 |
| C1 - F26 | Skin | 1 | neg | neg | neg | neg | 21,1 |
| C1 - F27 | Skin | 1 | neg | neg | neg | neg | 20,6 |
| C1 - F28 | Skin | 1 | neg | neg | neg | neg | 19,9 |
| C1 - F1 | Gill | 1 | neg | neg | neg | neg | 15,6 |
| C1 - F2 | Gill | 1 | neg | neg | neg | neg | 15,6 |
| C1 - F3 | Gill | 1 | neg | neg | neg | neg | 16,1 |
| C1 - F4 | Gill | 1 | neg | neg | neg | neg | 15,4 |
| C1 - F5 | Gill | 1 | neg | neg | neg | neg | 15,5 |
| C1 - F6 | Gill | 1 | neg | neg | neg | neg | 15,8 |
| C1 - F7 | Gill | 1 | neg | neg | neg | neg | 15,7 |
| C1 - F8 | Gill | 1 | neg | neg | neg | neg | 16,4 |
| C1 - F9 | Gill | 1 | neg | neg | neg | neg | 14,8 |
| C1 - F10 | Gill | 1 | neg | neg | neg | neg | 14,9 |
| C1 - F11 | Gill | 1 | neg | neg | neg | neg | 14,4 |
| C1 - F12 | Gill | 1 | neg | neg | neg | neg | 15,5 |
| C1 - F13 | Gill | 1 | neg | neg | neg | neg | 14,5 |
| C1 - F14 | Gill | 1 | neg | neg | neg | neg | 14,8 |
| C1 - F15 | Gill | 1 | neg | neg | neg | neg | 15,8 |
| C1 - F16 | Gill | 1 | neg | neg | neg | neg | 14,9 |
| C1 - F17 | Gill | 1 | neg | neg | neg | neg | 14,7 |
| C1 - F18 | Gill | 1 | neg | neg | neg | neg | 15,0 |
| C1 - F19 | Gill | 1 | neg | neg | neg | neg | 15,5 |
| C1 - F20 | Gill | 1 | neg | neg | neg | neg | 15,6 |
| C1 - F21 | Gill | 1 | neg | neg | neg | neg | 17,1 |
| C1 - F22 | Gill | 1 | neg | neg | neg | neg | 19,6 |
| C1 - F23 | Gill | 1 | neg | neg | neg | neg | 16,9 |
| C1 - F24 | Gill | 1 | neg | neg | neg | neg | 20,1 |
| C1 - F25 | Gill | 1 | neg | neg | neg | neg | 18,1 |
| C1 - F26 | Gill | 1 | neg | neg | neg | neg | 19,9 |
| C1 - F27 | Gill | 1 | neg | neg | neg | neg | 20,1 |
| C1 - F28 | Gill | 1 | neg | neg | neg | neg | 16,6 |
| C1 - F29 | Gill | 1 | neg | neg | neg | neg | 19,0 |
| C1 - F30 | Gill | 1 | neg | neg | neg | neg | 18,8 |

**Table 4.** Fish samples 1 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R1 - F1 | Skin | 1 | neg | neg | neg | neg | 19,6 |
| R1 - F2 | Skin | 1 | neg | neg | neg | neg | 19,8 |
| R1 - F3 | Skin | 1 | neg | neg | neg | neg | 18,0 |
| R1 - F4 | Skin | 1 | neg | 36,6 | neg | neg | 18,3 |
| R1 - F5 | Skin | 1 | neg | neg | neg | neg | 17,9 |
| R1 - F6 | Skin | 1 | neg | neg | neg | neg | 18,3 |
| R1 - F7 | Skin | 1 | neg | neg | neg | neg | 17,9 |
| R1 - F8 | Skin | 1 | neg | neg | neg | neg | 19,5 |
| R1 - F9 | Skin | 1 | neg | neg | neg | neg | 17,9 |
| R1 - F10 | Skin | 1 | neg | neg | neg | neg | 18,4 |
| R1 - F11 | Skin | 1 | neg | neg | neg | neg | 20,7 |
| R1 - F12 | Skin | 1 | neg | 39,8 | neg | neg | 18,8 |
| R1 - F13 | Skin | 1 | neg | neg | neg | neg | 19,7 |
| R1 - F14 | Skin | 1 | neg | neg | neg | neg | 19,1 |
| R1 - F15 | Skin | 1 | neg | neg | neg | neg | 18,7 |
| R1 - F16 | Skin | 1 | neg | neg | neg | neg | 19,1 |
| R1 - F17 | Skin | 1 | neg | neg | neg | neg | 17,2 |
| R1 - F18 | Skin | 1 | neg | neg | neg | neg | 18,4 |
| R1 - F19 | Skin | 1 | neg | neg | neg | neg | 19,0 |
| R1 - F20 | Skin | 1 | neg | neg | neg | neg | 18,1 |
| R1 - F21 | Skin | 1 | neg | neg | neg | neg | 18,9 |
| R1 - F22 | Skin | 1 | neg | neg | neg | neg | 18,3 |
| R1 - F23 | Skin | 1 | neg | neg | neg | neg | 18,7 |
| R1 - F24 | Skin | 1 | neg | neg | neg | neg | 17,7 |
| R1 - F25 | Skin | 1 | neg | neg | neg | neg | 19,7 |
| R1 - F26 | Skin | 1 | neg | neg | neg | neg | 19,7 |
| R1 - F27 | Skin | 1 | neg | neg | neg | neg | 18,1 |
| R1 - F28 | Skin | 1 | neg | neg | neg | neg | 18,7 |
| R1 - F29 | Skin | 1 | neg | neg | neg | neg | 17,9 |
| R1 - F30 | Skin | 1 | neg | neg | neg | neg | 19,7 |
| R1 - F1 | Gill | 1 | neg | neg | neg | neg | 16,3 |
| R1 - F2 | Gill | 1 | neg | neg | neg | neg | 16,8 |
| R1 - F3 | Gill | 1 | neg | neg | neg | neg | 17,4 |
| R1 - F4 | Gill | 1 | 37,1 | neg | neg | neg | 22,7 |
| R1 - F5 | Gill | 1 | neg | 36,2 | neg | neg | 19,4 |
| R1 - F6 | Gill | 1 | neg | neg | neg | neg | 17,6 |
| R1 - F7 | Gill | 1 | neg | neg | neg | neg | 18,5 |
| R1 - F8 | Gill | 1 | neg | neg | neg | neg | 17,4 |
| R1 - F9 | Gill | 1 | neg | neg | neg | neg | 16,5 |
| R1 - F10 | Gill | 1 | neg | neg | neg | neg | 20,4 |
| R1 - F11 | Gill | 1 | neg | neg | neg | neg | 18,1 |
| R1 - F12 | Gill | 1 | neg | neg | neg | neg | 17,3 |
| R1 - F13 | Gill | 1 | neg | neg | neg | neg | 17,5 |
| R1 - F14 | Gill | 1 | neg | neg | neg | neg | 19,0 |
| R1 - F15 | Gill | 1 | neg | neg | neg | neg | 19,5 |
| R1 - F16 | Gill | 1 | neg | neg | neg | neg | 17,9 |
| R1 - F17 | Gill | 1 | neg | neg | neg | neg | 20,0 |
| R1 - F18 | Gill | 1 | neg | neg | neg | neg | 19,6 |
| R1 - F19 | Gill | 1 | neg | neg | neg | neg | 15,8 |
| R1 - F20 | Gill | 1 | neg | neg | neg | neg | 17,9 |
| R1 - F21 | Gill | 1 | neg | neg | neg | neg | 18,2 |
| R1 - F22 | Gill | 1 | neg | neg | neg | neg | 17,9 |
| R1 - F23 | Gill | 1 | neg | neg | neg | neg | 16,8 |
| R1 - F24 | Gill | 1 | neg | neg | neg | neg | 20,0 |
| R1 - F25 | Gill | 1 | neg | neg | neg | neg | 17,6 |
| R1 - F26 | Gill | 1 | neg | neg | neg | neg | 17,6 |
| R1 - F27 | Gill | 1 | neg | neg | neg | neg | 17,2 |
| R1 - F28 | Gill | 1 | neg | neg | neg | neg | 17,6 |
| R1 - F29 | Gill | 1 | neg | neg | neg | neg | 16,2 |
| R1 - F30 | Gill | 1 | neg | neg | neg | neg | 18,8 |

**Table 5**. Fish samples 8 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C2 - F1 | Skin | 8 | neg | neg | NT | neg | 18,4 |
| C2 - F2 | Skin | 8 | neg | neg | NT | neg | 19,5 |
| C2 - F3 | Skin | 8 | neg | neg | NT | neg | 19,0 |
| C2 - F4 | Skin | 8 | neg | neg | NT | neg | 18,8 |
| C2 - F5 | Skin | 8 | neg | neg | NT | neg | 18,5 |
| C2 - F6 | Skin | 8 | neg | neg | NT | neg | 19,4 |
| C2 - F7 | Skin | 8 | neg | neg | NT | neg | 19,5 |
| C2 - F8 | Skin | 8 | neg | neg | NT | neg | 19,2 |
| C2 - F9 | Skin | 8 | neg | neg | NT | neg | 19,3 |
| C2 - F10 | Skin | 8 | neg | neg | NT | neg | 19,4 |
| C2 - F11 | Skin | 8 | neg | neg | NT | neg | 18,8 |
| C2 - F12 | Skin | 8 | neg | neg | NT | neg | 18,4 |
| C2 - F13 | Skin | 8 | neg | neg | NT | neg | 18,9 |
| C2 - F14 | Skin | 8 | neg | neg | NT | neg | 18,0 |
| C2 - F15 | Skin | 8 | neg | neg | NT | neg | 18,8 |
| C2 - F16 | Skin | 8 | neg | neg | NT | neg | 19,2 |
| C2 - F17 | Skin | 8 | neg | neg | NT | neg | 18,1 |
| C2 - F18 | Skin | 8 | neg | neg | NT | neg | 18,7 |
| C2 - F19 | Skin | 8 | neg | neg | NT | neg | 19,6 |
| C2 - F20 | Skin | 8 | neg | neg | NT | neg | 17,8 |
| C2 - F21 | Skin | 8 | neg | neg | NT | neg | 18,7 |
| C2 - F22 | Skin | 8 | neg | neg | NT | neg | 18,0 |
| C2 - F23 | Skin | 8 | neg | neg | NT | neg | 17,7 |
| C2 - F24 | Skin | 8 | neg | neg | NT | neg | 18,3 |
| C2 - F25 | Skin | 8 | neg | neg | NT | neg | 17,3 |
| C2 - F26 | Skin | 8 | neg | neg | NT | neg | 16,3 |
| C2 - F27 | Skin | 8 | neg | neg | NT | neg | 17,9 |
| C2 - F28 | Skin | 8 | neg | neg | NT | neg | 16,1 |
| C2 - F29 | Skin | 8 | neg | neg | NT | neg | 17,4 |
| C2 - F30 | Skin | 8 | neg | neg | NT | neg | 17,5 |
| C2 - F1 | Gill | 8 | neg | neg | NT | neg | 17,9 |
| C2 - F2 | Gill | 8 | neg | neg | NT | neg | 22,8 |
| C2 - F3 | Gill | 8 | neg | neg | NT | neg | 21,4 |
| C2 - F4 | Gill | 8 | neg | neg | NT | neg | 18,6 |
| C2 - F5 | Gill | 8 | neg | neg | NT | neg | 20,2 |
| C2 - F6 | Gill | 8 | neg | neg | NT | neg | 19,4 |
| C2 - F7 | Gill | 8 | neg | neg | NT | neg | 18,2 |
| C2 - F8 | Gill | 8 | neg | neg | NT | neg | 19,1 |
| C2 - F9 | Gill | 8 | neg | neg | NT | neg | 21,8 |
| C2 - F10 | Gill | 8 | neg | neg | NT | neg | 17,6 |
| C2 - F11 | Gill | 8 | neg | neg | NT | neg | 31,1 |
| C2 - F12 | Gill | 8 | neg | neg | NT | neg | 26,6 |
| C2 - F13 | Gill | 8 | neg | neg | NT | neg | 16,7 |
| C2 - F14 | Gill | 8 | neg | neg | NT | neg | 17,1 |
| C2 - F15 | Gill | 8 | neg | neg | NT | neg | 20,0 |
| C2 - F16 | Gill | 8 | neg | neg | NT | neg | 16,9 |
| C2 - F17 | Gill | 8 | neg | neg | NT | neg | 20,1 |
| C2 - F18 | Gill | 8 | neg | neg | NT | neg | 19,1 |
| C2 - F19 | Gill | 8 | neg | neg | NT | neg | 16,4 |
| C2 - F20 | Gill | 8 | neg | neg | NT | neg | 20,2 |
| C2 - F21 | Gill | 8 | neg | neg | NT | neg | 19,6 |
| C2 - F22 | Gill | 8 | 37,2 | neg | NT | neg | 18,1 |
| C2 - F23 | Gill | 8 | neg | neg | NT | neg | 20,0 |
| C2 - F24 | Gill | 8 | 37,0 | neg | NT | neg | 17,9 |
| C2 - F25 | Gill | 8 | neg | neg | NT | neg | 19,0 |
| C2 - F26 | Gill | 8 | neg | neg | NT | neg | 18,3 |
| C2 - F27 | Gill | 8 | neg | neg | NT | neg | 23,8 |
| C2 - F28 | Gill | 8 | neg | neg | NT | neg | 20,3 |
| C2 - F29 | Gill | 8 | neg | neg | NT | neg | 24,1 |
| C2 - F30 | Gill | 8 | neg | neg | NT | neg | 20,1 |

