Norwegian University of Life Sciences
Faculty of Science and Technology (REALTEK)

Philosophiae Doctor (PhD)
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Drivers behind variation in welfare, quality, and production performance in Atlantic salmon farming production data

Årsaker til variasjon i velferdstilstand, kvalitet, og prestasjon i produksjonsdata fra oppdrett av atlanterhavslaks

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1 Abbreviations and definitions

The main abbreviations used throughout this thesis are:

0+: Under-yearling smolt (less than 1 year old)
1+: Yearling smolt (one year old or more)
CMS: Cardiomyopathy syndrome
ERP: Enterprise resource planning
FCR: Feed conversion ratio
GA(M)M: Generalised additive (mixed) model
HSMI: Heart and skeletal muscle inflammation
IPNV: Infectious pancreatic necrosis virus
PMCV: Piscine myocarditis virus
PD: Pancreas disease
PRV: Piscine orthoreovirus
RAS: Recirculating aquaculture system
RDA: Redundancy analysis
SGR: Specific growth rate
tb-RDA: Transformation based redundancy analysis
TGC: Thermal growth coefficient

2 List of papers

I. Alvestad, R., Måge, I., Noble, C., Liland, K. H. The effect of freshwater production strategy on post-seawater transfer growth, total mortality, mortalities attributed to HSMI/CMS in Atlantic salmon (*Salmo salar* L.) aquaculture and the associated effects of delousing treatments. Manuscript.

II. Alvestad, R., Noble, C., Måge, I., Liland, K.H. The dynamics and prevalence of ulcerative diseases and mouth rot in Atlantic salmon (*Salmo salar* L.) farms in Northern Norway. Manuscript.

III. Alvestad, R., Noble, C., Måge, I., Liland, K. H. Principal causes for quality downgrading in Norwegian arctic Atlantic salmon (*Salmo salar* L.) farming and their welfare and production related drivers. Manuscript.

3 Abstract

Atlantic salmon aquaculture is an important industry in Norway and farmed salmon is among the most economically important global aquaculture species. Despite this, the growth of the industry has stagnated in Norway and it is affected by severe challenges to fish health and welfare. This thesis presents the findings from analyses of production data from one large Atlantic salmon production company with operations in Northern Norway. The aim was to identify risk factors for total and cause specific mortality, quality downgrading, and reduced production performance.

We found that smolt weight did not significantly affect subsequent growth during the seawater stage, but a higher specific growth rate (SGR) during the freshwater stage resulted in a somewhat reduced thermal growth coefficient (TGC) during the seawater stage. We found that mechanical delousing treatments were significantly associated with increased total mortalities and mortalities due to heart and skeletal muscle inflammation (HSMI) or cardiomyopathy syndrome (CMS), while bathing treatments were not.

A higher smolt weight was associated with both significantly higher mortalities due to HSMI or CMS and to mouth rot. The effect on mortalities due to mouth rot is strong but not easily explainable with the available dataset and should be subject to further investigation.

Mortalities attributed to mouth rot predominantly occurred during the first 7 months post seawater transfer. We found a significantly increased mortality due to mouth rot in fish transferred to seawater with lower and falling temperatures, which agrees with previously reported observations from the industry. We also found that 1+ smolts had significantly lower mortality attributed to mouth rot.

The presence of ulcers was the most important cause for downgrading during primary processing in the dataset from Northern Norway, affecting 7.4 % of the processed fish. Ulcers were also a persistent cause for mortalities over several production cycles. The prevalence of ulcers, both as a cause for downgrading and mortalities, were highest during the winter months and conformed with what is commonly called winter ulcer disease. Elevated mortalities due to mouth rot earlier in the production cycle significantly contributed to increased mortalities due to ulcers during the first winter at sea. Lower water temperatures significantly contributed to both increased mortalities due to ulcers during the first winter at sea and increased downgrading due to ulcers. Production cycles originating from one specific smolt

supplier experienced significantly lower mortalities due to ulcers during the first winter at sea.

Other important causes for downgrading were the presence of dark spots, sexual maturation, and deformities, affecting 3.7 %, 2.4 %, and 1.5 % of the slaughtered fish, respectively. A higher prevalence of dark spots was significantly associated with a higher mortality due to HSMI or CMS, which agrees with previously published results.

This thesis demonstrates the utility of collecting and analysing production data from commercial Atlantic salmon producers in identifying risk factors for reduced production performance, poor fish welfare and increased mortality, as well as reduced quality. There is a considerable need for standardising and quality control of data in the industry, but also the development of methods and analytical frameworks that can accommodate the challenges unique to analysing such datasets.

4 Norsk sammendrag

Lakseoppdrett er en viktig næring i Norge og laks er en av de mest økonomisk betydningsfulle fiskeartene i akvakultur globalt. Næringen er likevel preget av en stagnerende produksjonsvekst og store utfordringer med fiskehelse og -velferd. Denne avhandlingen presenter resultatene av analyser gjort på produksjonsdata fra en stor lakseprodusent hvis produksjon er lokalisert i Nord-Norge. Formålet var å identifisere risikofaktorer for overordnet og årsaksspesifikk dødelighet, kvalitetsnedgradering ved slakt, og redusert prestasjon.

Vi fant at smoltvekt ikke påvirket tilvekst i den etterfølgende sjøfasen, mens høyere spesifikk vekstrate (SGR) i ferskvannsfasen medførte en noe redusert termisk vekstkoeffisient (TGC) i sjøfasen. Mekanisk avlusning, men ikke badebehandling mot lus, bidro til signifikant forøket overordnet dødelighet og dødelighet grunnet hjerte- og skjellettmuskelbetennelse (HSMB) eller kardiomyopatisyndrom (CMS).

Høyere smoltvekt var signifikant forbundet med både forøket dødelighet grunnet HSMB eller CMS og munnråte. Denne effekten på munnråte er vanskelig å forklare og fordrer videre undersøkelser. Dødelighet på grunn av munnråte forekom nesten utelukkende under de første 7 månedene etter overføring til sjø. I tråd med tidligere rapporterte observasjoner fra næringen fant vi en forøket dødelighet grunnet munnråte når fisk ble satt i sjø ved lavere og fallende temperaturer. Vi fant også at 1+ smolt opplevde en signifikant lavere dødelighet grunnet munnråte.

Forekomst av sår var den viktigste årsaken til nedklassing ved slakt og påvirket 7,4 % av den prosesserte fisken. Sår forekom også som en vedvarende dødsårsak i mange produksjonssykluser. Forekomsten av sår, både som dødelighets- og nedklassingsårsak, var høyest gjennom vinteren og er nok derfor overveiende sammenfallende med diagnosen kjent som vintersår. Foregående dødelighet grunnet munnråte medførte signifikant forøket sår-relatert dødelighet under den første vinteren i sjø. Lave vanntemperaturer bidro til både signifikant forøket sår-relatert dødelighet under den første vinteren i sjø og forøket forekomst av sår som nedklassingsårsak ved slakt. Produksjonssykluser som stammet fra én enkelt smoltleverandør opplevde signifikant lavere dødelighet på grunn av sår under den første vinteren i sjø.

Andre viktige nedklassingsårsaker var mørke flekker, kjønnsmodning, og deformiteter, som påvirket henholdsvis 3,7 %, 2,4 %, og 1,5 % av den prosesserte fisken. Forekomsten av mørke flekker var signifikant forbundet med høyere dødelighet tilskrevet HSMB eller CMS, hvilket er i overensstemmelse med tidligere rapporterte funn.

Denne avhandlingen demonstrerer at innsamling og analyse av produksjonsdata fra kommersielle produsenter kan være et viktig verktøy for å utlede risikofaktorer for nedsatt produksjonsprestasjon, dårlig fiskevelferd og forøket dødelighet, samt kvalitetsreduksjon i oppdrettet laks. Det er likevel et behov for standardisering og kvalitetssikring av data i næringen, samt utvikling av prosedyrer og metoder som kan imøtekomme utfordringene ved å analysere slike datasett.

5 General introduction

5.1 Atlantic salmon farming

In 2018, Atlantic salmon (*Salmo salar* L.) accounted for 4.5 % of the world's finfish aquaculture production in terms of produced volume, and salmonids overall accounted for 19 % of total traded value of fish products, internationally (FAO, 2020). Most of the output volume is produced in Norway, Chile, Scotland, and Canada (Bachmann-Vargas et al., 2021). At the global level, Norway has been the leader of the salmon farming industry since its inception in the late 1960's, accounting for 53 % of global salmon production in 2015. At a national level Atlantic salmon aquaculture is a key strategic industry and one of the most important industries in rural parts of the country (Olaussen, 2018). Norwegian Atlantic salmon production volumes increased rapidly until about 2012, but have somewhat plateaued at around 1.20 to 1.35 million tonnes annual output since (Norwegian Directorate of Fisheries, 2021). Productivity growth rates have similarly declined, concomitantly with a rise in production costs (Asche et al., 2013; Iversen et al., 2020).

The constrained growth during the last decade has been attributed to an exhaustion of the available production space and a general decline in productivity growth associated with the maturing of the industry (Asche et al., 2013). To abate the environmental risks associated with Atlantic salmon farming, the government of Norway has imposed restrictions on awarding production licenses as well as regulations limiting the allowed biomass, stocking numbers, and production densities in cages. These have mostly been motivated by concerns regarding the impact of salmon farms on surrounding wildlife, especially the spread of the salmon louse (*Lepeophtheirus salmonis* L.) from farms to wild populations (Hersoug et al., 2019; Larsen and Vormedal, 2021). However, there is also a growing concern for the welfare of farmed fish, the threat posed by disease (Kristiansen et al., 2020; MOWI, 2020; Pettersen et al., 2015) and the economic implications thereof (Iversen et al., 2020).

Median mortality rates varied between 12 % and 16 % for the marine stage of Atlantic salmon production cycles completed during the years 2015-2019 in Norway. The 3rd quartiles similarly varied between 22 % and 27 %, indicating high variability across production units and sites. Precise data on cause specific mortalities are not available, particularly as not all diseases are notifiable, but infectious diseases, parasite infestations, adverse environmental conditions, and challenging husbandry operations likely all contribute considerably to the numbers (Sommerset et al., 2020).

Intensive Atlantic salmon culture has traditionally relied on the hatching of eggs and growing of fry to smolts, weighing between 30-50 g, in a freshwater hatchery, followed by transfer to a seawater net cage for ongrowing until the fish reaches market size (Bergheim et al., 2009). Recent years' constrained growth and health challenges has led to producers employing a much wider range of production strategies and technologies to avoid or abate some of the problems. For example, smolt sizes have been steadily increasing, and the on-growing of post-smolts in land based or closed containment systems are now being utilised by some producers, meaning that fish transferred to seawater can range from 90-120 g up to 1000 g (Bergheim et al., 2009; Dam, 2020; Karlsen et al., 2018). During the seawater stage, a wider range of strategies are being utilised for treating and controlling parasites, especially sea lice. These treatments now include non-medicinal treatments, such as mechanical and thermal treatments, with some being associated with increased mortalities and adverse welfare states (Overton et al., 2019). In such times of rapid change there is a need for documenting and analysing the impacts of different production strategies and technologies on Atlantic salmon during the production cycle, to identify the ones that benefit fish health and welfare and move the industry in the right direction, and those that do not.

5.2 Cardiovascular disease

In intensive salmonid culture, diseases affecting the cardiovascular system constitute a widespread challenge to fish welfare and contribute to significant annual losses due to mortalities (Dalum et al., 2017; Garseth et al., 2018; Hjeltnes et al., 2018; Jansen et al., 2017; Sommerset et al., 2020). While much of these losses are attributable to infectious diseases, chronic afflictions such as malformed hearts and misalignment of central blood vessels, compromising the functioning of the cardiovascular system, are widespread in intensive fish culture (Pombo et al., 2012; Poppe et al., 2003). Domesticated salmonids have been demonstrated to have aberrant heart morphologies when compared with their wild conspecifics. The most widely observed difference is the rounded shape of the ventricle, which is normally triangular, and that is reminiscent of that seen in sedentary wild fish species (Gamperl and Farrell, 2004; Poppe et al., 2003).