**Table 6.** Fish samples 8 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R2 - F1 | Skin | 8 | neg | neg | NT | neg | 17,9 |
| R2 - F2 | Skin | 8 | neg | neg | NT | neg | 17,8 |
| R2 - F3 | Skin | 8 | neg | neg | NT | neg | 18,6 |
| R2 - F4 | Skin | 8 | neg | neg | NT | neg | 18,5 |
| R2 - F5 | Skin | 8 | neg | neg | NT | neg | 18,2 |
| R2 - F6 | Skin | 8 | neg | neg | NT | neg | 18,4 |
| R2 - F7 | Skin | 8 | neg | neg | NT | neg | 18,3 |
| R2 - F8 | Skin | 8 | neg | neg | NT | neg | 17,9 |
| R2 - F9 | Skin | 8 | neg | neg | NT | neg | 18,3 |
| R2 - F10 | Skin | 8 | neg | neg | NT | neg | 18,9 |
| R2 - F11 | Skin | 8 | neg | neg | NT | neg | 18,1 |
| R2 - F12 | Skin | 8 | 35,3 | neg | NT | neg | 17,8 |
| R2 - F13 | Skin | 8 | 37,4 | neg | NT | neg | 18,4 |
| R2 - F14 | Skin | 8 | neg | neg | NT | neg | 17,9 |
| R2 - F15 | Skin | 8 | neg | neg | NT | neg | 17,8 |
| R2 - F16 | Skin | 8 | neg | neg | NT | neg | 17,6 |
| R2 - F17 | Skin | 8 | neg | neg | NT | neg | 17,3 |
| R2 - F18 | Skin | 8 | neg | neg | NT | neg | 19,7 |
| R2 - F19 | Skin | 8 | neg | neg | NT | neg | 18,0 |
| R2 - F20 | Skin | 8 | neg | neg | NT | neg | 18,6 |
| R2 - F21 | Skin | 8 | neg | neg | NT | neg | 18,7 |
| R2 - F22 | Skin | 8 | neg | neg | NT | neg | 18,0 |
| R2 - F23 | Skin | 8 | neg | neg | NT | neg | 18,3 |
| R2 - F24 | Skin | 8 | neg | neg | NT | neg | 18,1 |
| R2 - F25 | Skin | 8 | 37,7 | neg | NT | neg | 18,0 |
| R2 - F26 | Skin | 8 | 37,7 | neg | NT | neg | 17,5 |
| R2 - F27 | Skin | 8 | 28,9 | neg | NT | neg | 15,7 |
| R2 - F28 | Skin | 8 | neg | neg | NT | neg | 17,5 |
| R2 - F29 | Skin | 8 | neg | neg | NT | neg | 17,4 |
| R2 - F30 | Skin | 8 | 38,2 | neg | NT | neg | 18,1 |
| R2 - F1 | Gill | 8 | neg | 36,1 | NT | neg | 18,0 |
| R2 - F2 | Gill | 8 | neg | 36,2 | NT | neg | 19,7 |
| R2 - F3 | Gill | 8 | neg | neg | NT | neg | 17,7 |
| R2 - F4 | Gill | 8 | neg | neg | NT | neg | 17,8 |
| R2 - F5 | Gill | 8 | neg | neg | NT | neg | 17,9 |
| R2 - F6 | Gill | 8 | neg | neg | NT | neg | 18,6 |
| R2 - F7 | Gill | 8 | neg | neg | NT | neg | 17,4 |
| R2 - F8 | Gill | 8 | neg | neg | NT | neg | 17,5 |
| R2 - F9 | Gill | 8 | neg | neg | NT | neg | 17,7 |
| R2 - F10 | Gill | 8 | neg | neg | NT | neg | 17,4 |
| R2 - F11 | Gill | 8 | 37,4 | neg | NT | neg | 19,6 |
| R2 - F12 | Gill | 8 | 38,0 | 36,3 | NT | neg | 16,5 |
| R2 - F13 | Gill | 8 | neg | neg | NT | neg | 17,8 |
| R2 - F14 | Gill | 8 | neg | 36,4 | NT | neg | 16,7 |
| R2 - F15 | Gill | 8 | neg | neg | NT | neg | 17,2 |
| R2 - F16 | Gill | 8 | neg | neg | NT | neg | 15,5 |
| R2 - F17 | Gill | 8 | 37,1 | neg | NT | neg | 19,4 |
| R2 - F18 | Gill | 8 | 36,8 | neg | NT | neg | 16,6 |
| R2 - F19 | Gill | 8 | neg | neg | NT | neg | 19,5 |
| R2 - F20 | Gill | 8 | neg | neg | NT | neg | 17,7 |
| R2 - F21 | Gill | 8 | neg | neg | NT | neg | 18,1 |
| R2 - F22 | Gill | 8 | neg | neg | NT | neg | 16,6 |
| R2 - F23 | Gill | 8 | neg | neg | NT | neg | 17,5 |
| R2 - F24 | Gill | 8 | neg | 36,4 | NT | neg | 18,8 |
| R2 - F25 | Gill | 8 | 34,7 | neg | NT | neg | 17,7 |
| R2 - F26 | Gill | 8 | 34,5 | neg | NT | neg | 17,4 |
| R2 - F27 | Gill | 8 | neg | neg | NT | neg | 16,5 |
| R2 - F28 | Gill | 8 | 36,6 | 37,1 | NT | neg | 14,2 |
| R2 - F29 | Gill | 8 | neg | neg | NT | neg | 14,1 |
| R2 - F30 | Gill | 8 | neg | neg | NT | neg | 34,2 |

**Table 7.** Fish samples 15 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C3 - F1 | Skin | 15 | neg | neg | NT | neg | 18,1 |
| C3 - F2 | Skin | 15 | neg | neg | NT | neg | 16,8 |
| C3 - F3 | Skin | 15 | neg | neg | NT | neg | 18,1 |
| C3 - F4 | Skin | 15 | neg | neg | NT | neg | 17,0 |
| C3 - F5 | Skin | 15 | neg | neg | NT | neg | 18,7 |
| C3 - F6 | Skin | 15 | neg | neg | NT | neg | 18,9 |
| C3 - F7 | Skin | 15 | neg | neg | NT | neg | 16,9 |
| C3 - F8 | Skin | 15 | neg | neg | NT | neg | 17,6 |
| C3 - F9 | Skin | 15 | neg | neg | NT | neg | 17,6 |
| C3 - F10 | Skin | 15 | neg | neg | NT | neg | 17,6 |
| C3 - F11 | Skin | 15 | neg | neg | NT | neg | 18,4 |
| C3 - F12 | Skin | 15 | neg | neg | NT | neg | 17,8 |
| C3 - F13 | Skin | 15 | neg | neg | NT | neg | 17,8 |
| C3 - F14 | Skin | 15 | neg | neg | NT | neg | 18,9 |
| C3 - F15 | Skin | 15 | 35,8 | neg | NT | neg | 17,9 |
| C3 - F16 | Skin | 15 | neg | neg | NT | neg | 17,4 |
| C3 - F17 | Skin | 15 | 32,5 | neg | NT | neg | 18,1 |
| C3 - F18 | Skin | 15 | neg | neg | NT | neg | 17,5 |
| C3 - F19 | Skin | 15 | neg | neg | NT | neg | 17,0 |
| C3 - F20 | Skin | 15 | 37,8 | neg | NT | neg | 18,1 |
| C3 - F21 | Skin | 15 | neg | neg | NT | neg | 17,7 |
| C3 - F22 | Skin | 15 | neg | neg | NT | neg | 17,4 |
| C3 - F23 | Skin | 15 | neg | neg | NT | neg | 16,3 |
| C3 - F24 | Skin | 15 | neg | neg | NT | neg | 18,5 |
| C3 - F25 | Skin | 15 | neg | neg | NT | neg | 17,7 |
| C3 - F26 | Skin | 15 | neg | neg | NT | neg | 16,3 |
| C3 - F27 | Skin | 15 | neg | neg | NT | neg | 18,8 |
| C3 - F28 | Skin | 15 | neg | neg | NT | neg | 18,0 |
| C3 - F29 | Skin | 15 | neg | neg | NT | neg | 17,4 |
| C3 - F30 | Skin | 15 | neg | neg | NT | neg | 17,3 |
| C3 - F1 | Gill | 15 | neg | 36,3 | NT | neg | 22,5 |
| C3 - F2 | Gill | 15 | neg | neg | NT | neg | 15,7 |
| C3 - F3 | Gill | 15 | neg | neg | NT | neg | 14,7 |
| C3 - F4 | Gill | 15 | neg | neg | NT | neg | 14,4 |
| C3 - F5 | Gill | 15 | 35,7 | neg | NT | neg | 14,3 |
| C3 - F6 | Gill | 15 | neg | neg | NT | neg | 15,5 |
| C3 - F7 | Gill | 15 | neg | neg | NT | neg | 15,8 |
| C3 - F8 | Gill | 15 | neg | neg | NT | neg | 15,9 |
| C3 - F9 | Gill | 15 | neg | neg | NT | neg | 13,8 |
| C3 - F10 | Gill | 15 | neg | neg | NT | neg | 14,7 |
| C3 - F11 | Gill | 15 | neg | neg | NT | neg | 14,0 |
| C3 - F12 | Gill | 15 | neg | neg | NT | neg | 14,2 |
| C3 - F13 | Gill | 15 | neg | neg | NT | neg | 14,1 |
| C3 - F14 | Gill | 15 | neg | neg | NT | neg | 13,5 |
| C3 - F15 | Gill | 15 | neg | neg | NT | neg | 14,1 |
| C3 - F16 | Gill | 15 | neg | neg | NT | neg | 14,2 |
| C3 - F17 | Gill | 15 | 37,2 | 36,4 | NT | neg | 14,6 |
| C3 - F18 | Gill | 15 | neg | neg | NT | neg | 13,7 |
| C3 - F19 | Gill | 15 | neg | neg | NT | neg | 15,9 |
| C3 - F20 | Gill | 15 | neg | neg | NT | neg | 16,5 |
| C3 - F21 | Gill | 15 | neg | neg | NT | neg | 16,0 |
| C3 - F22 | Gill | 15 | neg | neg | NT | neg | 15,1 |
| C3 - F23 | Gill | 15 | neg | neg | NT | neg | 14,8 |
| C3 - F24 | Gill | 15 | neg | 36,4 | NT | neg | 14,5 |
| C3 - F25 | Gill | 15 | neg | neg | NT | neg | 15,7 |
| C3 - F26 | Gill | 15 | neg | neg | NT | neg | 14,9 |
| C3 - F27 | Gill | 15 | neg | neg | NT | neg | 13,6 |
| C3 - F28 | Gill | 15 | neg | neg | NT | neg | 14,8 |
| C3 - F29 | Gill | 15 | neg | neg | NT | neg | 15,4 |
| C3 - F30 | Gill | 15 | neg | neg | NT | neg | 13,8 |