Malformations and misalignments between central organs in the cardiovascular system likely predispose the fish to cardiac failure when exposed to stressful situations such as handling, e.g. crowding, pumping, netting, and delousing treatments (Dalum et al., 2017). Reduced cardiac output may also inhibit growth (Poppe et al., 2003). As these afflictions are diverse and complex, there are likely several causes at play. Malformations of the heart, correlated with higher mortalities during disease outbreaks, have been associated with high temperatures during incubation and early life stages, but also during on-growing (Brocklebank and Raverty, 2001; Mercier et al., 2000; Poppe et al., 2003; Rodger and Mitchell, 2011; Takle et al., 2005). Arteriosclerosis has been associated with high growth rates (Farrell, 2002; Poppe et al., 2003) and coronary arteriosclerosis seem to vary with hatchery of origin in the on-growing phase of rainbow trout production (Brijs et al., 2020). Faster smolt growth has been found to pathologically affect cardiac morphology and predispose the fish to cardiac related mortality, also affecting subsequent growth (Frisk et al., 2020). Thus, production strategies are important in explaining the variation in mortalities and reduced growth rates associated with cardiac dysfunction in intensive salmonid culture.

Among the more severe and widespread infectious diseases affecting the cardiovascular system in Atlantic salmon farming in Norway are Cardiomyopathy Syndrome (CMS) and Heart and Skeletal Muscle Inflammation (HSMI) (Sommerset et al., 2020). CMS was first diagnosed and recognised as a rapidly increasing problem in the mid-1980's. In recent years it affected over 100 sites annually and it is recognised as among the most significant contributors to mortalities in Norwegian Atlantic salmon farming. It is not a notifiable disease in Norway and it is likely underreported (Sommerset et al., 2020). The disease is also found in Scotland, Ireland, and the Faroe Islands (Brun et al., 2003; Rodger et al., 2014) and is caused by the piscine myocarditis virus (PMCV) (Garseth et al., 2018; Løvoll et al., 2010).

Fish that are affected by CMS appear to be in good external morphological condition before death. The disease manifests as severe cardiac lesions, mainly in the spongy myocardium of the ventricle and atrium, accompanied by chronic myocardial inflammation and rupture of the atrial wall (Ferguson et al., 1990). While infection happens between 1 and 7 months after seawater transfer, outbreaks typically occur after around 12 to 18 months (Brun et al., 2003; Svendsen et al., 2019). It is likely a chronic disease, resulting in elevated mortalities over time with occasional acute outbreaks (Brun et al., 2003). Time at sea, transferring fish to seawater during autumn, infection pressure, cohort size, and previously recorded HSMI, PD, or CMS infections have been identified as risk factors for CMS (Bang Jensen et al., 2020, 2013). The characteristics and severity of the lesions that develop during disease are correlated with viral loads (Wiik-Nielsen et al., 2016).

HSMI appeared in farmed Atlantic salmon in 1999 and has predominantly been a problem in Norway (Kongtorp et al., 2004). It has not been a notifiable disease in Norway since 2014, but there were at least 75 affected seawater sites reported in 2019 and the disease has also been observed in hatcheries (Sommerset et al., 2020). HSMI is likely caused by a particularly virulent strain of piscine orthoreovirus (PRV), which targets erythrocytes. As PRV is ubiquitous, also in the wild, phenotypic differences between strains and viral loads likely determine the characteristics of disease development (Dhamotharan et al., 2019; Di Cicco et al., 2017; Finstad et al., 2014; Mikalsen et al., 2012; Wessel et al., 2020, 2017).

HSMI is associated with myocarditis in both spongy and compact layers of the ventricle, as well as epicarditis. A severe inflammatory response is seen in the heart but is also often observed in red muscle (Kongtorp et al., 2004). During outbreaks mortality numbers range from negligible up to 20 % in affected cages, while morbidity can be close to 100 % (Di Cicco et al., 2017; Kongtorp et al., 2006). The most severe symptoms and highest mortality figures are seen in May through July (Kongtorp et al., 2006). Risk factors for HSMI include infection pressure, cohort size, and cohort lifespan (time spent in seawater). Despite an even distribution of viral load, there are regional differences in risk of disease development (Kristoffersen et al., 2013).

5.3 Ulcerative disease and mouth rot

Fish skin and its associated mucus layer is an important barrier, protecting the fish against the external environment and its associated pathogens. Lesions, and particularly ulcers, break this barrier, compromising the welfare of the fish and exposes it to the risk of infection, disease, and mortality.

Ulcerative disease is a challenge to Atlantic salmon farming in Norway and according to conservative estimates, up to 2.5 % of farmed Atlantic salmon are affected by ulcers at any one time. Ulcers are recognised as a major welfare and reputational problem for the industry, but is also a cause for economic losses due to elevated mortalities and quality downgrading of slaughtered fish (Sommerset et al., 2020; Takle et al., 2015). Common causes for ulcers in fish are mechanical trauma, environmental stress, pathogens, and pollutants. Causal mechanisms are poorly understood (Noga, 2000).

In Norwegian Atlantic salmon farming, particularly in the northern regions, ulcerative disease is often associated with a condition called winter ulcer disease, which appeared in the 1980’s (Lunder et al., 1995). It is manifest as circular epidermal ulcers, ranging in severity from superficial to penetrating the dermis and exposing muscle (Bruno et al., 1998; Lunder et al., 1995). Winter ulcer disease has been associated with the bacterium *Moritella viscosa*, but it is not clear if systemic infection with the bacterium causes the disease or if it opportunistically infects existent lesions and ulcers. *Tenacibaculum* spp. and *Aliivibrio wodanis* are commonly found in infected ulcers, alongside *M. viscosa*, and all have been proposed to have driving or modulating roles in the development of ulcers. However, it is presently unclear if winter ulcer disease is caused by a specific pathogen or some perturbation to the host-microbiota (Karlsen et al., 2017b, 2017a, 2014; Olsen et al., 2011). It is also unclear if the ulcers themselves or systemic infection cause mortalities.

Winter ulcer disease is predominantly found during the winter months in Norwegian Atlantic salmon farming, when water temperatures are below 8-10 °C (Greger and Goodrich, 1999; Løvoll et al., 2009). Its prevalence ranges from 1 % to 50 % and its severity also varies, with cumulative mortality rates ranging from negligible to over 50 % in some outbreaks (Bruno et al., 1998; Lunder et al., 1995). To various degrees, ulcers can heal when temperatures rise above 8 °C (Lunder et al., 1995). Possible risk factors are vaccine type, prior mechanical trauma, smolt supplier, salmon lice infestation, and fish size, with smaller fish being more at risk for severe disease (Coyne et al., 2006, 2004; Salte et al., 1994; Vågsholm and Djupvik, 1998b). Fish skin is generally more sensitive in Atlantic salmon following seawater transfer, and adverse production conditions, e.g. high densities, are also known to inhibit wound healing (Karlsen et al., 2018; Sveen et al., 2018).

Mouth rot, commonly attributed to *Tenacibaculum* spp. infections and referred to as tenacibaculosis, is a less common ulcerative disease in Norwegian Atlantic salmon farming than winter ulcer disease. However, it is associated with severe mortality events when it occurs. The disease manifests itself as ulcers around the jaw, and sometimes around the tail, and fins (Sommerset et al., 2020). Tenacibaculosis in northern Norway is mostly associated with the "*Tenacibaculum finnmarkense*" strains as causative agents (Småge et al., 2018). Risk factors for developing mouth rot in Norwegian salmon farming have not been thoroughly explored. However, the condition is more commonly observed in warmer water temperatures than winter ulcer disease and it is particularly observed following transfer to seawater, whether it is in sea cages or land-based systems (Småge et al., 2018, 2017).

5.4 Quality grading

Quality grading according to the scheme specified in the industry standard NBS 10-01 (Industry Standards for Fish, 1999) is a common feature of the primary processing of farmed Atlantic salmon in Norway. While fish quality is a complex concept, involving such characteristics as taste, nutritional value, and the ethical standards according to which the fish was raised (Nordtvedt et al., 2007), the NBS 10-01 industry standard specifically focuses on the externally observable characteristics of gutted fish. The slaughtered fish are assigned to one of the quality grades Superior, Ordinary, and Production according to the type and severity of observed quality defects. Defects associated with the Ordinary category include dark spots and damage to the skin or fins. More severe defects, such as pronounced dark spots, ulcers, and indications that the fish are sexually mature, result in the fish being downgraded to the Production category.

These quality traits do not necessarily affect product safety or edibility. For example, a sexually mature Atlantic salmon is fully edible and qualitatively different, rather than inferior, to immature ones. Nevertheless, industry and consumers demands for homogenous product quality results in these fish being downgraded according to the quality scheme (Nordtvedt et al., 2007).

Superior fish receive a higher price than Ordinary fish. A production fish require considerable processing to be permitted for sale, although it may also be discarded (Forskrift om kvalitet på fisk og fiskevarer, 2013; Michie, 2001). Thus, obtaining fish of good quality is economically desirable.

Specific quality defects, such as dark spots, have been recognised as a considerable cause for economic losses around slaughter. Even less severe and easily removable dark spots have been associated with a 5-10 % loss, and severe spots, leading to quality downgrading have been associated with a 30-100 % loss (Mørkøre, 2017). In general, publicly available data on the distribution of quality grades and causes for downgrading in the industry is piecemeal.

6 Objectives

The underlying foundation of this thesis was having access to the production and primary processing records of a large, Atlantic salmon producer with operations in northern Norway. The framing of the research was therefore contingent on data availability and quality. The specific objectives chosen for of each of the studies in this thesis were to:

- Evaluate the impact of smolt production strategies and lice treatments on seawater growth performance, general mortalities, and mortalities due to HSMI or CMS (**Paper I**).
- Characterise the temporal dynamics of and find production related drivers behind skin ulcer and mouth rot prevalence (**Paper II**).
- Establish the relative importance of the principal, proximate causes for downgrading during primary processing of harvested salmon and find possible associations with production related drivers (**Paper III**).

7 Methodology

This thesis comprises of analyses of production data from one large production company. In addition to descriptive analyses, we used two types of models to elucidate relationships between the target variables and various production related explanatory variables: generalised additive models (GAMs), or generalised additive mixed models (GAMMs), and transformation based redundancy analysis (tb-RDA). Both types of models are commonly applied to analyse complex data, e.g. in ecology and epidemiology. GAM(M)s are described in section 7.2 and tb-RDA is described in section 7.3.

7.1 The dataset and its pre-processing

The main dataset used for all analyses in this thesis was provided by one large and vertically integrated production company with operations in northern Norway. The sites studied are in production regions 9 and 12, between latitudes 67 °N and 71 °N (Figure 1). The data covered all aspects of fish rearing, from the egg stage until and including primary processing. Data from all hatcheries and seawater net cages, and from one processing plant, owned and operated by the company were available to us. Little to no data from relevant external actors, e.g. smolt suppliers or seawater production sites not owned and operated by the company, were available. Some additional data regarding delousing events were extracted from BarentsWatch (www.barentswatch.no), a website hosting publicly available data relevant to disease and disease treatments in Norwegian aquaculture, and data on wind conditions were downloaded from the website of the Meteorological Institute of Norway (www.met.no).

Figure 1 : A map of Norway with the 13 marine aquaculture production regions indicated. The data analysed in this thesis originated from farms in regions 9 and 12. Source: https://www.lovdata.no.

The production records in the company's database were available as time series with daily entries. For example, temperatures, amounts of feed fed per cage, and cause-specific mortality counts were available as time series. For the analyses we calculated aggregate values, e.g. specific growth rates or cumulative mortalities, ourselves. Contextual data, e.g. name of site, production unit, county, year class, the supplier of brood stock or smolts, was also available in the production records.

The company has recorded cause specific mortalities according to clearly defined and standardised guidelines since 2014, although some mortality causes have been recorded since the start of the dataset. While the guidelines have been developed by the company's fish health experts, the farmers themselves assign the collected mortalities to a specific cause.

The slaughter records from the processing plant were based on the quality evaluations of random samples of about 100 fish. Such evaluations are done several times per day, and usually multiple times per processed production cycle, by the processing plant workers.

Most of the data made available by the company were extracted from a centralised database, accessed through the company's stock management system, FishTalk™ (AKVA group, Bryne, Norway). The exception to this were the slaughter records from the processing plant, which were provided as a single spreadsheet. While data in the database were available from at least as far back as the early 2000's, we chose to only extract data from seawater production cycles commencing after the beginning of 2010 and ending before the end of 2018. This was due both to practical difficulties associated with extracting data from the company's database, and that changes in the company's structure and operations during this time made older data less relevant.

7.2 Generalised additive (mixed) models

GAMs are models that describe the relationship between a response variable and one or more explanatory variables, or covariates, by smooth functions, commonly called smoothers. As with generalised linear models (GLMs), the models are general in the sense that distributions beyond the Gaussian, e.g. other distributions in the exponential family, may be assumed for the response. However, they represent a more flexible framework than GLMs by allowing the modelling of non-linear patterns or relationships in the data (Wood, 2017; Zuur et al., 2007). A generic formulation of an additive model is:

$$y_i = \alpha + f_1(x_{i1}) + \cdots + f_p(x_{ip})$$

For p explanatory variables and i observations, where α is the population intercept. The functions f_i is a population smoothing function of which several varieties are available. In this thesis thin plate regression splines and cyclic cubic regression splines are used. Thin plate regression splines are versatile and computationally inexpensive. A cyclic cubic regression spline is a function penalised to produce a smooth with the same value for the lower and upper boundaries. It is therefore well suited to represent cyclic phenomena, e.g. the day of year (Wood, 2017; Zuur et al., 2009).

GAMs can be extended to account for intra-group variability by including the grouping variable, a factor, as a random effect. Such models are commonly referred to as GAMMs, or hierarchical generalized additive models. In the case of the dataset analysed in this thesis, a hierarchical structure is already present in the data. Fish groups are cultivated in production units, i.e. tanks or cages, that are part of groups of production units that constitute production sites, i.e. hatcheries or seawater cage sites. Each group of production units will necessarily be subject to similar management practices and certain environmental conditions, e.g. influent water qualities. Such grouping variables, e.g. production site, may not be variables of interest in themselves and including them as fixed effects may claim too many degrees of freedom in the model. Nevertheless, one may be interested in characterising the underlying population while still accounting for the grouping structure in the data. In this case, including the variables as random effects allows the relationship between the explanatory variables and responses to vary between groups, by allowing for a random intercept, while pooling the functions representing the relationship toward a common shape (Gelman and Hill, 2007; Pedersen et al., 2019; Zuur et al., 2009).

When several reasonable candidate models for a certain response can be specified, model selection tools can be used to find the model that best explains variability in the response given some criteria. Additive modelling allows for model selection using either information-theoretic tools, such as the Akaike Information Criterion (AIC), or hypothesis testing, using an appropriate statistic, such as the *t*-statistic, the *F*-statistic, or the likelihood ratio test. In practice, model selection proceeds from fitting a global model, including all explanatory variables and interactions relevant to answering the research question at hand, and then sequentially removing non-significant variables and interactions until a parsimonious model is obtained. Model validation proceeds by inspecting plots of residuals versus fitted values, and versus each of the explanatory variables, for systematic variation. This is also necessary for each of the grouping levels of a random effect in mixed modelling. When using non-Normal error structures, it is also necessary to check for overdispersion, i.e. whether the variance of the data exceeds that predicted by the model's error structure. Finally, problematic dependencies, or collinearity, among the explanatory variables should be evaluated through variance inflation factors or concurvity (Harrison et al., 2018; Ramsay et al., 2003; Zuur et al., 2009).

7.3 Transformation based Redundancy Analysis

Redundancy analysis (RDA) is commonly employed in ecological studies for ordination, i.e. the ordering of species or observations according to some gradient.

Ordination methods provide an opportunity for visualising complex multivariate relationships in a way that is easy to interpret (Legendre and Legendre, 2012).

RDA is a method for the simultaneous analysis of two, or more, data tables. Of these, one, a matrix **Y**, is a table of response variables and the other, a matrix **X**, is a table of explanatory variables. Each variable in **Y** is regressed on **X** to compute the fitted values, contained in a matrix $\bar{\mathbf{Y}}$. The canonical relationship between **X** and **Y** is then tested for its significance, i.e. to find if more of the variation in **Y** is explained by the **X** variables than randomly generated data. If significant, $\bar{\mathbf{Y}}$ is subjected to a principal component analysis (PCA) to produce a matrix **U** of canonical eigenvectors and a vector of canonical eigenvalues. $\bar{\mathbf{Y}}\mathbf{U}$ is computed to produce an ordination of the fitted values in the space of **X** which is used to visualise the relationships between the responses, explanatory variables, and observations (Borcard et al., 2011; Legendre and Legendre, 2012). Thus, RDA resembles an extension of multiple linear regression to cases where there are multiple response variables, e.g. compositional data (Zuur et al., 2007).

To accommodate response data tables where one or more of the vectors contain many zeroes, or overall lower counts than the other vectors, a transformation of the **Y** matrix may be necessary prior to RDA, yielding a so-called tb-RDA (Legendre and Legendre, 2012). While many transformations are available, we opted to use the Hellinger distance transformation, which is calculated as:

$$D_H(x_1, x_2) = \sqrt{\sum_{j=1}^{p} \left[\sqrt{\frac{y_{1j}}{y_{1+}}} - \sqrt{\frac{y_{2j}}{y_{2+}}} \right]^2}$$

For a table with two observations, where y_{ij} are the entries in the table represented by the matrix **Y** = $[y_{ij}]$ with rows *i* and columns *j*. Thus, y_{1+} and y_{2+} denote row sums and *p* the number of columns (Legendre and Gallagher, 2001).

Forward variable selection can be used with RDA to obtain a parsimonious model, starting from a model with only an intercept and sequentially adding the available candidate variables, remodelling and testing the new model at each step. Variables are added in order of decreasing *F*-values and permutation tests are used at each step to evaluate whether the added variable is significant according to a predefined significance level (Borcard et al., 2011).

8 Paper summaries

8.1.1 Paper I

In **paper I** we wanted to discover the effects of i) growth during the freshwater hatchery stage, ii) fish weight at seawater transfer, and iii) the timing of seawater transfer upon the growth, total mortality rate and mortality rate attributed to HSMI or CMS during the seawater stage. Additionally, we included the presence of bath and mechanical delousing treatments as binary covariates when modelling mortality. To represent seawater growth, seawater SGR, TGC, and a growth index were used as responses. GAMMs were used with seawater production site and smolt supplier as random effects.

We found that a higher SGR obtained during the freshwater stage significantly decreased subsequent seawater growth, while smolt weight did not have a significant effect, within the range of weights studied. Total mortalities significantly decreased with increasing freshwater stage SGR and increased with the presence of mechanical delousing treatments. Total mortalities were significantly lower when mean temperatures were in the range between 7.5 °C and 8.5 °C. Mortalities attributed to HSMI or CMS increased significantly with increasing smolt weight, decreasing mean seawater temperature, and the presence of mechanical delousing treatments. Overall mortalities and mortalities attributed to HSMI or CMS were significantly lower in fish transferred to seawater during spring through early summer.

8.1.2 Paper II

The purpose of **paper II** was to describe the temporal variation in mortalities due to skin ulcers and mouth rot, and to find production related drivers behind variation in mortalities attributed to these causes. We found that the monthly mortality rate attributed to ulcers were highest during the winter months, peaking in February through April, and that it was seemingly higher during the first winter at sea. Mortality rate attributed to mouth rot seemed to be negligible past the first 7 months of the seawater stage.

We set up a GAMM with mortality rate attributed to ulcers during the first winter at sea, specifically the months February through April, as a response and seawater production site as a response. The covariates included smolt weight, mean seawater temperature during the first 31 days at sea, mean seawater temperature and production density during the winter months, mortalities attributed to mouth rot, and the number of fish originally stocked in each cage. Smolt production strategy (0+ or 1+), year class, and smolt supplier were included as factors.

We also set up a GAM with mortality rate attributed to mouth rot during the first 200 days in seawater as a response. The covariates included smolt weight, the number of fish originally stocked in each cage, mean seawater temperature during the first 31 days following seawater transfer, and sea lice level, production density, and mortality rate attributed to ulcers during the first 200 days after seawater transfer. We also included the difference between mean seawater temperature during the first and fourth weeks following seawater transfer, to indicate whether the water temperatures were decreasing or increasing when the fish were transferred. Smolt production strategy (0+ or 1+) was included as a factor.

We found that 1+ smolts have a higher incidence of mortalities attributed to ulcers but a lower incidence attributed to mouth rot. There was little significant variation between smolt suppliers with regards to ulcers, except for one smolt supplier that was associated with a lower incidence of mortalities due to ulcers and one that was associated with a higher incidence. Mortalities attributed to ulcers significantly increased with increasing mean seawater temperature during the first 31 days following transfer and with the number of fish stocked per cage. A higher incidence of mortalities attributed to mouth rot was associated with an increase in mortalities due to ulcers, but not vice versa. The incidence of mortalities attributed to mouth rot increased with smolt weight and the number of fish stocked per cage but decreased with a higher mean seawater temperature during the first 31 days. A higher incidence of mortalities attributed to mouth rot was associated with transferring fish to seawater when water temperatures were falling.

8.1.3 Paper III

In **paper III** we analysed the records from a processing plant, describing the amount of sampled fish downgraded due to a specific, pre-defined quality defect in each processed batch. The records describe raw material quality traits in gutted fish. Furthermore, we collated these records with the production records for the seawater cage production cycles from which these processed batches originated. This was done to find production related drivers behind the variation in quality downgrading. For this we used tb-RDA as an exploratory method to visualise the relationships between the multivariate response, comprising of the counts of the four most common downgrading causes, and the explanatory variables. The tb-RDA variable selection was used to find candidate exploratory variables for further modelling using GAMs. A GAM was fit for each of the four most common downgrading causes.

The presence of ulcers was the main cause for downgrading, accounting for 7.4 % of processed and 39 % of downgraded fish, followed by dark spots, accounting for 17 % of downgraded fish, deformities, accounting for 12 % of downgraded fish, and early sexual maturation, accounting for 10 % of downgraded fish. Ulcers contributed the most to fish being downgraded to production grade and thus represent the most severe challenge to quality in our study. Overall, growth (SGR) during the seawater stage, mean and variation in seawater temperature, mortalities due to HSMI or CMS, and the timing of harvest (day of year) were important explanatory variables behind quality variation. Ulcers were more prevalent when seawater temperatures were lower and less variable, and in production cycles that were harvested during winter and spring. Dark spots were more prevalent in production cycles that experienced higher mean seawater temperatures and that had a higher incidence of mortalities due to HSMI or CMS. Deformities were more prevalent in production cycles experiencing mean seawater temperatures between 6.5 °C and 7.0 °C and that were transferred to seawater at higher temperatures and shorter daylengths. A higher prevalence of early sexual maturation was associated with a higher harvest weight, lower sea lice levels, and production cycles transferred to seawater during shorter daylengths.

9 General discussion

9.1 Findings

This thesis is a study of the impact of production strategies and variables on production performance, including cause specific mortalities and quality outcomes, in Atlantic salmon aquaculture. The data covers a single company's operations, with a focus on northern Norway, over a limited time period (8 years).

In **paper I** we attempted to test the effect of smolt production strategies on subsequent performance during the seawater stage, with a particular focus on cardiac health, by including mortalities due to HSMI or CMS as a response. A particular challenge was presented by the modelling of the effect of hatchery growth and smolt weight upon seawater growth, due to its natural, non-linear variation throughout the production cycle and dependence on body weight. Comparing three response variables to circumvent this problem may not be an ideal solution but gives an indicative result. A further limitation is the limited range of smolt weights studied, where only a few observations had smolt weights exceeding 250 g, as weights of smolts or post-smolts transferred to seawater cages now can exceed 500 g (Dam, 2020). While we did not find an effect of smolt weight upon subsequent seawater growth, we did find that increased freshwater SGR resulted in a lower seawater TGC, which agrees with previous findings (Frisk et al., 2020).

Higher smolt weight appears as a cause for the increased prevalence of HSMI or CMS in **paper I**, although the effect is relatively small, and more clearly mouth rot, in **paper II**. A negative impact of intensive smolt production on cardiac health has been observed previously (Frisk et al., 2020), and a higher smolt weight could be indicative of this in our dataset. However, as far as I know, no such effect has been discussed regarding mouth rot. The strength of the effect for smolt weight and an opposite effect of producing 1+ smolts, which are typically larger as they are older upon smoltification and transfer, supports the notion that the effect is not due to a confounding variable. As increasing smolt weights seems to be a trend (Bergheim et al., 2009; Dam, 2020), conducting further investigations on the effects of producing large smolts upon long-term fish health and welfare is imperative.