**Table 8.** Fish samples 15 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R3 - F1 | Skin | 15 | neg | neg | NT | neg | 19,1 |
| R3 - F2 | Skin | 15 | neg | neg | NT | neg | 18,4 |
| R3 - F3 | Skin | 15 | neg | neg | NT | neg | 19,5 |
| R3 - F4 | Skin | 15 | neg | neg | NT | neg | 18,6 |
| R3 - F5 | Skin | 15 | neg | neg | NT | neg | 18,8 |
| R3 - F6 | Skin | 15 | neg | neg | NT | neg | 18,2 |
| R3 - F7 | Skin | 15 | neg | neg | NT | neg | 18,0 |
| R3 - F8 | Skin | 15 | neg | neg | NT | neg | 17,6 |
| R3 - F9 | Skin | 15 | neg | neg | NT | neg | 17,3 |
| R3 - F10 | Skin | 15 | neg | neg | NT | neg | 16,9 |
| R3 - F11 | Skin | 15 | neg | neg | NT | neg | 17,8 |
| R3 - F12 | Skin | 15 | neg | neg | NT | neg | 17,6 |
| R3 - F13 | Skin | 15 | neg | neg | NT | neg | 18,0 |
| R3 - F14 | Skin | 15 | neg | neg | NT | neg | 18,2 |
| R3 - F15 | Skin | 15 | neg | neg | NT | neg | 17,6 |
| R3 - F16 | Skin | 15 | neg | neg | NT | neg | 17,3 |
| R3 - F17 | Skin | 15 | neg | neg | NT | neg | 17,8 |
| R3 - F18 | Skin | 15 | neg | neg | NT | neg | 18,4 |
| R3 - F19 | Skin | 15 | neg | neg | NT | neg | 18,2 |
| R3 - F20 | Skin | 15 | neg | neg | NT | neg | 17,5 |
| R3 - F21 | Skin | 15 | neg | neg | NT | neg | 18,5 |
| R3 - F22 | Skin | 15 | neg | neg | NT | neg | 17,1 |
| R3 - F23 | Skin | 15 | neg | neg | NT | neg | 18,1 |
| R3 - F24 | Skin | 15 | neg | neg | NT | neg | 18,8 |
| R3 - F25 | Skin | 15 | neg | neg | NT | neg | 17,7 |
| R3 - F26 | Skin | 15 | neg | neg | NT | neg | 18,7 |
| R3 - F27 | Skin | 15 | neg | neg | NT | neg | 17,5 |
| R3 - F28 | Skin | 15 | neg | neg | NT | neg | 18,2 |
| R3 - F29 | Skin | 15 | neg | neg | NT | neg | 17,5 |
| R3 - F30 | Skin | 15 | neg | neg | NT | neg | 18,2 |
| R3 - F1 | Gill | 15 | neg | neg | NT | neg | 16,4 |
| R3 - F2 | Gill | 15 | neg | neg | NT | neg | 16,5 |
| R3 - F3 | Gill | 15 | neg | neg | NT | neg | 16,3 |
| R3 - F4 | Gill | 15 | neg | neg | NT | neg | 15,9 |
| R3 - F5 | Gill | 15 | neg | neg | NT | neg | 17,0 |
| R3 - F6 | Gill | 15 | neg | neg | NT | neg | 15,1 |
| R3 - F7 | Gill | 15 | neg | neg | NT | neg | 16,7 |
| R3 - F8 | Gill | 15 | neg | neg | NT | neg | 15,9 |
| R3 - F9 | Gill | 15 | neg | neg | NT | neg | 16,4 |
| R3 - F10 | Gill | 15 | neg | neg | NT | neg | 15,4 |
| R3 - F11 | Gill | 15 | neg | neg | NT | neg | 15,9 |
| R3 - F12 | Gill | 15 | neg | neg | NT | neg | 15,7 |
| R3 - F13 | Gill | 15 | neg | neg | NT | neg | 16,0 |
| R3 - F14 | Gill | 15 | neg | neg | NT | neg | 14,7 |
| R3 - F15 | Gill | 15 | neg | neg | NT | neg | 14,9 |
| R3 - F16 | Gill | 15 | neg | neg | NT | neg | 15,9 |
| R3 - F17 | Gill | 15 | neg | neg | NT | neg | 16,2 |
| R3 - F18 | Gill | 15 | neg | neg | NT | neg | 16,3 |
| R3 - F19 | Gill | 15 | neg | neg | NT | neg | 15,9 |
| R3 - F20 | Gill | 15 | neg | neg | NT | neg | 14,9 |
| R3 - F21 | Gill | 15 | neg | neg | NT | neg | 14,0 |
| R3 - F22 | Gill | 15 | neg | neg | NT | neg | 15,7 |
| R3 - F23 | Gill | 15 | neg | neg | NT | neg | 15,3 |
| R3 - F24 | Gill | 15 | neg | neg | NT | neg | 15,5 |
| R3 - F25 | Gill | 15 | neg | neg | NT | neg | 15,6 |
| R3 - F26 | Gill | 15 | neg | neg | NT | neg | 15,0 |
| R3 - F27 | Gill | 15 | neg | neg | NT | neg | 14,8 |
| R3 - F28 | Gill | 15 | 36,4 | neg | NT | neg | 14,0 |
| R3 - F29 | Gill | 15 | neg | neg | NT | neg | 14,3 |
| R3 - F30 | Gill | 15 | 36,4 | neg | NT | neg | 15,6 |

**Table 9.** Fish samples 24 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C4 - F1 | Skin | 24 | neg | neg | NT | neg | 16,4 |
| C4 - F2 | Skin | 24 | neg | neg | NT | neg | 16,6 |
| C4 - F3 | Skin | 24 | neg | neg | NT | neg | 16,3 |
| C4 - F4 | Skin | 24 | neg | neg | NT | neg | 16,9 |
| C4 - F5 | Skin | 24 | neg | neg | NT | neg | 16,5 |
| C4 - F6 | Skin | 24 | neg | neg | NT | neg | 16,5 |
| C4 - F7 | Skin | 24 | 37,0 | neg | NT | neg | 16,2 |
| C4 - F8 | Skin | 24 | neg | neg | NT | neg | 16,3 |
| C4 - F9 | Skin | 24 | neg | neg | NT | neg | 17,0 |
| C4 - F10 | Skin | 24 | 36,9 | neg | NT | neg | 16,3 |
| C4 - F11 | Skin | 24 | neg | neg | NT | neg | 16,4 |
| C4 - F12 | Skin | 24 | neg | neg | NT | neg | 16,9 |
| C4 - F13 | Skin | 24 | neg | neg | NT | neg | 17,1 |
| C4 - F14 | Skin | 24 | neg | neg | NT | neg | 16,8 |
| C4 - F15 | Skin | 24 | neg | neg | NT | neg | 16,6 |
| C4 - F16 | Skin | 24 | neg | neg | NT | neg | 16,7 |
| C4 - F17 | Skin | 24 | neg | neg | NT | neg | 17,0 |
| C4 - F18 | Skin | 24 | neg | neg | NT | neg | 16,5 |
| C4 - F19 | Skin | 24 | neg | neg | NT | neg | 15,9 |
| C4 - F20 | Skin | 24 | 39,3 | neg | NT | neg | 16,3 |
| C4 - F21 | Skin | 24 | neg | neg | NT | neg | neg |
| C4 - F22 | Skin | 24 | neg | neg | NT | neg | 16,3 |
| C4 - F23 | Skin | 24 | neg | neg | NT | neg | 16,5 |
| C4 - F24 | Skin | 24 | neg | neg | NT | neg | 17,7 |
| C4 - F25 | Skin | 24 | neg | neg | NT | neg | 16,9 |
| C4 - F26 | Skin | 24 | neg | neg | NT | neg | 16,8 |
| C4 - F27 | Skin | 24 | 37,3 | neg | NT | neg | 17,1 |
| C4 - F28 | Skin | 24 | neg | neg | NT | neg | 16,6 |
| C4 - F29 | Skin | 24 | neg | neg | NT | neg | 16,9 |
| C4 - F30 | Skin | 24 | neg | neg | NT | neg | 16,4 |
| C4 - F1 | Gill | 24 | neg | neg | NT | neg | 13,1 |
| C4 - F2 | Gill | 24 | neg | neg | NT | neg | 13,6 |
| C4 - F3 | Gill | 24 | neg | neg | NT | neg | 14,0 |
| C4 - F4 | Gill | 24 | neg | neg | NT | neg | 13,5 |
| C4 - F5 | Gill | 24 | neg | neg | NT | neg | 14,0 |
| C4 - F6 | Gill | 24 | neg | neg | NT | neg | 13,6 |
| C4 - F7 | Gill | 24 | neg | neg | NT | neg | 13,6 |
| C4 - F8 | Gill | 24 | neg | neg | NT | neg | 13,7 |
| C4 - F9 | Gill | 24 | 37,5 | neg | NT | neg | 13,2 |
| C4 - F10 | Gill | 24 | neg | neg | NT | neg | 13,3 |
| C4 - F11 | Gill | 24 | neg | neg | NT | neg | 13,1 |
| C4 - F12 | Gill | 24 | neg | neg | NT | neg | 13,4 |
| C4 - F13 | Gill | 24 | neg | neg | NT | neg | 13,3 |
| C4 - F14 | Gill | 24 | neg | neg | NT | neg | 12,8 |
| C4 - F15 | Gill | 24 | neg | neg | NT | neg | 12,8 |
| C4 - F16 | Gill | 24 | 36,9 | neg | NT | neg | 14,1 |
| C4 - F17 | Gill | 24 | neg | neg | NT | neg | 13,1 |
| C4 - F18 | Gill | 24 | neg | neg | NT | neg | 12,1 |
| C4 - F19 | Gill | 24 | neg | neg | NT | neg | 12,8 |
| C4 - F20 | Gill | 24 | neg | neg | NT | neg | 12,9 |
| C4 - F21 | Gill | 24 | neg | neg | NT | neg | 13,1 |
| C4 - F22 | Gill | 24 | neg | neg | NT | neg | 13,2 |
| C4 - F23 | Gill | 24 | neg | neg | NT | neg | 14,2 |
| C4 - F24 | Gill | 24 | neg | neg | NT | neg | 13,9 |
| C4 - F25 | Gill | 24 | neg | neg | NT | neg | 13,2 |
| C4 - F26 | Gill | 24 | neg | neg | NT | neg | 14,9 |
| C4 - F27 | Gill | 24 | neg | neg | NT | neg | 14,4 |
| C4 - F28 | Gill | 24 | neg | neg | NT | neg | 13,5 |
| C4 - F29 | Gill | 24 | neg | neg | NT | neg | 14,4 |
| C4 - F30 | Gill | 24 | neg | neg | NT | neg | 13,9 |

**Table 10.** Fish samples 24 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R4 - F1 | Skin | 24 | neg | 38,6 | NT | neg | 16,5 |
| R4 - F2 | Skin | 24 | neg | neg | NT | neg | 16,6 |
| R4 - F3 | Skin | 24 | neg | neg | NT | neg | 16,8 |
| R4 - F4 | Skin | 24 | neg | neg | NT | neg | 17,4 |
| R4 - F5 | Skin | 24 | neg | neg | NT | neg | 17,0 |
| R4 - F6 | Skin | 24 | neg | neg | NT | neg | 17,5 |
| R4 - F7 | Skin | 24 | neg | neg | NT | neg | 16,9 |
| R4 - F8 | Skin | 24 | neg | neg | NT | neg | 17,3 |
| R4 - F9 | Skin | 24 | neg | 36,7 | NT | neg | 17,1 |
| R4 - F10 | Skin | 24 | neg | neg | NT | neg | 16,4 |
| R4 - F11 | Skin | 24 | neg | neg | NT | neg | 17,4 |
| R4 - F12 | Skin | 24 | neg | neg | NT | neg | 18,3 |
| R4 - F13 | Skin | 24 | neg | neg | NT | neg | 17,3 |
| R4 - F14 | Skin | 24 | neg | neg | NT | neg | 16,9 |
| R4 - F15 | Skin | 24 | neg | neg | NT | neg | 17,7 |
| R4 - F16 | Skin | 24 | neg | neg | NT | neg | 16,8 |
| R4 - F17 | Skin | 24 | neg | neg | NT | neg | 16,6 |
| R4 - F18 | Skin | 24 | neg | neg | NT | neg | 17,2 |
| R4 - F19 | Skin | 24 | neg | neg | NT | neg | 17,0 |
| R4 - F20 | Skin | 24 | neg | neg | NT | neg | 17,5 |
| R4 - F21 | Skin | 24 | neg | neg | NT | neg | 17,6 |
| R4 - F22 | Skin | 24 | neg | neg | NT | neg | 16,6 |
| R4 - F23 | Skin | 24 | neg | neg | NT | neg | 16,8 |
| R4 - F24 | Skin | 24 | neg | neg | NT | neg | 17,7 |
| R4 - F25 | Skin | 24 | neg | neg | NT | neg | 17,4 |
| R4 - F26 | Skin | 24 | neg | neg | NT | neg | 17,5 |
| R4 - F27 | Skin | 24 | neg | neg | NT | neg | 17,3 |
| R4 - F28 | Skin | 24 | neg | neg | NT | neg | 17,0 |
| R4 - F29 | Skin | 24 | neg | neg | NT | neg | 16,7 |
| R4 - F30 | Skin | 24 | neg | neg | NT | neg | 17,3 |
| R4 - F1 | Gill | 24 | neg | 35,2 | NT | neg | 13,2 |
| R4 - F2 | Gill | 24 | neg | neg | NT | neg | 13,6 |
| R4 - F3 | Gill | 24 | neg | neg | NT | neg | 13,8 |
| R4 - F4 | Gill | 24 | neg | neg | NT | neg | 14,3 |
| R4 - F5 | Gill | 24 | neg | neg | NT | neg | 13,8 |
| R4 - F6 | Gill | 24 | neg | neg | NT | neg | 14,4 |
| R4 - F7 | Gill | 24 | neg | neg | NT | neg | 13,1 |
| R4 - F8 | Gill | 24 | neg | 36,7 | NT | neg | 13,7 |
| R4 - F9 | Gill | 24 | neg | neg | NT | neg | 13,8 |
| R4 - F10 | Gill | 24 | neg | neg | NT | neg | 13,7 |
| R4 - F11 | Gill | 24 | neg | neg | NT | neg | 13,8 |
| R4 - F12 | Gill | 24 | neg | neg | NT | neg | 14,2 |
| R4 - F13 | Gill | 24 | neg | neg | NT | neg | 12,9 |
| R4 - F14 | Gill | 24 | neg | 36,3 | NT | neg | 13,4 |
| R4 - F15 | Gill | 24 | neg | 36,5 | NT | neg | 13,7 |
| R4 - F16 | Gill | 24 | neg | neg | NT | neg | 13,5 |
| R4 - F17 | Gill | 24 | neg | 35,4 | NT | neg | 13,3 |
| R4 - F18 | Gill | 24 | neg | 36,6 | NT | neg | 13,3 |
| R4 - F19 | Gill | 24 | neg | 35,2 | NT | neg | 13,8 |
| R4 - F20 | Gill | 24 | neg | 36,0 | NT | neg | 13,4 |
| R4 - F21 | Gill | 24 | neg | neg | NT | neg | 13,5 |
| R4 - F22 | Gill | 24 | 36,6 | neg | NT | neg | 13,5 |
| R4 - F23 | Gill | 24 | neg | 35,3 | NT | neg | 13,7 |
| R4 - F24 | Gill | 24 | neg | neg | NT | neg | 13,7 |
| R4 - F25 | Gill | 24 | neg | 36,2 | NT | neg | 13,1 |
| R4 - F26 | Gill | 24 | neg | neg | NT | neg | 12,3 |
| R4 - F27 | Gill | 24 | neg | neg | NT | neg | 13,5 |
| R4 - F28 | Gill | 24 | neg | neg | NT | neg | 13,5 |
| R4 - F29 | Gill | 24 | neg | neg | NT | neg | 13,3 |
| R4 - F30 | Gill | 24 | neg | 36,0 | NT | neg | 13,7 |