A clear effect of mechanical delousing treatments, but not bathing treatments, leading to increased total mortalities and mortalities attributed to HSMI or CMS was found in **paper I**. In general, delousing treatments are known to be stressful to the fish and affect their behaviour (Føre et al., 2018). Bathing treatments using higher concentrations of hydrogen peroxide have been shown to result in elevated mortalities (Overton et al., 2018), although the magnitude of the effect is uncertain. Mechanical treatments typically involve low pressure washing or flushing of the fish skin with water to remove the lice. Little data on associated mortalities or welfare impacts exist, although farmers have reported that scale loss and mortalities are common (Overton et al., 2019). Our study presents novel evidence that mechanical delousing treatments lead to elevated mortality levels during a production cycle, although we cannot elaborate on the mechanisms behind this elevation or the temporal dynamics of such mortalities. Some of the mortality risk associated with delousing may be connected to crowding, pumping and handling procedures during the process, rather than the delousing itself. However, we had no data with which to isolate such an effect. There is a potential for employing preventive measures against sea lice infestations, that could alleviate or circumvent the potential negative effects associated with reactive treatments (Barrett et al., 2020).

Outbreaks of mouth rot, or tenacibaculosis, are clearly temporally restricted to the first 7 months following seawater transfer, as shown in **paper II**, and seems to gradually diminish after the first 4 to 5 months in the dataset we studied. This is in accordance with previous studies (Småge et al., 2018, 2017). Mouth rot occurs for the first time in mid-2015 in our dataset, well over a year after the recording of cause-specific mortalities commenced, and it is seemingly a recent occurrence in this region. This is also supported by recent publications describing the phenomenon (Olsen et al., 2011; Småge et al., 2017, 2016). Comparable afflictions, associated with *Tenacibaculum* spp., have a longer history in Canada and Tasmania (Frisch et al., 2018). Given the novelty of the diagnosis in Norway, few studies and little comparable data exist with which to explain and compare our findings.

A higher number of fish stocked per cage is associated with a higher prevalence of ulcers and mouth rot in our study. As the size of a cage is the main determinant of the amount of fish stocked in it and different sites employ different cage sizes, there could be a confounding effect here. However, a linear association is seen and stocking numbers are known to impact infection dynamics in aquaculture production units (Murray, 2009).

There are, to my knowledge, few studies summarising the variation in raw material quality in commercial production plants, especially using a comparable quality grading scheme. It is therefore difficult to evaluate the representativeness of the figures reported by the processing plant studied in **paper III**. A survey on the amount of fish downgraded due to ulcers, with three anonymised Norwegian processing plants participating, show the average proportion of fish downgraded for this reason varying between 0.7 % and 3.8 % in 2012 and 2013 (Takle et al., 2015). This is considerably lower than the 7.4 % observed in our study but may be due to the northerly location of the production sites we studied. Dark spots have been reported to be present in up to 19 % of slaughtered fish (Mørkøre, 2017). However, fish with less severe incidences of dark spots are not downgraded and it is therefore not meaningful to compare this reported prevalence with our results. A report from a Scottish processing plant shows matures, processing damage, deformities, and sea lice to be the most common causes for downgrading during primary processing (Michie, 2001). This may be due to differences in climatic conditions and advances in production and processing practices and techniques.

The dependency of ulcer prevalence in slaughtered fish upon the timing of harvest is not surprising given the temporal distribution of ulcer-related mortality seen in **paper II**. The temporal variation in the prevalence of ulcers in slaughtered fish could also indicate that some ulcerated fish heal, although this cannot be quantified with our dataset. While our results show a potential for abating the problem of downgrading due to ulcers by adjusting harvest times according to estimated prevalence, the high mortality or culling rates during winter makes extending the production an unreasonable proposition. However, the fact that the majority of fish do not develop ulcers to the extent that they are culled or downgraded, or die naturally, shows that the problem can likely be avoided if the risk factors for this variation are identified and acted upon. The levels of mortality attributed to ulcers in our study, combined with the fact that all production cycles were likely vaccinated against *M. viscosa*, is evidence that the vaccine is not 100 % effective in our study, which is to be expected, and this has also been noted by other authors (Karlsen et al., 2017b). Thus, obtaining a wider range of proactive tools to avoid ulcer development is desirable.

The overall importance of ulcers, both as a cause of mortalities and of downgrading, in these studies can to some extent be explained by the northerly location and the associated prolonged periods with cold seawater temperatures experienced by fish cultivated at these latitudes. 80 % of all detected cases of *M. viscosa* and *Tenacibaculum* spp. reported in Norway are found in the northern part of the country (Sommerset et al., 2020). Both in **paper II** and **paper III** a clear association between ulcers and low seawater temperatures is seen. This agrees with the literature reporting on winter ulcer disease as mainly being a problem when temperatures are below 8 °C to 10 °C (Greger and Goodrich, 1999; Løvoll et al., 2009). The proportion of fish downgraded due to ulcers and the severity of the downgrades, as found in **paper III**, demonstrate the extent to which some diseases and adverse welfare conditions have consequences beyond causing elevated mortality levels. This is not captured by official statistics, nor publications on the various conditions that cause these mortalities or quality downgrades, and highlight the need for including data from multiple stages of the production process when assessing the overall welfare impact of the production strategies used.

The presence of dark spots has been strongly associated with piscine orthoreovirus (PRV), the causative agent behind HSMI (Bjørgen et al., 2015). However, the extent that it is a causative agent, or acts as an opportunist, is unclear, and the aetiology of dark spots remains unknown (Bjørgen et al., 2019). It has been proposed that dark spots form due to chronic inflammation following trauma or infection and an associated immune response to opportunistic pathogens (Krasnov et al., 2016). Our results show clearly that an increase in the prevalence of dark spots is associated with increasing mortalities attributed to HSMI or CMS. As in **paper I**, it is possible that an even stronger association could have been seen if mortalities due to HSMI and CMS were accurately differentiated in the dataset. In **paper I,** increased prevalence of HSMI or CMS was associated with the use of mechanical delousing treatments, lower mean seawater temperatures, and increased smolt weight. In **paper III** no significant effect of delousing treatments or smolt weight were found on the prevalence of dark spots. It is possible that the linearity constraints imposed by the RDA, or the Hellinger transformation, may have contributed to the elimination of these variables and that they would have been significant if included in the GAM. Similarly, in **paper III** the prevalence of dark spots is shown to increase with mean seawater temperature, whereas in **paper I** the prevalence of HSMI or CMS is shown to decrease with increasing mean seawater temperature. While such an effect may be attributed to the lack of differentiation between HSMI and CMS in the dataset, **paper III** also relies on a more geographically restricted subset of the dataset, i.e. only including production region 12, than does **paper I**. Thus, the results in **paper III** may be affected by a regional dynamic that is not picked up in the analyses in **paper I**.

It is less straightforward to interpret the significant explanatory variables found for deformities and sexual maturation. Deformities have been connected to high incubation and larval rearing temperatures and including smolt supplier as a fixed effect could possibly have increased the explanatory power of the model. Other observed risk factors include lower smolt weights, low growth and low salinity (Vågsholm and Djupvik, 1998a). The increasing prevalence of deformities with increasing seawater temperature upon transfer could possibly be associated with smolt weight in our study.

9.2 Methodological considerations

The tenet of this thesis was that something could be learned from analysing a dataset from commercial salmon farming operations. Since the extent and quality of the said dataset limits what hypotheses can be meaningfully and satisfactorily answered, this has also represented the main risk and challenge throughout this work.

The data used for the analyses is mainly used within the company's enterprise resource planning (ERP) system to record and give projections of e.g. mortalities, feed usage, and associated costs. Furthermore, some figures are regularly reported to select government institutions for monitoring compliance with e.g. allowable biomass and density restrictions. Thus, there is a minimum of data that will be available for all sites covered by the dataset. Beyond this, however, the extent and regularity with which the various sites record certain variables, especially environmental ones, varies considerably. Amongst environmental variables, only temperature registrations are complete for nearly all seawater sites. A few sites record only this variable, while others also record oxygen and/or salinity at regular intervals. Thus, if two or more of these environmental variables are to be included in a model, the number of observations for which all explanatory variables are available is small and confined to a few sites only. Data from well boat transports and holding cages at the processing plants are completely missing from the dataset, and these situations are known to impact fish welfare and slaughter quality (Ådland Hansen et al., 2012; Erikson et al., 2016; Iversen et al., 2005, 1998).

Further compromising the availability of data for modelling in this thesis is the difficulty of collating data from different production stages. The database lacked an identifier with which e.g. a hatchery production cycle could be connected to its subsequent seawater stage production cycle. This was also a challenge when attempting to collate the seawater stage production records with the slaughter records, which required much manual inspection. Adding to this, fish from several hatchery tanks may be mixed during the stocking of sea cages, which severely diminishes the ability to identify and trace fish and fish groups through the production chain, i.e. from egg to slaughter. Also, some variables, such as fish weight, only exist as aggregate values, e.g. means, on the group level. Thus, when fish groups are merged the information on mean weight in the group is lost, as means of means cannot reliably be calculated, until new representative weight samples are taken. These problems may be abated if weights can be estimated for each individual fish upon or after stocking, or if recognition and tracking of individual fish, e.g. through tagging or appearance-based video tracking, becomes a possibility (e.g. Yang et al., 2020; Zhang et al., 2020).

The veracity, or accuracy, of the data used represent another constraint to the analyses. The data are historical, cover several sites, and have been collected by numerous workers over the years. Without established and standardised procedures for taking measurements and estimating uncertainty, or the availability of reference data, the veracity of data originating from a company's legacy database cannot be ascertained beyond identifying clearly unrealistic data entries. In the analyses presented in this thesis the amount of data somewhat abates this problem and the relationships found between responses and explanatory variables likely hold. There is nevertheless a need for standardised procedures for data collection and data quality control in aquaculture which is particularly pressing given the reliance upon such data in everything from day to day farm management to governance (Lien, 2015, 2007).

Another challenge with using production data from Atlantic salmon farming is the lack of variation in the data. For example, producers typically follow set procedures in rearing the fish based on their knowledge of the fishes' biological requirements, e.g. by maintaining certain temperatures during incubation, hatching, and start feeding or using feed with a certain dietary protein to lipid ratio for a given weight range. Also, for practical, contractual, or economic reasons, only a limited range of feeds or vaccines may be in use and only a limited weight range of smolts may be produced. This limits the scope of potential analyses on the data. The lack of variation also makes isolating the effects of confounding variables difficult. For example, in our dataset, smolt weight was highly correlated with day of seawater transfer, which again is inherently related to daylength and seawater temperatures. There was also an uneven distribution of smolt weights, e.g. with very few production cycles with weights above 200 g. The strength of designed experiments is that variation can be produced through the systematic manipulation of relevant explanatory variables. Nevertheless, production data have an advantage in the volume of available data, both in terms of replicates and observations, and in representing the real conditions under which salmon farming takes place; conditions which in some cases would be prohibitively expensive to replicate in an experimental setting. Also, where an analysis of production data and an experiment may answer the same question equally well, production data analysis has the ethical advantage of not relying on the use of experimental animals.

In our analyses, we did not make full use of the information contained in the time series data available to us. Instead, we relied on aggregate values, e.g. averages of temperature registrations over a period or sums of mortality counts. Given the lengths of Atlantic salmon seawater production cycles in the region, some exceeding two years, much information is likely lost by relying on aggregate values. A lot of the variation in ulcerative disease development, for example, could likely have been better explained by the daily or weekly variation in temperatures. Generalised additive models can be extended to model such relationships (Zuur et al., 2009). Specialised methods for analysing exposure-response relationships in time series are also available, such as distributed lag models and distributed lag nonlinear models. These models account for temporal lags between exposure and response, which may have been relevant in our case, and have been used to estimate human mortality risk associated with e.g. high ambient air temperatures (Bhaskaran et al., 2013; Gasparrini et al., 2010; Gasparrini and Armstrong, 2011). However, such models are generally challenging to specify and have not yet, to my knowledge, been developed for cases with time series of varying length and highly time-varying population sizes. Functional data analysis, where time series are used as covariates to model a single response variable (Wood, 2017) could also be applied to model e.g. the impact of the daily ambient seawater temperatures experienced by the fish on the prevalence of downgrading due to ulcers.