**Table 11.** Fish samples 29 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C5 - F1 | Skin | 29 | neg | neg | NT | neg | 17,3 |
| C5 - F2 | Skin | 29 | neg | neg | NT | neg | 16,6 |
| C5 - F3 | Skin | 29 | neg | neg | NT | neg | 16,9 |
| C5 - F4 | Skin | 29 | neg | neg | NT | neg | 16,5 |
| C5 - F5 | Skin | 29 | neg | neg | NT | neg | 17,8 |
| C5 - F6 | Skin | 29 | neg | neg | NT | neg | 16,7 |
| C5 - F7 | Skin | 29 | neg | neg | NT | neg | 16,6 |
| C5 - F8 | Skin | 29 | neg | neg | NT | neg | 17,3 |
| C5 - F9 | Skin | 29 | neg | neg | NT | neg | 16,8 |
| C5 - F10 | Skin | 29 | 37,3 | neg | NT | neg | 16,4 |
| C5 - F11 | Skin | 29 | neg | neg | NT | neg | 16,8 |
| C5 - F12 | Skin | 29 | neg | neg | NT | neg | 16,8 |
| C5 - F13 | Skin | 29 | neg | neg | NT | neg | 17,3 |
| C5 - F14 | Skin | 29 | neg | neg | NT | neg | 18,3 |
| C5 - F15 | Skin | 29 | neg | neg | NT | neg | 18,5 |
| C5 - F16 | Skin | 29 | neg | neg | NT | neg | 17,4 |
| C5 - F17 | Skin | 29 | neg | neg | NT | neg | 17,6 |
| C5 - F18 | Skin | 29 | neg | neg | NT | neg | 17,1 |
| C5 - F19 | Skin | 29 | neg | neg | NT | neg | 15,9 |
| C5 - F20 | Skin | 29 | neg | neg | NT | neg | 19,7 |
| C5 - F21 | Skin | 29 | neg | neg | NT | neg | 18,4 |
| C5 - F22 | Skin | 29 | neg | neg | NT | neg | 17,6 |
| C5 - F23 | Skin | 29 | neg | neg | NT | neg | 18,6 |
| C5 - F24 | Skin | 29 | neg | neg | NT | neg | 18,6 |
| C5 - F25 | Skin | 29 | neg | neg | NT | neg | 18,2 |
| C5 - F26 | Skin | 29 | neg | neg | NT | neg | 18,1 |
| C5 - F27 | Skin | 29 | neg | neg | NT | neg | 19,4 |
| C5 - F28 | Skin | 29 | neg | neg | NT | neg | 17,7 |
| C5 - F29 | Skin | 29 | neg | neg | NT | neg | 17,9 |
| C5 - F30 | Skin | 29 | neg | neg | NT | neg | 20,1 |
| C5 - F1 | Gill | 29 | neg | neg | NT | neg | 13,9 |
| C5 - F2 | Gill | 29 | neg | neg | NT | neg | 13,6 |
| C5 - F3 | Gill | 29 | neg | neg | NT | neg | 12,8 |
| C5 - F4 | Gill | 29 | neg | neg | NT | neg | 12,6 |
| C5 - F5 | Gill | 29 | neg | neg | NT | neg | 13,4 |
| C5 - F6 | Gill | 29 | neg | neg | NT | neg | 12,9 |
| C5 - F7 | Gill | 29 | neg | neg | NT | neg | 12,5 |
| C5 - F8 | Gill | 29 | neg | neg | NT | neg | 13,2 |
| C5 - F9 | Gill | 29 | neg | neg | NT | neg | 14,5 |
| C5 - F10 | Gill | 29 | neg | neg | NT | neg | 13,5 |
| C5 - F11 | Gill | 29 | neg | neg | NT | neg | 13,9 |
| C5 - F12 | Gill | 29 | neg | neg | NT | neg | 13,8 |
| C5 - F13 | Gill | 29 | neg | neg | NT | neg | 12,9 |
| C5 - F14 | Gill | 29 | neg | neg | NT | neg | 14,9 |
| C5 - F15 | Gill | 29 | neg | neg | NT | neg | 13,5 |
| C5 - F16 | Gill | 29 | neg | neg | NT | neg | 13,4 |
| C5 - F17 | Gill | 29 | neg | neg | NT | neg | 13,2 |
| C5 - F18 | Gill | 29 | neg | 36,1 | NT | neg | 13,4 |
| C5 - F19 | Gill | 29 | neg | neg | NT | neg | 14,5 |
| C5 - F20 | Gill | 29 | neg | neg | NT | neg | 14,4 |
| C5 - F21 | Gill | 29 | neg | neg | NT | neg | 13,6 |
| C5 - F22 | Gill | 29 | neg | neg | NT | neg | 13,9 |
| C5 - F23 | Gill | 29 | 36,9 | neg | NT | neg | 13,8 |
| C5 - F24 | Gill | 29 | neg | neg | NT | neg | 14,2 |
| C5 - F25 | Gill | 29 | neg | neg | NT | neg | 13,5 |
| C5 - F26 | Gill | 29 | neg | neg | NT | neg | 14,3 |
| C5 - F27 | Gill | 29 | neg | neg | NT | neg | 13,7 |
| C5 - F28 | Gill | 29 | neg | neg | NT | neg | 13,3 |
| C5 - F29 | Gill | 29 | neg | neg | NT | neg | 13,5 |
| C5 - F30 | Gill | 29 | neg | neg | NT | neg | 13,1 |

**Table 12.** Fish samples 29 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R5 - F1 | Skin | 29 | neg | neg | NT | neg | 18,8 |
| R5 - F2 | Skin | 29 | neg | neg | NT | neg | 19,3 |
| R5 - F3 | Skin | 29 | neg | neg | NT | neg | 19,1 |
| R5 - F4 | Skin | 29 | neg | neg | NT | neg | 18,3 |
| R5 - F5 | Skin | 29 | 37,7 | neg | NT | neg | 18,9 |
| R5 - F6 | Skin | 29 | neg | neg | NT | neg | 19,3 |
| R5 - F7 | Skin | 29 | neg | neg | NT | neg | 18,3 |
| R5 - F8 | Skin | 29 | neg | neg | NT | neg | 19,1 |
| R5 - F9 | Skin | 29 | neg | neg | NT | neg | 20,0 |
| R5 - F10 | Skin | 29 | neg | neg | NT | neg | 19,9 |
| R5 - F11 | Skin | 29 | neg | neg | NT | neg | 18,9 |
| R5 - F12 | Skin | 29 | neg | neg | NT | neg | 18,6 |
| R5 - F13 | Skin | 29 | neg | neg | NT | neg | 18,2 |
| R5 - F14 | Skin | 29 | neg | neg | NT | neg | 19,2 |
| R5 - F15 | Skin | 29 | neg | neg | NT | neg | 19,8 |
| R5 - F16 | Skin | 29 | neg | neg | NT | neg | 18,7 |
| R5 - F17 | Skin | 29 | neg | neg | NT | neg | 18,9 |
| R5 - F18 | Skin | 29 | neg | neg | NT | neg | 18,3 |
| R5 - F19 | Skin | 29 | neg | neg | NT | neg | 19,3 |
| R5 - F20 | Skin | 29 | neg | neg | NT | neg | 20,2 |
| R5 - F21 | Skin | 29 | neg | neg | NT | neg | 19,8 |
| R5 - F22 | Skin | 29 | neg | neg | NT | neg | 29,1 |
| R5 - F23 | Skin | 29 | neg | neg | NT | neg | 19,0 |
| R5 - F24 | Skin | 29 | neg | neg | NT | neg | 18,6 |
| R5 - F25 | Skin | 29 | neg | neg | NT | neg | 19,2 |
| R5 - F1 | Gill | 29 | neg | neg | NT | neg | 15,1 |
| R5 - F2 | Gill | 29 | neg | neg | NT | neg | 14,1 |
| R5 - F3 | Gill | 29 | neg | neg | NT | neg | 14,2 |
| R5 - F4 | Gill | 29 | neg | neg | NT | neg | 14,5 |
| R5 - F5 | Gill | 29 | neg | neg | NT | neg | 15,2 |
| R5 - F6 | Gill | 29 | neg | neg | NT | neg | 14,9 |
| R5 - F7 | Gill | 29 | neg | neg | NT | neg | neg |
| R5 - F8 | Gill | 29 | neg | neg | NT | neg | 14,1 |
| R5 - F9 | Gill | 29 | neg | neg | NT | neg | 30,3 |
| R5 - F10 | Gill | 29 | neg | neg | NT | neg | 34,9 |
| R5 - F11 | Gill | 29 | neg | neg | NT | neg | 13,5 |
| R5 - F12 | Gill | 29 | neg | neg | NT | neg | neg |
| R5 - F13 | Gill | 29 | neg | neg | NT | neg | 18,8 |
| R5 - F14 | Gill | 29 | neg | 36,2 | NT | neg | 13,5 |
| R5 - F15 | Gill | 29 | neg | neg | NT | neg | 13,5 |
| R5 - F16 | Gill | 29 | neg | neg | NT | neg | 15,2 |
| R5 - F17 | Gill | 29 | neg | neg | NT | neg | 20,0 |
| R5 - F18 | Gill | 29 | neg | neg | NT | neg | 14,3 |
| R5 - F19 | Gill | 29 | neg | neg | NT | neg | 14,8 |
| R5 - F20 | Gill | 29 | neg | neg | NT | neg | 14,7 |
| R5 - F21 | Gill | 29 | 37,2 | neg | NT | neg | 14,8 |
| R5 - F22 | Gill | 29 | neg | neg | NT | neg | 14,2 |
| R5 - F23 | Gill | 29 | neg | neg | NT | neg | 15,0 |
| R5 - F24 | Gill | 29 | neg | neg | NT | neg | 14,6 |
| R5 - F25 | Gill | 29 | 36,6 | 33,2 | NT | neg | 14,7 |

**Table 13.** Fish samples 55 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C6 - F1 | Skin | 55 | neg | neg | NT | neg | 19,0 |
| C6 - F2 | Skin | 55 | neg | neg | NT | neg | 20,0 |
| C6 - F3 | Skin | 55 | neg | neg | NT | neg | 20,8 |
| C6 - F4 | Skin | 55 | neg | neg | NT | neg | 18,7 |
| C6 - F5 | Skin | 55 | neg | neg | NT | neg | 18,4 |
| C6 - F6 | Skin | 55 | neg | neg | NT | neg | 17,2 |
| C6 - F7 | Skin | 55 | neg | neg | NT | neg | 17,9 |
| C6 - F8 | Skin | 55 | neg | neg | NT | neg | 20,0 |
| C6 - F9 | Skin | 55 | neg | neg | NT | neg | 18,4 |
| C6 - F10 | Skin | 55 | neg | neg | NT | neg | 17,4 |
| C6 - F11 | Skin | 55 | neg | neg | NT | neg | 18,1 |
| C6 - F12 | Skin | 55 | neg | neg | NT | neg | 17,4 |
| C6 - F13 | Skin | 55 | neg | neg | NT | neg | 17,0 |
| C6 - F14 | Skin | 55 | neg | neg | NT | neg | 17,8 |
| C6 - F15 | Skin | 55 | neg | neg | NT | neg | 20,6 |
| C6 - F16 | Skin | 55 | neg | neg | NT | neg | 16,7 |
| C6 - F17 | Skin | 55 | neg | neg | NT | neg | 16,9 |
| C6 - F18 | Skin | 55 | neg | neg | NT | neg | 17,5 |
| C6 - F19 | Skin | 55 | neg | neg | NT | neg | 16,9 |
| C6 - F20 | Skin | 55 | neg | neg | NT | neg | 17,1 |
| C6 - F21 | Skin | 55 | neg | neg | NT | neg | 18,3 |
| C6 - F22 | Skin | 55 | neg | neg | NT | neg | 19,7 |
| C6 - F23 | Skin | 55 | neg | neg | NT | neg | 16,3 |
| C6 - F24 | Skin | 55 | neg | neg | NT | neg | 17,7 |
| C6 - F25 | Skin | 55 | neg | neg | NT | neg | 18,5 |
| C6 - F26 | Skin | 55 | neg | neg | NT | neg | 16,8 |
| C6 - F27 | Skin | 55 | neg | neg | NT | neg | 16,9 |
| C6 - F28 | Skin | 55 | neg | neg | NT | neg | 18,9 |
| C6 - F29 | Skin | 55 | neg | neg | NT | neg | 17,8 |
| C6 - F30 | Skin | 55 | neg | neg | NT | neg | 17,5 |
| C6 - F1 | Gill | 55 | neg | neg | NT | neg | 14,6 |
| C6 - F2 | Gill | 55 | neg | neg | NT | neg | 14,2 |
| C6 - F3 | Gill | 55 | neg | neg | NT | neg | 15,0 |
| C6 - F4 | Gill | 55 | neg | neg | NT | neg | 15,1 |
| C6 - F5 | Gill | 55 | neg | neg | NT | neg | 14,6 |
| C6 - F6 | Gill | 55 | 30,8 | neg | NT | neg | 15,2 |
| C6 - F7 | Gill | 55 | neg | neg | NT | neg | 14,2 |
| C6 - F8 | Gill | 55 | neg | neg | NT | neg | 14,8 |
| C6 - F9 | Gill | 55 | neg | neg | NT | neg | 14,7 |
| C6 - F10 | Gill | 55 | neg | neg | NT | neg | 13,8 |
| C6 - F11 | Gill | 55 | neg | neg | NT | neg | 14,6 |
| C6 - F12 | Gill | 55 | neg | neg | NT | neg | 13,8 |
| C6 - F13 | Gill | 55 | neg | neg | NT | neg | 13,9 |
| C6 - F14 | Gill | 55 | neg | neg | NT | neg | 14,1 |
| C6 - F15 | Gill | 55 | neg | neg | NT | neg | neg |
| C6 - F16 | Gill | 55 | neg | neg | NT | neg | 18,5 |
| C6 - F17 | Gill | 55 | neg | neg | NT | neg | neg |
| C6 - F18 | Gill | 55 | neg | neg | NT | neg | 20,4 |
| C6 - F19 | Gill | 55 | neg | neg | NT | neg | neg |
| C6 - F20 | Gill | 55 | neg | neg | NT | neg | 23,1 |
| C6 - F21 | Gill | 55 | neg | neg | NT | neg | 15,2 |
| C6 - F22 | Gill | 55 | neg | neg | NT | neg | 23,5 |
| C6 - F23 | Gill | 55 | neg | neg | NT | neg | 16,1 |
| C6 - F24 | Gill | 55 | neg | neg | NT | neg | 18,2 |
| C6 - F25 | Gill | 55 | neg | neg | NT | neg | 14,7 |
| C6 - F26 | Gill | 55 | neg | neg | NT | neg | 13,8 |
| C6 - F27 | Gill | 55 | neg | neg | NT | neg | 14,3 |
| C6 - F28 | Gill | 55 | neg | neg | NT | neg | 14,2 |
| C6 - F29 | Gill | 55 | neg | neg | NT | neg | 19,2 |
| C6 - F30 | Gill | 55 | neg | neg | NT | neg | 15,1 |

**Table 14.** Fish samples 56 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R6 - F1 | Skin | 56 | neg | neg | NT | neg | 20,6 |
| R6 - F2 | Skin | 56 | neg | neg | NT | neg | 19,3 |
| R6 - F3 | Skin | 56 | neg | neg | NT | neg | 19,9 |
| R6 - F4 | Skin | 56 | neg | neg | NT | neg | 19,1 |
| R6 - F5 | Skin | 56 | neg | neg | NT | neg | 21,7 |
| R6 - F6 | Skin | 56 | neg | neg | NT | neg | 18,7 |
| R6 - F7 | Skin | 56 | neg | neg | NT | neg | 18,2 |
| R6 - F8 | Skin | 56 | neg | neg | NT | neg | 20,3 |
| R6 - F9 | Skin | 56 | neg | neg | NT | neg | 19,3 |
| R6 - F10 | Skin | 56 | neg | neg | NT | neg | 20,1 |
| R6 - F11 | Skin | 56 | neg | neg | NT | neg | 19,9 |
| R6 - F12 | Skin | 56 | neg | neg | NT | neg | 18,7 |
| R6 - F13 | Skin | 56 | neg | neg | NT | neg | 19,4 |
| R6 - F14 | Skin | 56 | neg | neg | NT | neg | 19,8 |
| R6 - F15 | Skin | 56 | neg | neg | NT | neg | 20,2 |
| R6 - F16 | Skin | 56 | neg | neg | NT | neg | 20,1 |
| R6 - F17 | Skin | 56 | neg | neg | NT | neg | 19,2 |
| R6 - F18 | Skin | 56 | neg | neg | NT | neg | 19,2 |
| R6 - F19 | Skin | 56 | neg | neg | NT | neg | 20,0 |
| R6 - F20 | Skin | 56 | neg | neg | NT | neg | 20,4 |
| R6 - F21 | Skin | 56 | neg | neg | NT | neg | 17,9 |
| R6 - F22 | Skin | 56 | neg | neg | NT | neg | 19,1 |
| R6 - F23 | Skin | 56 | neg | neg | NT | neg | 19,0 |
| R6 - F24 | Skin | 56 | neg | neg | NT | neg | 18,4 |
| R6 - F25 | Skin | 56 | neg | neg | NT | neg | 19,0 |
| R6 - F26 | Skin | 56 | neg | neg | NT | neg | 19,8 |
| R6 - F27 | Skin | 56 | neg | neg | NT | neg | 18,7 |
| R6 - F28 | Skin | 56 | neg | neg | NT | neg | 20,5 |
| R6 - F29 | Skin | 56 | neg | neg | NT | neg | 18,6 |
| R6 - F30 | Skin | 56 | neg | neg | NT | neg | 18,9 |
| R6 - F1 | Gill | 56 | neg | neg | NT | neg | 15,7 |
| R6 - F2 | Gill | 56 | neg | neg | NT | neg | 15,6 |
| R6 - F3 | Gill | 56 | neg | neg | NT | neg | 15,8 |
| R6 - F4 | Gill | 56 | neg | 36,3 | NT | neg | 15,5 |
| R6 - F5 | Gill | 56 | 37,0 | neg | NT | neg | 16,2 |
| R6 - F6 | Gill | 56 | neg | neg | NT | neg | 15,2 |
| R6 - F7 | Gill | 56 | neg | neg | NT | neg | 14,7 |
| R6 - F8 | Gill | 56 | neg | neg | NT | neg | 15,2 |
| R6 - F9 | Gill | 56 | neg | neg | NT | neg | 15,9 |
| R6 - F10 | Gill | 56 | neg | neg | NT | neg | 17,1 |
| R6 - F11 | Gill | 56 | neg | neg | NT | neg | 15,5 |
| R6 - F12 | Gill | 56 | neg | neg | NT | neg | 15,5 |
| R6 - F13 | Gill | 56 | neg | neg | NT | neg | 15,5 |
| R6 - F14 | Gill | 56 | neg | neg | NT | neg | 16,0 |
| R6 - F15 | Gill | 56 | neg | neg | NT | neg | 16,2 |
| R6 - F16 | Gill | 56 | neg | neg | NT | neg | 15,4 |
| R6 - F17 | Gill | 56 | 27,3 | 22,7 | NT | neg | 15,3 |
| R6 - F18 | Gill | 56 | neg | 36,2 | NT | neg | 16,4 |
| R6 - F19 | Gill | 56 | neg | neg | NT | neg | 15,6 |
| R6 - F20 | Gill | 56 | neg | neg | NT | neg | 15,7 |
| R6 - F21 | Gill | 56 | neg | neg | NT | neg | 15,4 |
| R6 - F22 | Gill | 56 | neg | neg | NT | neg | 15,8 |
| R6 - F23 | Gill | 56 | neg | 36,1 | NT | neg | 15,2 |
| R6 - F24 | Gill | 56 | neg | neg | NT | neg | 15,9 |
| R6 - F25 | Gill | 56 | neg | neg | NT | neg | 16,2 |
| R6 - F26 | Gill | 56 | neg | neg | NT | neg | 15,7 |
| R6 - F27 | Gill | 56 | neg | neg | NT | neg | 15,8 |
| R6 - F28 | Gill | 56 | neg | neg | NT | neg | 15,5 |
| R6 - F29 | Gill | 56 | neg | neg | NT | neg | 15,1 |
| R6 - F30 | Gill | 56 | 37,2 | neg | NT | neg | 15,7 |

**Table 15.** Fish samples 83 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C7 - F1 | Skin | 83 | neg | neg | NT | neg | 20,1 |
| C7 - F2 | Skin | 83 | neg | neg | NT | neg | 21,3 |
| C7 - F3 | Skin | 83 | neg | neg | NT | neg | 20,7 |
| C7 - F4 | Skin | 83 | neg | neg | NT | neg | 21,8 |
| C7 - F5 | Skin | 83 | neg | neg | NT | neg | 22,1 |
| C7 - F6 | Skin | 83 | neg | neg | NT | neg | 21,9 |
| C7 - F7 | Skin | 83 | neg | neg | NT | neg | 20,1 |
| C7 - F8 | Skin | 83 | 37,4 | neg | NT | neg | 19,5 |
| C7 - F9 | Skin | 83 | neg | neg | NT | neg | 20,1 |
| C7 - F10 | Skin | 83 | neg | neg | NT | neg | 20,0 |
| C7 - F11 | Skin | 83 | neg | neg | NT | neg | 19,6 |
| C7 - F12 | Skin | 83 | neg | neg | NT | neg | 19,4 |
| C7 - F13 | Skin | 83 | neg | neg | NT | neg | 19,5 |
| C7 - F14 | Skin | 83 | neg | neg | NT | neg | 20,6 |
| C7 - F15 | Skin | 83 | neg | neg | NT | neg | 19,9 |
| C7 - F16 | Skin | 83 | neg | neg | NT | neg | 19,7 |
| C7 - F17 | Skin | 83 | neg | neg | NT | neg | 19,4 |
| C7 - F18 | Skin | 83 | neg | neg | NT | neg | 19,1 |
| C7 - F19 | Skin | 83 | 36,3 | neg | NT | neg | 19,3 |
| C7 - F20 | Skin | 83 | neg | neg | NT | neg | 19,8 |
| C7 - F21 | Skin | 83 | neg | neg | NT | neg | 20,7 |
| C7 - F22 | Skin | 83 | 37,2 | neg | NT | neg | 20,7 |
| C7 - F23 | Skin | 83 | neg | neg | NT | neg | 20,0 |
| C7 - F24 | Skin | 83 | neg | neg | NT | neg | 19,5 |
| C7 - F25 | Skin | 83 | neg | neg | NT | neg | 20,1 |
| C7 - F26 | Skin | 83 | neg | neg | NT | neg | 19,2 |
| C7 - F27 | Skin | 83 | neg | neg | NT | neg | 19,1 |
| C7 - F28 | Skin | 83 | neg | neg | NT | neg | 19,4 |
| C7 - F29 | Skin | 83 | neg | neg | NT | neg | 19,0 |
| C7 - F30 | Skin | 83 | neg | neg | NT | neg | 20,3 |
| C7 - F1 | Gill | 83 | neg | neg | NT | neg | 17,8 |
| C7 - F2 | Gill | 83 | neg | neg | NT | neg | 17,4 |
| C7 - F3 | Gill | 83 | neg | neg | NT | neg | 17,9 |
| C7 - F4 | Gill | 83 | neg | 34,2 | NT | neg | 16,5 |
| C7 - F5 | Gill | 83 | neg | neg | NT | neg | 17,4 |
| C7 - F6 | Gill | 83 | neg | neg | NT | neg | 16,6 |
| C7 - F7 | Gill | 83 | neg | neg | NT | neg | 20,1 |
| C7 - F8 | Gill | 83 | neg | neg | NT | neg | 18,0 |
| C7 - F9 | Gill | 83 | neg | neg | NT | neg | 17,3 |
| C7 - F10 | Gill | 83 | neg | 35,8 | NT | neg | 17,2 |
| C7 - F11 | Gill | 83 | neg | 35,7 | NT | neg | 17,0 |
| C7 - F12 | Gill | 83 | neg | 36,5 | NT | neg | 18,4 |
| C7 - F13 | Gill | 83 | neg | 38,0 | NT | neg | 17,8 |
| C7 - F14 | Gill | 83 | 36,8 | neg | NT | neg | 16,7 |
| C7 - F15 | Gill | 83 | neg | neg | NT | neg | 15,8 |
| C7 - F16 | Gill | 83 | neg | neg | NT | neg | 16,5 |
| C7 - F17 | Gill | 83 | neg | neg | NT | neg | 16,4 |
| C7 - F18 | Gill | 83 | neg | neg | NT | neg | 16,4 |
| C7 - F19 | Gill | 83 | neg | 37,8 | NT | neg | 16,4 |
| C7 - F20 | Gill | 83 | neg | 36,8 | NT | neg | 17,0 |
| C7 - F21 | Gill | 83 | neg | neg | NT | neg | 16,4 |
| C7 - F22 | Gill | 83 | 37,0 | neg | NT | neg | 20,1 |
| C7 - F23 | Gill | 83 | neg | neg | NT | neg | 16,3 |
| C7 - F24 | Gill | 83 | neg | neg | NT | neg | 17,8 |
| C7 - F25 | Gill | 83 | neg | 36,7 | NT | neg | 16,1 |
| C7 - F26 | Gill | 83 | 35,9 | 37,1 | NT | neg | 15,3 |
| C7 - F27 | Gill | 83 | neg | neg | NT | neg | 16,7 |
| C7 - F28 | Gill | 83 | neg | neg | NT | neg | 16,3 |
| C7 - F29 | Gill | 83 | 37,1 | neg | NT | neg | 17,7 |
| C7 - F30 | Gill | 83 | neg | neg | NT | neg | 18,3 |