Record keeping has long been recognised as an important tool for performance monitoring in aquaculture production management (James, 1935). The utility of sophisticated multivariate analysis for assessing production performance and identifying possible causes or risk factors for inferior performance, have long been recognized in pond aquaculture (Milstein and Hulata, 1993; Prein et al., 1993). In sea cage aquaculture, where fewer mechanisms are realistically available for exerting control over the production than in pond aquaculture, the use of production data has predominantly been epidemiological. In this regard, mortality records that include cause attribution have proven particularly valuable in finding risk factors for disease and adverse health states (e.g. Aldrin et al., 2011; Aunsmo et al., 2008; Bang Jensen et al., 2020; Kilburn et al., 2012; Soares et al., 2013, 2012, 2011), as has also been demonstrated in this thesis. Mortality has been recognised as an important retrospective welfare indicator in farmed fish (Ellis et al., 2012) and this thesis demonstrates that primary processing quality should also be recognized as such.

While much attention is presently given to novel data collection methods in aquaculture, most notably camera and machine vision technologies, more general aspects of data use and structuring has seemingly not been extensively studied in recent decades (El-Gayar, 1997; Prein et al., 1993; Prein and Milstein, 1988; Saberioon et al., 2017; Zhang et al., 2020; Zion, 2012). With multiple, multivariate and variable length time series and complex intrinsic underlying hierarchies, aquacultural datasets present a particularly complex modelling scenario. Development of specialised procedures and methods for handling and analysing such datasets will be necessary for more refined analyses without extensive loss of information.

10 Concluding remarks

The findings in this thesis demonstrate that several production characteristics and technologies are associated with increased mortalities and inferior production performance in Atlantic salmon aquaculture. This reveals a potential for improving fish health and welfare and production output by modifying production strategies.

We found that mechanical delousing treatments were significantly associated with increased total mortalities and mortalities due to HSMI or CMS, while bathing treatments were not.

A higher smolt weight was associated with both significantly higher mortalities due to HSMI or CMS and to mouth rot. The effect on mortalities due to HSMI or CMS may be attributed to an adverse effect on cardiac health, thereby predisposing the fish to these pathologies. The effect on mortalities due to mouth rot is strong but not easily explainable with the available dataset. This association should be investigated further.

This thesis has shown that ulcers, dark spots, deformities, and early sexual maturation are important causes for downgrading fish during slaughter in the studied region. 7.4 % of the processed fish were downgraded due to ulcers. Out of these, over half were downgraded to Production grade. Ulcerative disease is also a cause for persistent mortalities throughout many of the production cycles analysed, being most severe during the winter months. The overall impact of ulcers on production performance in the region is likely underreported. Prior incidence of mouth rot is a risk factor for increased mortality due to ulcers. Low temperature is a risk factor for both mortality and downgrading due to ulcers. Production cycles stemming from one smolt supplier were associated with significantly lower prevalence of mortalities due to ulcers, indicating a potential for reducing ulcer prevalence through optimising smolt production.

A higher prevalence of mortalities due to HSMI or CMS was associated with a significantly higher prevalence of dark spots. This conforms with previous studies finding a strong association between PRV infection and the prevalence of dark spots.

This thesis demonstrates the utility of analysing aquacultural production data in finding production related risk factors for cause specific mortality, quality downgrading, and overall inferior production performance. It also highlights the challenges associated with collating and analysing commercial production data. There is a need for studies that address the standardisation of data and improvement of data quality in aquaculture. There is also a need for developing analytical frameworks that can accommodate the specific challenges associated with aquacultural datasets, including the presence of variable length time series and hierarchical structures in the data.

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12 Papers

The effect of freshwater production strategy on post-seawater transfer growth, total mortality, mortalities attributed to HSMI/CMS in Atlantic salmon (*Salmo salar* L.) aquaculture and the associated effects of delousing treatments

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ABSTRACT

In Atlantic salmon farming, novel production methods and technologies incentivise changes in production strategies. Recent decades have seen increases in smolt sizes and freshwater hatchery growth rates, despite the impacts of these being poorly documented. In this study we analysed commercial production records from 40 sites in northern Norway to evaluate the impact of freshwater hatchery specific growth rate (SGR), smolt size, and the day of seawater transfer upon subsequent seawater stage growth performance. We also analysed the impact of these variables and the presence of bathing and mechanical delousing treatments upon total seawater mortalities and seawater mortalities attributable to HSMI or CMS. Our results show that increasing freshwater SGR significantly reduces the growth attained during the subsequent seawater stage. Smolt weight does not significantly affect subsequent growth within the range studied. Furthermore, total mortalities during the seawater stage increase with lower freshwater SGR, while mortalities attributed to HSMI or CMS increase with larger smolt sizes. Both total mortalities and mortalities attributed to HSMI or CMS are significantly higher when mechanical delousing treatments have been applied, while neither are significantly impacted when using bathing treatments. Our results may contribute to improving production planning and scheduling in the studied region.

Key words: Atlantic salmon, aquaculture, growth, mortality, HSMI, CMS, delousing treatments

1 Introduction

Atlantic salmon production can be considered a two-stage process in which the first stage traditionally encompasses the rearing of eggs to smolts in fresh/brackish water land-based hatcheries and the second stage encompasses the on-growing of post-smolts in marine-based net cages until slaughter, with this latter stage seeing the bulk of the biomass growth. Both stages of the production process are undergoing refinement and development, and the range of production systems and strategies being developed, tested, and used commercially has increased markedly over recent years. Examples include sea-based closed containment systems and land-based, brackish- or saltwater systems for ongrowing. The dominant sea-based production system, however, remains open net cages (Asche et al., 2018; Lekang et al., 2016).

Net cage sea based production (hereafter termed seawater production) exposes salmon to numerous risks including viral diseases (e.g. Cardiomyopathy syndrome, CMS or Pancreas disease, PD), and bacterial diseases (e.g. winter ulcer), ectoparasites including sea lice, *Lepeophtheirus salmonis* and *Caligus elongatus,* and the ectoprotozoan *Neoparamoeba perurans* that causes amoebic gill disease (AGD), predation, harmful algal blooms (HABs) and potentially challenging environmental conditions (Noble et al., 2018; Pincinato et al., 2021). Cage based production systems generally allow for little control over the fishes' environment and farmed Atlantic salmon are subjected to daily and seasonal variations in weather, temperature, oxygen and photoperiod (Oppedal et al., 2011), especially in northern latitudes. The quality of fish transferred to seawater and the timing of transfer are therefore two ways, or managerial factors, in which farmers can initially influence the biological performance of the fish in the sea.

To reduce the fishes' exposure to risk in the seawater cage-based on-growing phase, recent decades have seen an increase in the average body weight of smolts transferred to seawater, with incentives for this increase including a desire for reducing the time the fish spends in seawater net cages and thereby reducing the overall risk of disease and parasite infestation. This is not to be confused with the concurrent interest in keeping smoltified fish in land-based systems for prolonged periods, although the motivation behind this is similar. The mean sea water transfer weight for 0+ and 1+ smolts collectively approached 140 g in Norway in 2009, and mean weights for 1+ smolts have exceeded 200 g on some farms globally (Bergheim et al., 2009; Ellis et al., 2016). In conjunction with this increase, concerns have been raised that either increasing growth rates during the freshwater stage or increased smolt sizes, or both, may have adverse effects on subsequent seawater performance (Frisk et al., 2020).

The viral disease CMS, caused by Piscine Myocarditis virus (PMCV), is a major problem for Norwegian aquaculture, affecting over 100 farms per year, and it is, alongside heart and skeletal muscle inflammation (HSMI), one of the most serious cardiovascular conditions to affect farmed Atlantic salmon (Bang Jensen et al., 2020; Finstad et al., 2012). HSMI, caused by Piscine orthoreovirus 1, PRV-1 (Wessel et al., 2020) is at least a risk factor for CMS and their pathologies are similar (Bang Jensen et al., 2013; Kongtorp et al., 2004, 2006). A recently published paper (Frisk et al., 2020) suggests that increased growth rates during the smolt production phase and increased smolt sizes may result in slower growth and higher susceptibility to cardiac deformities associated with cardiac rupture in CMS outbreaks during the ongrowing stage in seawater. Such cardiac rupture is associated with stressful events, examples of which include delousing treatments using hydrogen peroxide or other chemotherapeutic baths or mechanical removal of lice (Meyer et al., 2019; Overton et al., 2019). In comparing small and large cohorts of 0+ and 1+ smolts, Roth et al. (2005) observed that the smaller cohorts for both smolt production strategies had higher overall growth rates in seawater compared to the larger cohorts, particularly during their second year in seawater, and both cohorts of 1+ smolts grew faster in seawater than their respective 0+ counterparts. Upon comparing salmon reared in a recirculating aquaculture system (RAS) under different salinity and smoltification configurations and transferred to seawater at 100, 200, and 600 g as post-smolts, Ytrestøyl et al. (2018) found that growth rates in seawater were significantly lower for the 600 g post-smolt group, while no significant difference was seen between the 100 g and 200 g groups. These studies, however, relied on a limited number of experimental cohorts to draw conclusions and do not consider the relative effects of freshwater growth rate, smolt size, or timing of seawater transfer together.

In this study, we analysed commercial production data from a large, commercial Atlantic salmon producer in Norway, with the aim of evaluating the effects of growth during the freshwater hatchery phase, smolt size, and transfer day on growth, total mortalities, and mortalities attributed to HSMI or CMS during the seawater phase. We also studied the effect of delousing treatments upon total and HSMI or CMS related mortalities.

2 Materials and Methods

2.1 Data handling

The dataset used in this study originated from a single, large production company and utilised production records from 40 seawater net cage production sites in northern Norway (from 67 °N to 71 °N). The original dataset comprised of time series with daily recordings of several production related variables for each cage, or production cycle, as collected by the workers on each farming site. As the production cycle represented the smallest identifiable unit in the dataset, we chose these as our subject of analysis. We excluded production cycles that took place in more than one cage, i.e. by fish being transferred to another cage during the production process, and production cycles that did not result in harvest, e.g. by being sold or experiencing excessive mortalities. Additionally, production cycles with recorded seawater SGRs above 2.5 % and/or smolt weights above 220 g were removed, as these constituted few but influential observations. Production cycles with missing values for variables included in the different models were also removed. This data cleaning affected the models for each response variable differently, and they therefore have different numbers of observations, which are provided along with the results.

To prepare the dataset for modelling, the relevant variables were selected and aggregated per production cycle. Specific growth rate per production cycle was calculated as $SGR\ (\%) = (\ln W_F - \ln W_I) \times \left({}^{100}/_{\Delta t}\right)$, where W_F is the final weight of the fish, W_I is the initial weight of the fish, and Δt is the duration, separately for the freshwater hatchery and seawater net cage stages. Similarly, for the seawater stage, the thermal growth coefficient (TGC) was calculated as $TGC = 1000 \times (\sqrt[3]{W_F} - \sqrt[3]{W_I}) \times D^{-1})$, where D is the sum of day degrees (°C) for the production cycle. A relative growth index (RGI) was calculated by dividing the obtained SGR by a theoretical SGR, calculated by the company's stock management system (FishTalk$^{\mathrm{TM}}$, AKVA group, Bryne, Norway) based on growth tables. The dataset did not include initial weights of fry from external smolt suppliers, and this was therefore set to 0.2 g for all production cycles. For the final weight of the seawater production cycle, i.e. slaughter weight, we used the weight recorded for the harvest day in which the highest number of fish were harvested. This was because production cycles were often harvested over several days, with only average weights being recorded for each day, without data on variation. This made the calculation of an average harvest weight for each whole production cycle impossible. However, recorded weights generally varied little over consecutive days. For the freshwater production stage, Δt was calculated as the duration, in days, between hatching and seawater transfer. For the seawater cage production stage, Δt was chosen as the duration, in days, between seawater transfer and the day from which the W_F value was chosen.

Mechanical and bathing treatments against lice were included as explanatory variables when modelling HSMI and CMS related mortalities, due to their perceived relevance to the pathologies. As there were few such treatments per production cycle, making modelling of the number of treatments unwieldy, we binary coded these variables, indicating the presence/absence of either type of treatment in each production cycle.