**Table 16.** Fish samples 83 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R7 - F1 | Skin | 83 | neg | neg | NT | neg | 22,5 |
| R7 - F2 | Skin | 83 | neg | neg | NT | neg | 21,1 |
| R7 - F3 | Skin | 83 | 36,6 | neg | NT | neg | 20,7 |
| R7 - F4 | Skin | 83 | neg | neg | NT | neg | 21,5 |
| R7 - F5 | Skin | 83 | neg | neg | NT | neg | 20,8 |
| R7 - F6 | Skin | 83 | neg | neg | NT | neg | 21,7 |
| R7 - F7 | Skin | 83 | neg | neg | NT | neg | 20,7 |
| R7 - F8 | Skin | 83 | neg | neg | NT | neg | 20,8 |
| R7 - F9 | Skin | 83 | neg | neg | NT | neg | 20,3 |
| R7 - F10 | Skin | 83 | neg | neg | NT | neg | 22,8 |
| R7 - F11 | Skin | 83 | neg | neg | NT | neg | 22,5 |
| R7 - F12 | Skin | 83 | neg | 35,9 | NT | neg | 21,1 |
| R7 - F13 | Skin | 83 | neg | neg | NT | neg | 21,7 |
| R7 - F14 | Skin | 83 | 32,8 | neg | NT | neg | 21,0 |
| R7 - F15 | Skin | 83 | 36,9 | neg | NT | neg | 22,0 |
| R7 - F16 | Skin | 83 | neg | neg | NT | neg | 21,6 |
| R7 - F17 | Skin | 83 | neg | neg | NT | neg | 21,7 |
| R7 - F18 | Skin | 83 | 36,5 | neg | NT | neg | 23,8 |
| R7 - F19 | Skin | 83 | neg | neg | NT | neg | 21,9 |
| R7 - F20 | Skin | 83 | neg | neg | NT | neg | 18,9 |
| R7 - F21 | Skin | 83 | neg | neg | NT | neg | 22,6 |
| R7 - F22 | Skin | 83 | neg | neg | NT | neg | 22,0 |
| R7 - F23 | Skin | 83 | neg | 36,1 | NT | neg | 24,3 |
| R7 - F24 | Skin | 83 | neg | neg | NT | neg | 21,8 |
| R7 - F25 | Skin | 83 | neg | neg | NT | neg | 22,0 |
| R7 - F26 | Skin | 83 | neg | neg | NT | neg | 22,6 |
| R7 - F27 | Skin | 83 | neg | 38,2 | NT | neg | 22,2 |
| R7 - F28 | Skin | 83 | neg | neg | NT | neg | 22,3 |
| R7 - F29 | Skin | 83 | neg | neg | NT | neg | 21,8 |
| R7 - F30 | Skin | 83 | neg | neg | NT | neg | 21,9 |
| R7 - F1 | Gill | 83 | neg | neg | NT | neg | 17,8 |
| R7 - F2 | Gill | 83 | neg | neg | NT | neg | 18,4 |
| R7 - F3 | Gill | 83 | neg | neg | NT | neg | 20,5 |
| R7 - F4 | Gill | 83 | neg | neg | NT | neg | 18,0 |
| R7 - F5 | Gill | 83 | neg | neg | NT | neg | 17,9 |
| R7 - F6 | Gill | 83 | neg | neg | NT | neg | 17,6 |
| R7 - F7 | Gill | 83 | neg | neg | NT | neg | 17,7 |
| R7 - F8 | Gill | 83 | neg | neg | NT | neg | 17,3 |
| R7 - F9 | Gill | 83 | neg | neg | NT | neg | 19,0 |
| R7 - F10 | Gill | 83 | neg | neg | NT | neg | 16,9 |
| R7 - F11 | Gill | 83 | neg | neg | NT | neg | 18,3 |
| R7 - F12 | Gill | 83 | neg | 37,0 | NT | neg | 18,1 |
| R7 - F13 | Gill | 83 | neg | neg | NT | neg | 19,3 |
| R7 - F14 | Gill | 83 | 34,2 | neg | NT | neg | 17,5 |
| R7 - F15 | Gill | 83 | neg | neg | NT | neg | 18,0 |
| R7 - F16 | Gill | 83 | neg | neg | NT | neg | 18,0 |
| R7 - F17 | Gill | 83 | neg | neg | NT | neg | 18,3 |
| R7 - F18 | Gill | 83 | neg | neg | NT | neg | 19,7 |
| R7 - F19 | Gill | 83 | 36,6 | neg | NT | neg | 18,5 |
| R7 - F20 | Gill | 83 | neg | neg | NT | neg | 19,2 |
| R7 - F21 | Gill | 83 | neg | neg | NT | neg | 19,8 |
| R7 - F22 | Gill | 83 | neg | neg | NT | neg | 18,2 |
| R7 - F23 | Gill | 83 | neg | neg | NT | neg | 18,8 |
| R7 - F24 | Gill | 83 | neg | neg | NT | neg | 18,0 |
| R7 - F25 | Gill | 83 | neg | neg | NT | neg | 19,4 |
| R7 - F26 | Gill | 83 | neg | neg | NT | neg | 18,6 |
| R7 - F27 | Gill | 83 | 40,2 | neg | NT | neg | 18,5 |
| R7 - F28 | Gill | 83 | 35,7 | neg | NT | neg | 18,7 |
| R7 - F29 | Gill | 83 | neg | neg | NT | neg | 18,3 |
| R7 - F30 | Gill | 83 | neg | 37,8 | NT | neg | 18,1 |

**Table 17.** Fish samples 111 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| C8 - F1 | Skin | 111 | neg | neg | NT | neg | 18,9 |
| C8 - F2 | Skin | 111 | neg | neg | NT | neg | 20,2 |
| C8 - F3 | Skin | 111 | neg | neg | NT | neg | 19,4 |
| C8 - F4 | Skin | 111 | neg | neg | NT | neg | 21,4 |
| C8 - F5 | Skin | 111 | neg | neg | NT | neg | 19,8 |
| C8 - F6 | Skin | 111 | neg | neg | NT | neg | 19,5 |
| C8 - F7 | Skin | 111 | neg | neg | NT | neg | 23,5 |
| C8 - F8 | Skin | 111 | neg | neg | NT | neg | 19,9 |
| C8 - F9 | Skin | 111 | neg | neg | NT | neg | 21,2 |
| C8 - F10 | Skin | 111 | neg | neg | NT | neg | 19,8 |
| C8 - F11 | Skin | 111 | neg | neg | NT | neg | 23,3 |
| C8 - F12 | Skin | 111 | neg | neg | NT | neg | 19,9 |
| C8 - F13 | Skin | 111 | neg | neg | NT | neg | 18,6 |
| C8 - F14 | Skin | 111 | neg | neg | NT | neg | 19,4 |
| C8 - F15 | Skin | 111 | neg | neg | NT | neg | 22,6 |
| C8 - F16 | Skin | 111 | neg | neg | NT | neg | 19,5 |
| C8 - F17 | Skin | 111 | neg | neg | NT | neg | 19,7 |
| C8 - F18 | Skin | 111 | 36,9 | neg | NT | neg | 19,6 |
| C8 - F19 | Skin | 111 | neg | neg | NT | neg | 19,3 |
| C8 - F20 | Skin | 111 | neg | neg | NT | neg | 20,5 |
| C8 - F21 | Skin | 111 | neg | neg | NT | neg | 19,6 |
| C8 - F22 | Skin | 111 | neg | neg | NT | neg | 18,4 |
| C8 - F23 | Skin | 111 | neg | neg | NT | neg | 19,0 |
| C8 - F24 | Skin | 111 | neg | neg | NT | neg | 21,1 |
| C8 - F25 | Skin | 111 | neg | neg | NT | neg | 18,3 |
| C8 - F26 | Skin | 111 | neg | neg | NT | neg | 19,2 |
| C8 - F27 | Skin | 111 | neg | neg | NT | neg | 21,2 |
| C8 - F28 | Skin | 111 | neg | neg | NT | neg | 20,5 |
| C8 - F29 | Skin | 111 | neg | neg | NT | neg | 21,1 |
| C8 - F30 | Skin | 111 | neg | neg | NT | neg | 20,9 |
| C8 - F1 | Gill | 111 | neg | neg | NT | neg | 15,8 |
| C8 - F2 | Gill | 111 | neg | neg | NT | neg | 16,1 |
| C8 - F3 | Gill | 111 | neg | neg | NT | neg | 15,3 |
| C8 - F4 | Gill | 111 | neg | neg | NT | neg | 16,8 |
| C8 - F5 | Gill | 111 | neg | neg | NT | neg | 15,2 |
| C8 - F6 | Gill | 111 | neg | neg | NT | neg | 16,0 |
| C8 - F7 | Gill | 111 | neg | neg | NT | neg | 15,6 |
| C8 - F8 | Gill | 111 | neg | neg | NT | neg | 16,3 |
| C8 - F9 | Gill | 111 | neg | neg | NT | neg | 15,8 |
| C8 - F10 | Gill | 111 | neg | neg | NT | neg | 16,2 |
| C8 - F11 | Gill | 111 | neg | neg | NT | neg | 17,4 |
| C8 - F12 | Gill | 111 | neg | neg | NT | neg | 15,2 |
| C8 - F13 | Gill | 111 | neg | neg | NT | neg | 14,9 |
| C8 - F14 | Gill | 111 | neg | neg | NT | neg | 14,8 |
| C8 - F15 | Gill | 111 | neg | neg | NT | neg | 17,9 |
| C8 - F16 | Gill | 111 | neg | neg | NT | neg | 15,7 |
| C8 - F17 | Gill | 111 | neg | neg | NT | neg | 16,5 |
| C8 - F18 | Gill | 111 | neg | neg | NT | neg | 16,1 |
| C8 - F19 | Gill | 111 | neg | neg | NT | neg | 16,5 |
| C8 - F20 | Gill | 111 | neg | neg | NT | neg | 15,6 |
| C8 - F21 | Gill | 111 | neg | neg | NT | neg | 15,7 |
| C8 - F22 | Gill | 111 | neg | neg | NT | neg | 15,3 |
| C8 - F23 | Gill | 111 | 37,1 | neg | NT | neg | 14,9 |
| C8 - F24 | Gill | 111 | neg | neg | NT | neg | 16,5 |
| C8 - F25 | Gill | 111 | neg | neg | NT | neg | 16,4 |
| C8 - F26 | Gill | 111 | neg | neg | NT | neg | 16,0 |
| C8 - F27 | Gill | 111 | neg | neg | NT | neg | 15,2 |
| C8 - F28 | Gill | 111 | neg | neg | NT | neg | 14,3 |
| C8 - F29 | Gill | 111 | neg | neg | NT | neg | 13,6 |
| C8 - F30 | Gill | 111 | neg | neg | NT | neg | 14,0 |