2.2 Modelling

The hypotheses we tested were (1) whether growth in the hatchery phase, smolt size, and timing of seawater transfer influences subsequent seawater growth. We then tested whether freshwater growth in the hatchery phase, smolt size, the timing of seawater transfer, and the use of either mechanical or bathing delousing treatments influences (2) total mortalities, and (3) mortalities ascribed to HSMI and CMS. We used generalized additive models (GAMs) and generalized additive mixed models (GAMMs) (Wood, 2006) to evaluate all hypotheses. Additive modelling was chosen due to the presence of non-linear relationships and, particularly, the ability of the cyclic cubic regression spline smoother to capture the cyclic nature of seawater transfer day (as day of year) as a covariate. All other smoothers for fixed effects were thin plate regression splines, while those for random effects were ridge penalty smoothers. For the SGR and total mortality models, smolt supplier (freshwater hatchery site) and seawater production site were included as random effects to account for site specific sources of variation, e.g. operative and environmental differences, without consuming too many degrees of freedom.

As the number of potential explanatory variables was large compared to the number of observations, only the variables deemed most relevant were included in the models. Backward selection of variables, i.e. starting from a full model and sequentially removing the non-significant variables with the highest p-value and refitting the model until all terms were significant ($p < 0.01$), was used to for model selection along with Akaike's information criterion adjusted for small sample sizes (AICc). Models were further validated by checking residuals' independence, homogeneity, fit, and normality, variance inflation factors, and linear dependence among explanatory variables (concurvity). Concurvity values above 0.80 and variance inflation factors above 4 warranted closer model inspection.

2.2.1 Growth

To analyse the impact of freshwater SGR and smolt size at transfer upon seawater growth we opted to compare three models using different responses, namely seawater TGC, SGR, and RGI. This is due to SGR being heavily dependent on size and using smolt size as a covariate with SGR as a response necessarily will show an effect. To analyse the impact of freshwater SGR and smolt size at transfer on seawater SGR we fitted a model assuming a Gaussian distribution of the response and an identity-link function on the data. The fixed effects model specification was:

$$SW\_SGR_c = \alpha + f_1(FW\_SGR_c) + f_2(WS_c) + f_3(DT_c) + f_4(MT_c) + \epsilon_c$$

Where SW_SGR is the seawater SGR, FW_SGR is the freshwater SGR, *WS* is the smolt weight, *DT* is the day of transfer, and *MT* is the mean seawater temperature. f indicates a smoothing function; c denotes the individual production cycle and α is the intercept. Seawater cage site and freshwater hatchery site were included as random effects. Whilst mean seawater temperature was not among the main variables of interest, it was included to capture variation and to contrast the variables of interest with a known seawater production related influence on growth.

To analyse the impact of freshwater SGR and smolt size at transfer on seawater TGC we fitted a model assuming a Gaussian distribution of the response and an identity-link function on the data. The fixed effects model specification was:

$$SW\_TGC_c = \alpha + f_1(FW\_SGR_c) + f_2(WS_c) + f_3(DT_c) + f_4(MT_c) + \epsilon_c$$

Seawater cage site and freshwater hatchery site were included as random effects.

To analyse the impact of freshwater SGR and smolt size at transfer on seawater RGI we fitted a model assuming a Tweedie distribution of the response, due to overdispersion, and an identity-link function on the data. The fixed effects model specification was:

$$SW\_RGI_c = \alpha + f_1(FW\_SGR_c) + f_2(WS_c) + f_3(DT_c) + f_4(MT_c) + \epsilon_c$$

Seawater cage site and freshwater hatchery site were included as random effects.

2.2.2 Total mortalities

Total mortalities, as proportions of the number of fish originally stocked, were modelled assuming a Beta distribution and a logit-link function. The Beta distribution was chosen because attempts at fitting a model using Binomial or Quasi-Binomial distributions showed problematic overdispersion. The fixed effects model specification was:

$$logit(M_c) = \alpha + f_1(FW\_SGR_c) + f_2(WS_c) + f_3(DT_c) + f_4(MT_c) + TM_c + TB_c + o(log(DS_c)) + \epsilon_c$$

Where *M* is the total recorded mortalities per production cycle, *TM* is the presence of mechanical lice treatments, *TB* is the presence of bathing treatments against lice, and *o* denotes an offset variable, *DS,* which is the duration of the seawater phase. Duration was included as an offset variable to account for differences in mortality numbers due to differing durations of exposure to the open sea cage environment. Seawater cage site and freshwater hatchery site were included as random effects.

2.2.3 Mortality attributed to HSMI/CMS

Due to the uncertainty associated with differentiating the pathologies of HSMI and CMS on site, these were recorded as a single mortality cause in the dataset and had to be analysed as a combined response. We consider, however, that this combined response is a good indicator of poor cardiac status.

Due to difficulties fitting the model using any of distributions commonly used for proportional responses, the response was modelled assuming a Tweedie distribution with a logit-link function:

$$logit(MH_c) = \alpha + f_1(FW\_SGR_c) + f_2(WS_c) + f_3(DT_c) + f_4(MT_c) + TM_c + TB_c + YC_c + o(log(D_c)) + \epsilon_c$$

The model terms used here are the same as for total mortalities, except for the response, *MH*, indicating mortalities attributed to HSMI and CMS, and *YC* representing year class as a factor. Year class was included to account for possible annual variability in the incidence of the causative agents behind HSMI and CMS. As cause specific mortality recording was only fully implemented from 2014 onwards, the data used to model these mortalities comprised production cycles transferred to seawater from January 1st, 2014 and completed before December 31st, 2018. Due to the lower number of observations, only seawater cage site was included as a random effect in this model.

2.3 Software

The data, stored in a centralised database, was retrieved from the FishTalk^{TM} (AKVA group, Bryne, Norway) stock management system used by the company. All data cleansing and pre-processing was done using the Python programming language (version 3.7.3) and the associated Pandas (McKinney, 2010) and NumPy (van der Walt et al., 2011) libraries. Analyses were run in R (version 3.6.1) using the mgcv (Wood, 2011) package for fitting GAMs and MuMIn (version 1.43.17) for calculating AICc values, and car (version 3.0-10) for calculating VIF values. Visualisation of the results was done using the R packages ggplot2 (version 3.3.2), lattice (version 0.20), and gratia (version 0.4.1).

3 Results

The processed dataset covered 40 sites and, originally, 928 production cycles. Of these, 35 % were produced as 0+ smolts and 65 % as 1+ smolts. Summary statistics for the analysed, continuous variables of interest are provided in Table 1.

Table 1: Summary statistics, including 1st quartile, median or 2nd, and 3rd quartile, for the variables analysed in this study.

Variable	1st quartile	Median	3rd quartile
Freshwater SGR (%)	1.2	1.4	2.1
Seawater SGR (%)	0.7	0.8	0.9
Seawater TGC	3.1	3.3	3.4
Smolt weight (g)	62.8	76.0	102.3
Mortality rate (all causes) (%)	5.0	9.2	16.1
Mortality rate (HSMI/CMS) (%)	0.0	0.1	0.3
Seawater production duration (days)	474	521	598

3.1 Growth

When modelling seawater SGR as a response, all model terms were significant ($p < 0.01$). The AICc value of the full model was -2360.1. Mean temperature showed a high concurvity value (> 0.80) in relation to the site random effect. However, refitting the model without site (AICc = -2248.7) or smolt supplier (AICc = -2103.4) as random effects did not impact the shape of the smoothers. We therefore chose to retain the full model, which had an adjusted R^2 of 75 % and explained 77 % of deviance. It included 785 observations and the estimated degrees of freedom used was 62.8. Model diagnostics did not reveal any problems. The model terms and their significances are listed in Table 2.

Table 2: Intercept estimate and approximate significances of smooth terms, including F statistics and p-values, for the selected seawater SGR model.

Parametric coefficients	Estimate	Std. Error	Pr(>|t|)

Intercept	0.7797	0.0105	<0.001

Smooth term	**F**	**p-value**
Freshwater SGR	4.754	0.002
Smolt weight	25.752	<0.001
Mean seawater temperature	26.545	<0.001
Day of transfer	10.153	0.002
Smolt supplier	26.028	<0.001
Site	13.632	<0.001

The smoothing curves for the partial effects of the significant terms of the model are shown in Figure 1. We see SGR during the seawater stage consistently decrease with higher smolt weights and freshwater stage growth rates, up until a freshwater SGR of about 2. Increasing mean seawater temperature, up until a mean temperature of 8.5 °C, results in increased seawater stage SGR. Transferring fish to sea cages during the first ~150 days and the last ~60 days of the year is detrimental to seawater growth in northerly latitudes.

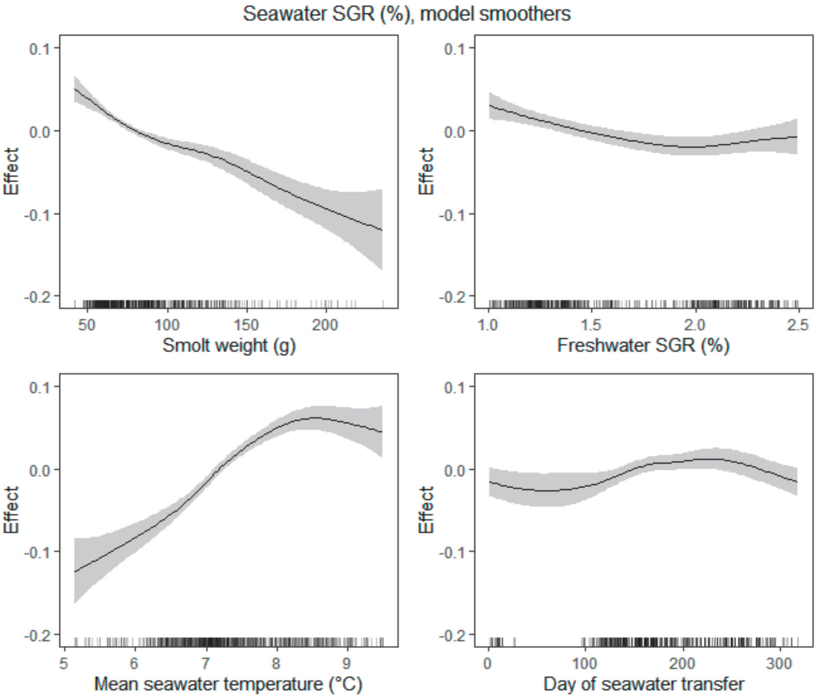

Figure 1: Smoothing curves with 95% confidence bands for the selected seawater stage SGR model, fitted to the partial effects of freshwater SGR, smolt weight at seawater transfer, the day the fish was transferred to the seawater cages, and mean temperature during the seawater stage. The marks along the horizontal axis indicate the data points used for model fitting.

For the seawater TGC model, smolt weight was not significant and AICc values did not differ considerably among the full and reduced models. We therefore chose to retain the reduced model, which had an adjusted R^2 of 61 % and explained 61 % of deviance. It included 794 observations. Model diagnostics did not reveal any problems. The candidate models and their specifications are listed in Table 3.

Table 3: Candidate models with seawater stage TGC as a response, including their fixed terms, degrees of freedom (Df), AICc, and adjusted R^2 values. FW_SGR: Specific growth rate during the freshwater hatchery stage, WS: Smolt weight, DT: Day of seawater transfer, MT: Mean seawater temperature.

Fixed model terms	**Df**	**AICc**	**Adj. R^2**
FW_SGR + WS + DT + MT	65.6	-418.9	0.58
FW_SGR + DT + MT	64.4	-418.7	0.58

The reduced TGC model terms and their significances are listed in Table 4.

Table 4: Intercept estimate and approximate significances of smooth terms, including F statistics and p-values, for the selected seawater TGC model.

Parametric coefficients	Estimate	Std. Error	Pr(>|t|)
Intercept	3.2513	0.0325	<0.001

Smooth term	F	p-value
Freshwater SGR	7.912	<0.001
Mean seawater temperature	15.672	<0.001
Day of transfer	34.601	<0.001
Smolt supplier	22.887	<0.001
Site	12.317	<0.001

The smoothing curves for the partial effects of the significant terms of the model are shown in Figure 2. We see TGC during the seawater stage decrease with higher freshwater SGR until it reaches a plateau, or increases slightly, above 2.0 %. As seen for the smoothers for seawater SGR, transferring fish to sea cages during the first ~150 days and the last ~60 days of the year is detrimental to seawater growth. Seawater TGC decreases with increasing temperature, which is as expected as the calculation of TGC involves the sum of seawater production temperature.

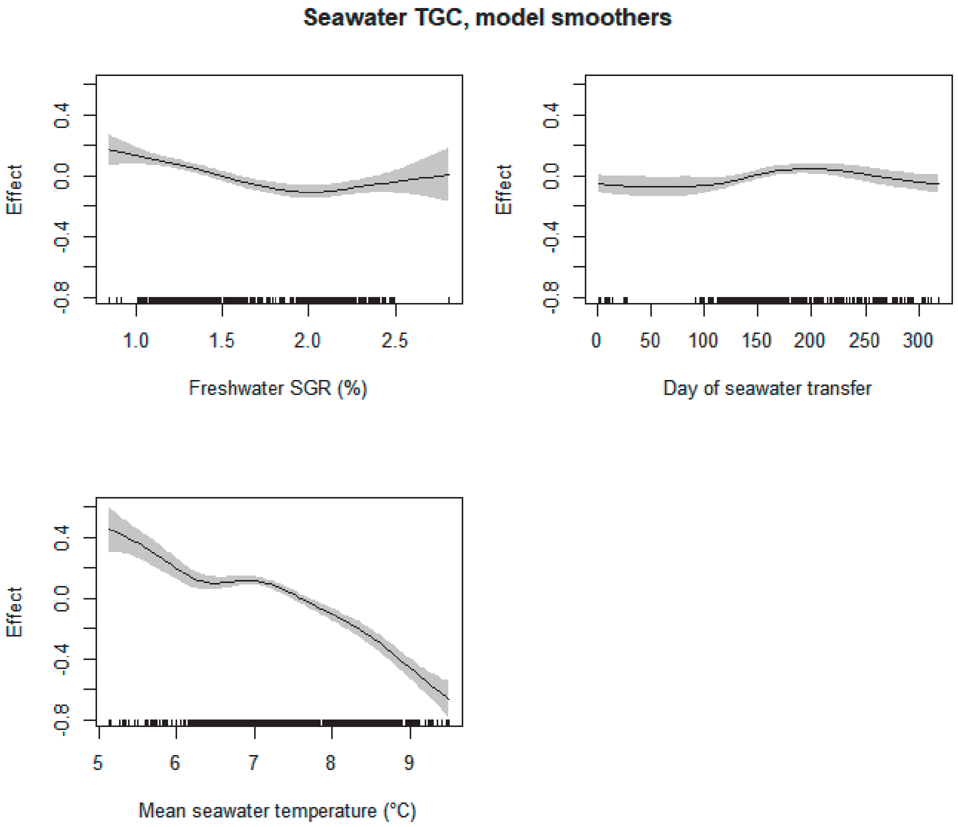

Figure 2: Smoothing curves with 95% confidence bands for the selected seawater stage TGC model, fitted to the partial effects of freshwater SGR, the day the fish was transferred to the seawater cages, and mean temperature during the seawater stage. The marks along the horizontal axis indicate the data points used for model fitting.

For the seawater RGI model only seawater transfer day and mean seawater temperature were significant among the fixed effects, and only seawater production site among the random effects. The reduced model had the lowest AICc value and we therefore chose to retain the reduced model, which had an adjusted R^2 of 17 % and explained 24 % of deviance. It included 870 observations. Model diagnostics revealed some overdispersion, which could not be resolved, but were otherwise acceptable. The candidate models and their specifications are listed in Table 5.

Table 5: Candidate models with seawater stage RGI as a response, including their fixed terms, degrees of freedom (Df), AICc, and adjusted R^2 values. FW_SGR: Specific growth rate during the freshwater hatchery stage, WS: Smolt weight, DT: Day of seawater transfer, MT: Mean seawater temperature, (S): site, (SS): smolt supplier.

Fixed and random model terms	**Df**	**AICc**	**Adj. R^2**

FW_SGR + WS + DT + MT + (S) + (SS)	52.5	-914.5	0.19
FW_SGR + DT + MT + (S) + (SS)	49.6	-912.7	0.19
FW_SGR + DT + MT + (S)	34.4	-930.0	0.17
DT + MT + (S)	38.1	-1028.9	0.17

The reduced RGI model terms and their significances are listed in Table 6.

Table 6: Intercept estimate and approximate significances of smooth terms, including F statistics and p-values, for the selected seawater RGI model.

Parametric coefficients	**Estimate**	**Std. Error**	**Pr(>|t|)**
Intercept	0.0441	0.0084	<0.001

Smooth term	**F**	**p-value**
Day of transfer	6.322	<0.001
Mean seawater temperature	6.798	<0.001
Site	2.264	<0.001

The smoothing curves for the partial effects of the significant terms of the model are shown in Figure 3. We see RGI increase with mean seawater temperature up until about 8 °C before sharply decreasing again. RGI is lowest for the early spring smolt, thereafter increasing through to winter.

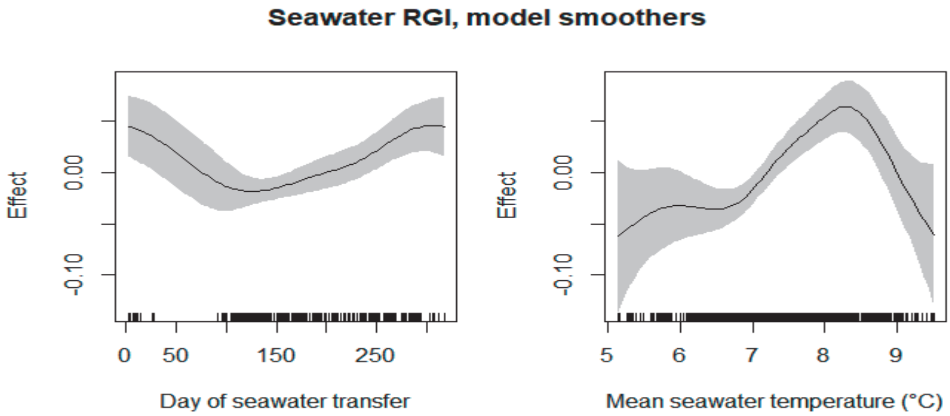

Figure 3: Smoothing curves with 95% confidence bands for the selected seawater stage RGI model, fitted to the partial effects the day the fish was transferred to the seawater cages and mean temperature during the seawater stage. The marks along the horizontal axis indicate the data points used for model fitting.

3.2 Mortality

When modelling total mortality during the seawater stage, not all fixed terms were significant terms and a stepwise, backward selection of variables resulted in four candidate models (Table 7). The terms for freshwater stage SGR, mean seawater temperature and the use of mechanical treatments were present in all the candidate models, while the day of seawater transfer was present in three out of the four. The estimated smoothing curves for these terms were similar and, therefore, the choice of model did not influence the interpretation of individual terms. Smolt supplier and site class were both significant as random effects. The model with the lowest AICc value included freshwater stage SGR, mean seawater temperature, and the presence of mechanical treatments as significant fixed terms, and the day of seawater transfer as a non-significant (*p≈0.05*) fixed term. It used 64.6 degrees of freedom on 811 observations. It explained 45.6 % of deviance. Model diagnostics did not reveal any problems, although plots of fitted vs. response values showed some residual spread for larger fitted values.

Table 7: Candidate models with seawater stage total mortalities as a response, including their fixed terms, degrees of freedom (Df), AICc, and adjusted R^2 values. FW_SGR: Specific growth rate during the freshwater hatchery stage, WS: Smolt weight, DT: Day of seawater transfer, MT: Mean seawater temperature, TM: The presence of mechanical treatments, TB: The presence of bathing treatments.

Fixed model terms	Df	AICc	Adj. R^2
FW_SGR + WS + DT + MT + TM + TB	65.8	-2322.9	0.39

FW_SGR + DT + MT + TM + TB	65.8	-2323.0	0.39
FW_SGR + DT + MT + TM	64.6	-2329.7	0.39
FW_SGR + MT + TM	55.4	-2324.9	0.38

Estimates and significance values for the parametric coefficients and smooth terms of the selected model for total mortalities during the seawater stage are shown in Table 8.

Table 8: Parametric coefficients and approximate significances of smooth terms, including F statistics and p-values, for the selected seawater stage mortality model.

Parametric coefficients	**Estimate**	**Std. Error**	**Pr(>|t|)**
Intercept	-8.2966	0.0931	<0.001
Mechanical treatments	0.3398	0.1180	0.004

Smooth terms	**χ^2**	**p-value**
Freshwater SGR	120.22	<0.001
Mean seawater temperature	255.97	0.003
Day of transfer	50.73	0.046
Smolt supplier	229.54	<0.001
Site	163.35	<0.001

The smoothing curves and the parametric effect for the partial effects of the covariates are shown in Figure 4. The smoothers reveal that a higher SGR during the freshwater rearing stage is associated with lower mortalities during the seawater stage. The effect of mean temperature during the seawater stage is variable, with some observations in the lower temperature range possibly having an undue effect, pulling the curve downwards, as seen in the wide confidence bands. Overall, mean seawater temperatures between 7.5 and 8.5 °C seem to be beneficial with regards to mortalities. Mortalities increase when the smolts are transferred to seawater during winter.

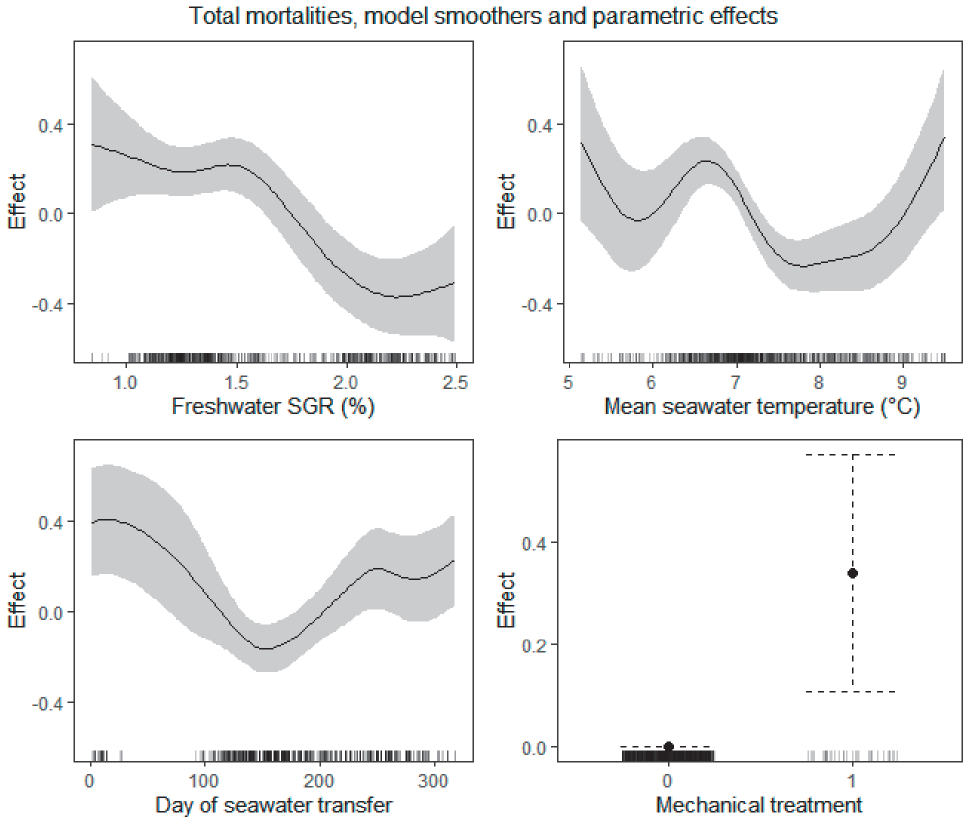

Figure 4: Smoothers for the parameters of the selected general mortality model, including freshwater stage specific growth rate (SGR), mean seawater temperature, and the day of the year the production cycle was transferred to seawater cages. The use of mechanical treatments, as a parametric effect, is shown with the horizontal label 1 indicating the use of mechanical treatments. The marks along the horizontal axis indicate the data points used for model fitting.