**Table 18.** Fish samples 83 dps from the open net pen and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| R8 - F1 | Skin | 111 | 37,8 | neg | NT | neg | 18,0 |
| R8 - F2 | Skin | 111 | 35,6 | 36,5 | NT | neg | 17,0 |
| R8 - F3 | Skin | 111 | neg | neg | NT | neg | 16,3 |
| R8 - F4 | Skin | 111 | neg | neg | NT | neg | 16,3 |
| R8 - F5 | Skin | 111 | 37,9 | neg | NT | neg | 15,8 |
| R8 - F6 | Skin | 111 | neg | neg | NT | neg | 17,7 |
| R8 - F7 | Skin | 111 | neg | neg | NT | neg | 18,5 |
| R8 - F8 | Skin | 111 | neg | neg | NT | neg | 20,5 |
| R8 - F9 | Skin | 111 | neg | neg | NT | neg | 21,3 |
| R8 - F10 | Skin | 111 | neg | neg | NT | neg | 21,7 |
| R8 - F11 | Skin | 111 | 36,7 | neg | NT | neg | 19,3 |
| R8 - F12 | Skin | 111 | neg | neg | NT | neg | 18,9 |
| R8 - F13 | Skin | 111 | neg | neg | NT | neg | 22,1 |
| R8 - F14 | Skin | 111 | neg | neg | NT | neg | 19,5 |
| R8 - F15 | Skin | 111 | neg | neg | NT | neg | 18,1 |
| R8 - F16 | Skin | 111 | neg | neg | NT | neg | neg |
| R8 - F17 | Skin | 111 | neg | neg | NT | neg | 21,5 |
| R8 - F18 | Skin | 111 | 36,8 | neg | NT | neg | 21,2 |
| R8 - F19 | Skin | 111 | neg | neg | NT | neg | 17,8 |
| R8 - F20 | Skin | 111 | neg | neg | NT | neg | 22,3 |
| R8 - F21 | Skin | 111 | neg | neg | NT | neg | 18,9 |
| R8 - F22 | Skin | 111 | neg | 35,9 | NT | neg | 22,5 |
| R8 - F23 | Skin | 111 | 35,0 | neg | NT | neg | 19,0 |
| R8 - F24 | Skin | 111 | neg | neg | NT | neg | 21,7 |
| R8 - F25 | Skin | 111 | neg | neg | NT | neg | 21,1 |
| R8 - F26 | Skin | 111 | neg | neg | NT | neg | 19,2 |
| R8 - F27 | Skin | 111 | neg | neg | NT | neg | 19,5 |
| R8 - F28 | Skin | 111 | neg | neg | NT | neg | 20,2 |
| R8 - F29 | Skin | 111 | neg | neg | NT | neg | 25,3 |
| R8 - F30 | Skin | 111 | neg | neg | NT | neg | 19,9 |
| R8 - F1 | Gill | 111 | neg | neg | NT | neg | 15,2 |
| R8 - F2 | Gill | 111 | neg | 36,0 | NT | neg | 14,7 |
| R8 - F3 | Gill | 111 | neg | neg | NT | neg | 15,3 |
| R8 - F4 | Gill | 111 | neg | 37,4 | NT | neg | 14,2 |
| R8 - F5 | Gill | 111 | neg | neg | NT | neg | 13,6 |
| R8 - F6 | Gill | 111 | 36,6 | 36,9 | NT | neg | 14,9 |
| R8 - F7 | Gill | 111 | neg | neg | NT | neg | 13,9 |
| R8 - F8 | Gill | 111 | neg | neg | NT | neg | 14,7 |
| R8 - F9 | Gill | 111 | neg | neg | NT | neg | 15,3 |
| R8 - F10 | Gill | 111 | neg | neg | NT | neg | 14,9 |
| R8 - F11 | Gill | 111 | neg | neg | NT | neg | 15,7 |
| R8 - F12 | Gill | 111 | neg | 36,1 | NT | neg | 14,9 |
| R8 - F13 | Gill | 111 | neg | neg | NT | neg | 14,7 |
| R8 - F14 | Gill | 111 | neg | neg | NT | neg | 14,8 |
| R8 - F15 | Gill | 111 | neg | neg | NT | neg | 15,8 |
| R8 - F16 | Gill | 111 | neg | neg | NT | neg | 15,9 |
| R8 - F17 | Gill | 111 | neg | neg | NT | neg | 15,4 |
| R8 - F18 | Gill | 111 | neg | neg | NT | neg | 14,6 |
| R8 - F19 | Gill | 111 | neg | neg | NT | neg | 14,4 |
| R8 - F20 | Gill | 111 | neg | 35,2 | NT | neg | 14,6 |
| R8 - F21 | Gill | 111 | neg | 35,7 | NT | neg | 14,8 |
| R8 - F22 | Gill | 111 | neg | neg | NT | neg | 16,5 |
| R8 - F23 | Gill | 111 | 38,1 | 35,1 | NT | neg | 14,3 |
| R8 - F24 | Gill | 111 | neg | 35,4 | NT | neg | 13,4 |
| R8 - F25 | Gill | 111 | neg | 36,9 | NT | neg | 14,2 |
| R8 - F26 | Gill | 111 | neg | neg | NT | neg | 16,3 |
| R8 - F27 | Gill | 111 | 37,9 | 34,2 | NT | neg | 13,7 |
| R8 - F28 | Gill | 111 | 39,4 | neg | NT | neg | 15,9 |
| R8 - F29 | Gill | 111 | 38,3 | neg | NT | neg | neg |
| R8 - F30 | Gill | 111 | neg | 36,2 | NT | neg | 16,3 |

## Fish samples 152 dps from the SCCS at the study site collected during an outbreak of ulcerative disease

**Table 19.** Fish samples 152 dps from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the TdC2 assay targeting *T. dicentrarchi* and the MvOmpA assay targeting *Moritella viscosa*. Samples were collected from the skin under the jaw when no lesions could be detected and the gills. Skin – U refers to the skin tissue sample collected from skin around ulcers in the mucle/filet of the fish and Skin – M is skin tissue samples collected from the area around the mouth. The fish collected shown in the table were suffering from ulcerative disease.

| FISH ID | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA | EF1A |
|---|---|---|---|---|---|---|---|
| F1 | Skin - U | 152 | 29,0 | 30,3 | neg | 22,4 | 18,2 |
| F2 | Skin - U | 152 | 21,9 | 30,1 | neg | 29,6 | 17,6 |
| F4 | Skin - U | 152 | 21,4 | 21,9 | neg | 25,5 | 16,7 |
| F5 | Skin - U | 152 | 23,1 | 26,9 | neg | 24,9 | 15,5 |
| F7 | Skin - U | 152 | 18,7 | 21,6 | neg | 24,2 | 18,5 |
| F8 | Skin - U | 152 | 20,5 | 20,6 | neg | 25,0 | 19,7 |
| F9 | Skin - U | 152 | 21,8 | 23,1 | neg | 31,0 | 19,7 |
| F10 | Skin - U | 152 | 23,2 | 24,6 | neg | 21,5 | 18,4 |
| F11 | Skin - U | 152 | 21,3 | 26,5 | neg | 22,3 | 18,2 |
| F12 | Skin - U | 152 | 28,6 | 26,6 | neg | 24,6 | 17,2 |
| F13 | Skin - U | 152 | 21,9 | 26,5 | neg | 27,9 | 16,8 |
| F14 | Skin - U | 152 | 22,7 | 21,9 | neg | 22,3 | 16,5 |
| F15 | Skin - U | 152 | 19,7 | 21,8 | neg | 28,3 | 18,0 |
| F16 | Skin - U | 152 | 22,0 | 21,5 | neg | 27,1 | 19,0 |
| F17 | Skin - U | 152 | 22,8 | 26,9 | neg | 23,7 | 18,2 |
| F18 | Skin - U | 152 | 20,8 | 21,6 | neg | 29,1 | 18,8 |
| F19 | Skin - U | 152 | 19,0 | 17,6 | neg | 27,9 | 19,3 |
| F20 | Skin - U | 152 | 18,0 | 26,1 | neg | 29,1 | 17,2 |
| F3 | Skin - M | 152 | 19,0 | 25,9 | neg | 34,8 | 18,7 |
| F5 | Skin - M | 152 | 23,2 | 22,7 | neg | 27,7 | 17,6 |
| F6 | Skin - M | 152 | 22,9 | 29,2 | neg | 34,8 | 18,3 |
| F7 | Skin - M | 152 | 22,6 | 31,3 | neg | 35,9 | 19,6 |
| F8 | Skin - M | 152 | 20,5 | 25,5 | neg | 32,8 | 22,8 |
| F9 | Skin - M | 152 | 21,6 | 25,4 | neg | 32,4 | 18,9 |
| F10 | Skin - M | 152 | 22,0 | 26,2 | neg | 36,8 | 16,9 |
| F15 | Skin - M | 152 | 22,2 | 26,4 | neg | 37,4 | 24,9 |
| F16 | Skin - M | 152 | 19,7 | 25,4 | neg | 34,8 | 18,6 |
| F17 | Skin - M | 152 | 18,5 | 25,7 | neg | 32,8 | 17,9 |
| F18 | Skin - M | 152 | 20,3 | 24,5 | neg | 32,7 | 22,1 |
| F19 | Skin - M | 152 | 21,6 | 18,8 | neg | 33,2 | 16,8 |
| F20 | Skin - M | 152 | 23,2 | 26,9 | neg | 34,6 | 18,1 |
| F1 | Gill | 152 | 25,9 | 27,3 | neg | 27,3 | 14,3 |
| F2 | Gill | 152 | 29,4 | 31,2 | neg | 31,3 | 14,4 |
| F3 | Gill | 152 | 27,4 | 28,5 | neg | 30,3 | 14,8 |
| F4 | Gill | 152 | 27,3 | 28,9 | neg | 28,2 | 15,0 |
| F5 | Gill | 152 | 25,1 | 26,7 | neg | 27,3 | 14,7 |
| F6 | Gill | 152 | 27,6 | 29,2 | neg | 29,7 | 15,6 |
| F7 | Gill | 152 | 24,9 | 26,8 | neg | 27,8 | 15,3 |
| F8 | Gill | 152 | 27,3 | 29,2 | neg | 29,6 | 16,6 |
| F9 | Gill | 152 | 26,8 | 28,5 | neg | 28,8 | 14,9 |
| F10 | Gill | 152 | 29,3 | 30,3 | neg | 30,6 | 14,8 |
| F11 | Gill | 152 | 27,8 | 28,8 | neg | 29,2 | 15,6 |
| F12 | Gill | 152 | 27,7 | 28,8 | neg | 29,6 | 14,3 |
| F13 | Gill | 152 | 26,3 | 27,5 | neg | 27,0 | 14,7 |
| F14 | Gill | 152 | 26,7 | 28,5 | neg | 27,9 | 14,8 |
| F15 | Gill | 152 | 27,6 | 29,3 | neg | 29,9 | 15,3 |
| F16 | Gill | 152 | 28,5 | 29,3 | neg | 30,2 | 15,6 |
| F17 | Gill | 152 | 28,2 | 30,2 | neg | 30,9 | 15,5 |
| F18 | Gill | 152 | 27,7 | 29,7 | neg | 28,6 | 15,3 |
| F19 | Gill | 152 | 25,8 | 27,7 | neg | 28,6 | 14,6 |
| F20 | Gill | 152 | 28,0 | 28,8 | neg | 29,6 | 15,6 |

## Fish samples 182 dps from the SCCS and open net pen at the study site collected during an outbreak of ulcerative disease

**Table 20.** Overview of the obtained Ct-values from fish from the SCCS and open net pen at the study site (182 dps) using Real Time RT-PCR and the TffC3 assay (targeting *T. finnmarkense* gen. *finnmarkense*), TfuC1 (targeting *T. finnmarkense* gen. *ulcerans*), TdC2 (targeting *T. dicentrarchi)*, and MvOmpA to target *M. viscosa.*

| FISH ID | SCCS/Open pen | TISSUE | Dps | TffC3 | TfuC1 | TdC2 | MvOmpA |
|---|---|---|---|---|---|---|---|
| F1 | Open pen | Skin | 182 | 16,6 | 32,5 | neg | 32,7 |
| F2 | Open pen | Skin | 182 | 18,6 | 30,9 | neg | 33,3 |
| F3 | Open pen | Skin | 182 | 25,6 | 34,4 | neg | 34,2 |
| F4 | SCCS | Skin | 182 | 29,9 | 35,1 | neg | 33,8 |
| F5 | SCCS | Skin | 182 | 24,4 | neg | neg | 35,0 |
| F6 | SCCS | Skin | 182 | 29,9 | 34,4 | neg | 36,6 |
| F1 | Open pen | Gill | 182 | 26,9 | 34,7 | neg | 32,8 |
| F2 | Open pen | Gill | 182 | 28,4 | 33,3 | neg | 32,4 |
| F3 | Open pen | Gill | 182 | 30,0 | 35,6 | neg | 33,2 |
| F4 | SCCS | Gill | 182 | 29,7 | 35,6 | neg | 33,2 |
| F5 | SCCS | Gill | 182 | 30,4 | 35,6 | neg | 34,1 |
| F6 | SCCS | Gill | 182 | 24,6 | 30,9 | neg | 30,2 |

## Water samples 1-170 dps in SCCS and open net pen at the study site

**Table 21.** Water samples -6 to 170 dps sampled from the SCCS and the Ct-values obtained by using the TffC3 assay targeting *T. finnmarkense* gen. *finnmarkense*, the TfuC1 assay targeting *T. finnmarkense* gen. *ulcerans*, the MvOmpA assay targeting *Moritella viscosa* and the HSAL assay targeting *H. salinarum*. NT = not tested, neg = negative.

| | | | SCCS | | | | OPEN NET PEN | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sampling | Date | Dps | TffC3 | TfuC1 | MvOmpA | HSAL | TffC3 | TfuC1 | MvOmpA | HSAL |
| 1* | 09.09.2020 | -6 | neg | neg | neg | 21,5 | NT | NT | NT | NT |
| 2 | 15.09.2020 | 0 | 35,9 | 36,2 | neg | 22,0 | 33,3 | 36,3 | neg | 21,9 |
| 3 | 17.09.2020 | 2 | 34,5 | 36,5 | neg | 22,2 | 34,7 | 31,8 | neg | 22,6 |
| 4 | 19.09.2020 | 4 | 35,4 | 35,1 | neg | 22,2 | 31,4 | 33,9 | neg | 22,3 |
| 5 | 21.09.2020 | 6 | 35,0 | 33,7 | neg | 22,1 | neg | 37,8 | neg | 23,8 |
| 6 | 23.09.2020 | 8 | 28,0 | 31,4 | neg | 22,3 | neg | 37,5 | neg | 21,8 |
| 7 | 25.09.2020 | 10 | 35,3 | 36,6 | neg | 22,0 | neg | neg | neg | 21,4 |
| 8 | 27.09.2020 | 12 | 36,1 | 33,4 | neg | 22,0 | neg | neg | neg | 22,0 |
| 9 | 29.09.2020 | 14 | 35,5 | neg | neg | 22,5 | neg | 35,2 | neg | 22,4 |
| 10 | 01.10.2020 | 16 | 35,5 | 36,4 | neg | 22,2 | 30,9 | 33,9 | neg | 22,5 |
| 11 | 03.10.2020 | 18 | 35,9 | 35,6 | neg | 22,5 | 38,2 | neg | neg | 22,2 |
| 12 | 05.10.2020 | 20 | 35,8 | 36,4 | neg | 22,5 | neg | neg | neg | 22,7 |
| 13 | 07.10.2020 | 22 | 34,6 | 35,3 | neg | 21,7 | 38,2 | 34,5 | neg | 22,3 |
| 14 | 09.10.2020 | 24 | 35,3 | neg | neg | 21,4 | neg | 36,6 | neg | 22,0 |
| 15 | 11.10.2020 | 26 | 37,5 | 35,5 | neg | 22,5 | 38,2 | 34,1 | neg | 23,4 |
| 16 | 13.10.2020 | 28 | 35,4 | 36,6 | neg | 22,1 | 37,2 | 36,6 | neg | 22,4 |
| 17 | 15.10.2020 | 30 | 36,6 | 36,7 | neg | 22,6 | neg | 33,9 | neg | 21,9 |
| 18 | 22.10.2020 | 37 | 36,2 | 36,8 | neg | 22,1 | neg | neg | neg | 22,2 |
| 19 | 25.10.2020 | 40 | 34,4 | 36,7 | neg | 22,1 | neg | 35,2 | neg | 22,3 |
| 20 | 29.10.2020 | 44 | 34,8 | 35,7 | neg | 22,1 | 37,5 | 33,1 | neg | 22,6 |
| 21 | 05.11.2020 | 51 | 36,1 | 35,1 | neg | 22,3 | neg | 36,6 | neg | 22,2 |
| 22 | 12.11.2020 | 58 | 33,8 | 34,8 | neg | 22,8 | 34,4 | 29,6 | neg | 22,3 |
| 23 | 19.11.2020 | 65 | 34,2 | neg | neg | 22,8 | 36,3 | 35,2 | neg | 22,4 |
| 24 | 20.11.2020 | 66 | 26,8 | 26,7 | 34,7 | 24,3 | 34,0 | 33,5 | 38,9 | 21,7 |
| 25 | 26.11.2020 | 72 | 35,0 | 35,1 | neg | 22,2 | 37,0 | 34,4 | neg | 22,2 |
| 26 | 03.12.2020 | 79 | 33,1 | 33,6 | neg | 22,3 | 31,4 | 32,1 | neg | 22,8 |
| 27 | 10.12.2020 | 86 | 32,5 | 31,7 | neg | 22,1 | 32,1 | 30,6 | neg | 22,6 |
| 28 | 17.12.2020 | 93 | 32,3 | 34,6 | 37,3 | 34,2 | 35,0 | 35,8 | 37,4 | 22,0 |
| 29 | 24.12.2020 | 100 | 32,8 | 32,5 | 35,0 | 22,0 | 32,5 | 30,9 | 35,3 | 22,3 |
| 30 | 31.12.2020 | 107 | 31,3 | 32,8 | 36,2 | 22,0 | 38,5 | neg | neg | 22,1 |
| 31 | 07.01.2021 | 114 | 32,0 | 33,6 | 36,2 | 22,3 | 38,5 | neg | neg | 22,3 |
| 32 | 14.01.2021 | 121 | 32,5 | neg | 36,7 | 22,0 | 36,5 | 34,8 | neg | 21,5 |
| 33 | 21.01.2021 | 128 | 31,1 | 32,6 | 34,4 | 22,0 | 34,7 | 34,8 | 36,0 | 21,9 |
| 34 | 28.01.2021 | 135 | 30,8 | 31,7 | 35,3 | 21,8 | 37,5 | 35,8 | neg | 21,9 |
| 35 | 04.02.2021 | 142 | 29,8 | 29,0 | 33,7 | 23,4 | 35,9 | 33,6 | neg | 27,7 |
| 36 | 11.02.2021 | 149 | 30,3 | 29,8 | 32,3 | 22,2 | 28,5 | 27,7 | 30,5 | 22,6 |
| 37 | 18.02.2021 | 156 | 28,2 | 31,4 | 32,6 | 22,3 | 33,1 | 30,1 | 35,2 | 21,7 |
| 38 | 25.02.2021 | 163 | 29,6 | 31,3 | 34,1 | 22,3 | neg | 30,5 | 35,4 | 22,6 |
| 39 | 04.03.2021 | 170 | 30,2 | 33,7 | 33,5 | 22,2 | 32,9 | 32,5 | 33,9 | 22,0 |

# Additional Real Time RT-PCR assay development data:

## Assay specificity testing Ct-values

**Table 22.** The assays developed in this study and the Ct-values obtained from the specificity testing using *Tenacibaculum* spp. field isolates mentioned in Table 2.2 as well as the type species of Clade I and II (*T. finnmarkense* gen. *ulcerans* TNO010$^{T}$ and *T. dicentrarchi* 35/09$^{T}$). *T. finnmarkense* gen. *finnmarkense* strain HFJ were used instead of the type species of Clade III. The red box indicates the annealing temperature that ended up making the assays most specific, whilst not weakening them (which happened at annealing temperatures above 62 °C). The Ct-values marked in green indicates the assay amplifying RNA from the right isolate, whilst pink Ct-value means the assay amplified RNA from the wrong *Tenacibaculum* spp. clade. NT = not tested

| Assay | Isolate | Clade | 60 °C | 62 °C | 64 °C | 63 °C |
|---|---|---|---|---|---|---|
| TffC3 | LIM056 | CI | 32,9 | 36,9 | neg | neg |
| TffC3 | LIM016 | CI | 32,5 | 36,0 | neg | neg |
| TffC3 | LIM075 | INTER | neg | neg | neg | neg |
| TffC3 | LIM063 | CII | neg | neg | neg | neg |
| TffC3 | LIM026 | CIII | 16,8 | 16,5 | 16,8 | 16,9 |
| TffC3 | LIM020 | CIII | 15,4 | 14,9 | 15,3 | 15,2 |
| TffC3 | LIM006 | CIII | 15,8 | 15,6 | 15,8 | 15,8 |
| TffC3 | LIM072 | CIV | neg | neg | neg | neg |
| TffC3 | HFJ | CIII | 16,1 | 16,3 | 16,1 | 16,7 |
| TffC3 | TNO010 | CI | NT | 36,4 | NT | NT |
| TffC3 | 35/09 | CII | neg | neg | neg | neg |
| TfuC1 | LIM056 | CI | 15,6 | 15,2 | 15,5 | 13,6 |
| TfuC1 | LIM016 | CI | 15,2 | 15,1 | 15,6 | 13,3 |
| TfuC1 | LIM075 | INTER | neg | neg | neg | neg |
| TfuC1 | LIM063 | CII | neg | neg | neg | neg |
| TfuC1 | LIM026 | CIII | 28,2 | neg | neg | neg |
| TfuC1 | LIM020 | CIII | 30,9 | neg | neg | neg |
| TfuC1 | LIM006 | CIII | 27,1 | neg | neg | neg |
| TfuC1 | LIM072 | CIV | neg | neg | neg | neg |
| TfuC1 | HFJ | CIII | 30,6 | neg | neg | neg |
| TfuC1 | TNO010 | CI | NT | 16,2 | NT | NT |
| TfuC1 | 35/09 | CII | neg | neg | neg | neg |
| TdC2 | LIM056 | CI | neg | neg | neg | neg |
| TdC2 | LIM016 | CI | neg | neg | neg | neg |
| TdC2 | LIM075 | INTER | neg | neg | neg | neg |
| TdC2 | LIM063 | CII | 16,1 | 15,8 | 20,9* | 18,4 |
| TdC2 | LIM026 | CIII | neg | neg | neg | neg |
| TdC2 | LIM020 | CIII | neg | neg | neg | neg |
| TdC2 | LIM006 | CIII | neg | neg | neg | neg |
| TdC2 | LIM072 | CIV | neg | neg | neg | neg |
| TdC2 | HFJ | CIII | neg | neg | neg | neg |
| TdC2 | TNO010 | CI | NT | 35,5 | NT | NT |
| TdC2 | 35/09 | CII | 16,9 | 16,7 | 22,1 | 18,3 |

## Assay testing using field cases of suspected tenacibaculosis

**Table 23.** The Real Time RT-PCR assays developed in this study tested using fish skin samples collected from field outbreaks of ulcerative disease using Real Time RT-PCR and assay TffC3 targeting *T. finnmarkense* gen. *finnmarkense*, TfuC1 targeting *T. finnmarkense* gen. *ulcerans*, Tb_rpoB targeting *Tenacibaculum* spp., TdC2 targeting *T. dicentrarchi* and MvOmpA targeting *M. viscosa*. 1TF, 2TF, 3TF and 4TF are four different sites in Troms and Finnmark where fish have been collected. Site CE is extracted RNA from fish of a challenge experiment conducted in 2019, challenged with *T. finnmarkense* gen. *finnmarkense* strain HFJ. *Only 1 out of 3 replicates tested positive. NT = Not tested. x = not applicable.

| FISH ID | SITE | TISSUE | TffC3 | Tb_rpoB | TfuC1 | TdC2 | MvOmpA |
|---|---|---|---|---|---|---|---|
| F1 | 1TF | Skin | NT | 22,5 | 29,5 | neg | 18,9 |
| F2 | 1TF | Skin | NT | 21,1 | 35,1 | neg | 19,9 |
| F3 | 1TF | Skin | NT | 28,0 | neg | neg | 19,7 |
| F4 | 1TF | Skin | NT | 25,5 | neg | neg | 20,0 |
| F5 | 1TF | Skin | NT | 30,7 | 27,4 | neg | 22,9 |
| F6 | 1TF | Skin | NT | 22,3 | 33,3 | neg | 22,4 |
| F7 | 1TF | Skin | NT | 25,8 | 28,6 | neg | 21,9 |
| F8 | 1TF | Skin | NT | 20,5 | 33,4 | neg | 21,7 |
| F9 | 1TF | Skin | NT | 19,9 | 24,3 | neg | 19,6 |
| F10 | 1TF | Skin | NT | 24,3 | 33,6 | neg | 24,3 |
| M6F7 | 2TF | Skin | 21,8 | 19,1 | 20,2 | neg | 22,1 |
| M6F3 | 2TF | Skin | 31,6 | 29,0 | 33,2 | neg | 19,3 |
| M7F6 | 2TF | Skin | 25,9 | 23,5 | 26,4 | neg | 21,0 |
| M7F10 | 2TF | Skin | 31,7 | 29,1 | 31,5 | 38,0* | 22,6 |
| M4F1 | 2TF | Skin | NT | 32,5 | neg | neg | 32,2 |
| M4F2 | 2TF | Skin | NT | 22,4 | neg | neg | 23,2 |
| M6F3 | 2TF | Skin | NT | 17,9 | 24,8 | neg | 30,5 |
| M6F4 | 2TF | Skin | NT | 25,4 | 35,6 | neg | 29,6 |
| M10F5 | 2TF | Skin | NT | 16,7 | 31,4 | neg | 32,5 |
| M10F6 | 2TF | Skin | NT | 27,4 | 26,5 | neg | x |
| F1 | 3TF | Skin | 18,9 | NT | 17,8 | neg | 18,0 |
| F2 | 3TF | Skin | 21,3 | NT | 22,2 | neg | 25,1 |
| F3 | 3TF | Skin | 20,5 | NT | 29,0 | neg | 26,7 |
| F4 | 3TF | Skin | 16,8 | NT | 22,3 | neg | 18,4 |
| F5 | 3TF | Skin | 20,0 | NT | 21,5 | neg | 20,6 |
| F6 | 3TF | Skin | 18,1 | NT | 23,6 | neg | 33,1 |
| F7 | 3TF | Skin | 23,6 | NT | 26,4 | neg | 28,9 |
| F8 | 3TF | Skin | 21,7 | NT | 29,8 | neg | 19,9 |
| F9 | 3TF | Skin | 21,2 | NT | 31,0 | neg | 20,3 |
| F1 | 4TF | Skin | 26,9 | NT | 31,3 | NT | 15,5 |
| F2 | 4TF | Skin | 22,5 | NT | 27,4 | NT | 12,8 |
| F3 | 4TF | Skin | 15,9 | NT | 21,1 | NT | 26,7 |
| F4 | 4TF | Skin | 20,8 | NT | 22,0 | NT | 15,8 |
| F5 | 4TF | Skin | 16,2 | NT | 24,8 | NT | 29,8 |
| F1 | 4TF | Eye | 37,0 | NT | 35,5 | NT | 17,5 |
| F2 | 4TF | Eye | 31,5 | NT | 28,1 | NT | 32,8 |
| F3 | 4TF | Eye | 30,7 | NT | 33,7 | NT | 33,3 |
| F4 | 4TF | Eye | neg | NT | neg | NT | neg |
| F5 | 4TF | Eye | 34,9 | NT | neg | NT | neg |
| K1F1 | CE | Skin | 17,6 | 15,4 | neg | neg | NT |
| K1F4 | CE | Skin | 16,4 | 12,2 | neg | neg | NT |
| K2F4 | CE | Skin | 14,9 | 10,7 | neg | neg | NT |
| K3F5 | CE | Skin | 27,3 | 24,6 | neg | neg | NT |
| K2F6 | CE | Skin | 37,4* | neg | neg | neg | NT |