3.3 Mortality attributed to HSMI/CMS

When modelling mortality due to HSMI or CMS, not all model terms were found to be significant and stepwise backward selection resulted in five candidate models. These models, their degrees of freedom, AICc, and adjusted R^2 values are shown in Table 9. The AICc values did not vary considerably among the first four candidate models. The model including smolt weight, day of transfer, mean seawater temperature, and the presence of mechanical treatments as fixed terms had the lowest AICc value, but only by 0.3 compared to the best competing models. However, as this was the simpler model, we consider it the better one in the interest of parsimony. The model explained 76.1 % of deviance. Model diagnostics did not reveal any considerable problems, although plots of fitted vs. response values showed high residual spread for larger fitted values. 395 observations were used for this model.

Table 9: Candidate models with seawater stage heart and skeletal muscle inflammation (HSMI) or cardiomyopathy syndrome (CMS) mortalities as a response, including their fixed terms, degrees of freedom (Df), AICc, and adjusted R^2 values. FW_SGR: Specific growth rate during the freshwater hatchery stage, WS: Smolt weight, DT: Day of seawater transfer, MT: Mean seawater temperature, TM: The presence of mechanical treatments, TB: The presence of bathing treatments, YC: Year class.

Fixed model terms	**Df**	**AICc**	**Adj. R^2**
FW_SGR + WS + DT + MT + TM + TB + YC	55.5	5438.4	0.63
FW_SGR + WS + DT + MT + TM + YC	54.3	5438.4	0.63
FW_SGR + WS + DT + MT + TM	52.9	5438.6	0.65
WS + DT + MT + TM	52.5	5438.1	0.65
WS + DT + MT	51.5	5444.2	0.68

Estimates and significance values for the parametric coefficients and smooth terms of the selected model for mortalities due to HSMI or CMS are presented in Table 10.

Table 10: Parametric coefficients and approximate significances of smooth terms, including F statistics and p-values, for the selected model for mortality attributed to heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS).

Parametric coefficients	**Estimate**	**Std. Error**	**Pr(>|t|)**
Intercept	0.0126	0.4559	0.978
Mechanical treatments	0.6435	0.2342	0.006

Smooth terms	**F**	**p-value**
Smolt weight	68.48	<0.001
Mean seawater temperature	1948.04	<0.001
Day of transfer	105.55	0.002

Site	16.41	<0.001

Figure 5 shows the smoothing curves for the partial effects of the significant, continuous terms of the model, along with the parametric effect indicating the use of mechanical delousing treatments. We see that mortalities attributed to HSMI or CMS increase with smolt weight and decrease with mean seawater temperature. Fish transferred to seawater in the latter half of the year seem to experience more mortalities due to HSMI or CMS, although the confidence bands are wide outside days 150 to about 250.

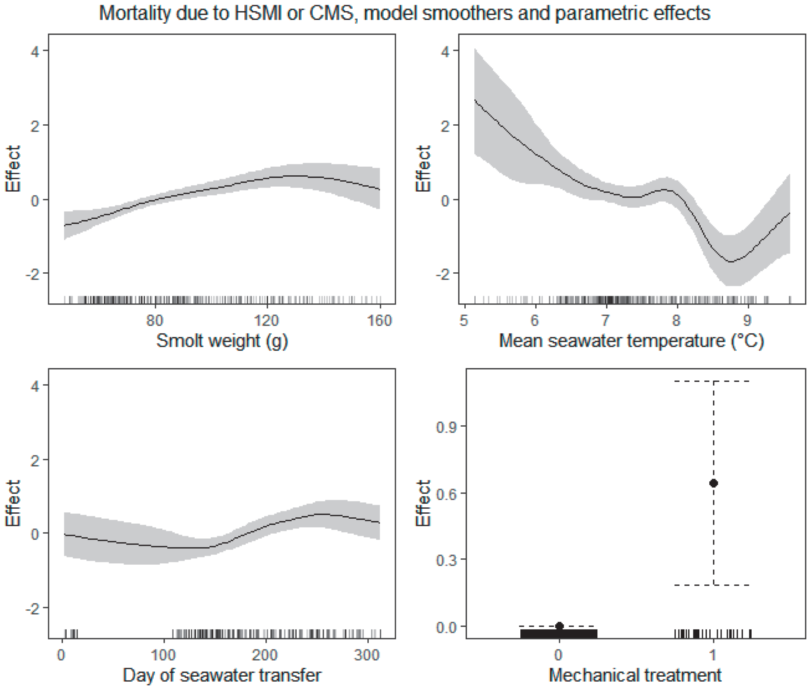

Figure 5: Smoothers for the parameters of the selected model for mortalities due to heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS), including as explanatory variables smolt weight, mean seawater temperature, and the day of the year the fish were transferred to seawater. The use of mechanical treatments, as a parametric effect, is shown with the horizontal label 1 indicating the use of mechanical treatments. The marks along the horizontal axis indicate the data points used for model fitting.

4 Discussion

4.1 Growth

Our results indicate a negative effect of increasing freshwater SGR upon seawater SGR and TGC at the latitudes our dataset covers (from 67 °N to 71 °N), for a freshwater SGR roughly below 2.0, and thereafter a slightly positive, but highly uncertain effect. The freshwater SGR values in our dataset are stratified and SGR values below 2.0 are predominantly transferred to seawater as 1+ smolts while those above 2.0 are predominantly transferred as 0+ smolts. Thus, it is possible that there is an effect of the timing of seawater transfer, whereby fish that have grown faster as 0+ smolts are transferred earlier during the autumn at a more favourable time point and, similarly, fish that have grown slower as 1+ smolts are transferred later in the spring, which is also a more favourable time point according to the smoother for transfer day shown in Figure 1. With respect to these variables, however, inspection of variance inflation factors and concurvity did not show a problematic degree of collinearity or dependence.

The day of seawater transfer has a significant effect upon seawater SGR and TGC in this dataset, although the confidence region for the smoother is wide, and the estimated effect is marginal. There is evidence to support previous findings that transferring smolts to low seawater temperatures, or to seawater holding temperatures that are markedly different to the freshwater rearing temperature from which the fish originate, has a negative effect upon the resumption of feeding and growth (Arnesen et al., 1998; Kristensen et al., 2012). A different pattern is shown by the RGI model. However, as this model relies on an estimated, theoretical SGR, this may just indicate an overestimation of growth potential in fish groups transferred to sea in spring or, vice versa, an underestimation of growth potential in groups transferred during the fall and winter.

While the temperature at transfer is not included as a covariate, our results indicate that June to October is the better period for transferring smolts to seawater with regards to subsequent SGR and TGC. This corresponds to the period of warmer seawater temperatures in this region, and the result is consistent with previous findings concerning performance after transfer, at similar latitudes in Norway (Toften et al., 2003). The day of seawater transfer also determines the body size at which the post-smolts first are exposed to parasites and pathogens that have higher prevalence during summer, e.g. sea lice (Abolofia et al., 2017), or that are more prevalent during winter, e.g. *Moritella viscosa* and *Tenacibaculum* spp. (Karlsen et al., 2017; Olsen et al., 2011), which may have a bearing upon initial post-transfer growth.

The period from June to October also corresponds to a period starting with continuous daylight and ending well into the period of declining daylight. While transferring smolts to seawater while daylength is declining has been shown to negatively affect immediate growth, overall growth may not be severely affected due to the compensatory growth capacity of the fish (Duncan et al., 1999, 2002, 1998; Mørkøre and Rørvik, 2001). Also, the use of artificial light may counter the effect of natural photoperiod in our case (Imsland et al., 2017). We used a cyclic cubic regression spline to represent the days of the year the various groups were transferred to seawater. Whilst daylength at each date will not vary throughout a timespan of eight years, as our analysis was constrained to, it would be interesting for future work to include data on natural daylength and artificial light regimes, in conjunction with temperature, in the analysis. With regard to investigating how environmental factors determine the optimal timing of transfer, a model that accounted for regional differences, or possibly included more covariates relevant to this, should perform better and give increased insight into the effects of environmental factors.

Determining the effect of smolt size upon seawater growth performance, especially when this is reported as SGR, is problematic due to the dependence of the latter upon the former. Nevertheless, when considered in tandem to our results from both the TGC and RGI models, which are less sensitive to this problem, there is evidence that smolt weight, within the range studied, does not in itself affect future growth. Our results, for smolt sizes up to 200 g, are in agreement with those presented by Dam (2020), relying on commercial production data from the Faroe Islands, who nevertheless demonstrated increasing TGC and Ewos Growth Index (EGI) values for increasing smolt sizes beyond 200 g. Note that our findings do not suggest an optimal size for transferring post-smolts from land-based or closed seawater systems to marine net cages. The extent to which it is beneficial to induce early smoltification in salmon that are to be kept in such systems, either in brackish or salt water, should be further investigated.

Mean temperature is the covariate that shows the clearest effect upon seawater SGR and RGI in our analyses and was included to capture some of the variation and enhance the model especially as the two models were largely in agreement. Atlantic salmon post-smolts generally avoid temperatures below 6 °C and above 18 °C (Johansson et al., 2009, 2006; Oppedal et al., 2011) and their optimal temperature for growth, while seemingly varying with fish size, has been shown to be in the range of 12-14 °C (Handeland et al., 2008). The positive effect of increasing mean temperature plateauing around 8.5 °C in our study may be associated with periods of high maximum temperatures in these production cycles having growth depressing effects.

4.2 Total mortality

The general mortality response variable includes all mortalities, cause notwithstanding, also those due to culling. Since the most common reason for the removal and culling of live fish in our dataset appears to be welfare related, i.e. associated with an expectation that the individuals removed already had a poor welfare status and culling was carried out to prevent further suffering, or that the fish would die at a later time point, or otherwise not contribute toward saleable biomass, we find this reasonable.

Total mortalities during the seawater stage of each production cycle decrease with increasing freshwater SGR, plateauing beyond a freshwater SGR of 2.0, according to our results. Although not significant, there is a weak indication of an effect of transfer time, as has also been reported in other studies (Pincinato et al., 2021). Our results suggest the optimal period for transferring smolts to seawater with regards to mortality may be May through July at the latitudes the salmon are farmed under in this study (from 67 °N to 71 °N). However, the stratification of freshwater SGR related to transfer season (that has been discussed earlier in relation to the seawater SGR model) could also influence this result. Thus, as the fish transferred during this period are almost exclusively 1+ smolts, who typically exhibit lower freshwater growth rates, they are also then generally associated with freshwater SGR values for which total mortalities are higher. The causes for this variation in mortality through varying transfer times could be due to the adverse effects of transferring smolts into cold seawater (Arnesen et al., 1998).

The use of mechanical treatments against lice seems to contribute significantly towards increased mortalities in the seawater production of Atlantic salmon, while bathing treatments do not. A previous analysis of the Norwegian Directorate of Fisheries' national database on delousing found that mechanically treating salmon against lice resulted in low mortalities in uncompromised fish, overall, in the month following treatment, and this was lower than for some types of bathing treatments (Overton et al., 2019). Another recent study has also reported elevated mortality during the two weeks following thermal, mechanical and bathing treatments against lice, with both mechanical and thermal delousing treatments having the greatest effects upon mortality (Sviland Walde et al., 2021). As our response variable is total mortality for the whole production cycle, it is conceivable that we capture more long-term relationships between explanatory variables and mortality. It is also possible, however, that the difference reflects particularities of the practices of the company or geographical region covered in our study. There is also the possibility that treatment frequency is correlated with a higher lice infestation pressure, which may be a driver behind mortalities. However, companies may also choose to implement a treatment before lice levels attain regulated levels, so treatment frequency per se may not be linked to increasing lice problems (Overton et al., 2019). It should also be noted that mechanical treatments against lice are a relatively recent phenomenon and the amount of production cycles and sites using mechanical treatments in our study were limited.

Seawater temperature, while significant, was not the main variable of interest and was included to capture some of the variation in the model and provide some reference for the other explanatory variables. Mean temperature seems to be associated with higher mortalities outside the range of about 7.5 °C to about 8.5 °C. The effect of mean temperature may well be circumstantial, e.g. reflecting temperatures associated with higher infection or infestation pressures, such as the previously mentioned winter ulcers, but may also reflect the influence of periods with extreme temperatures.

4.3 Mortality attributed to HSMI or CMS

Higher smolt weights, lower mean seawater temperatures and the use of mechanical delousing treatments have a significant, amplifying impact upon mortalities attributed to HSMI or CMS. Thus, mechanical treatments, and not bathing treatments, contribute to the increase of both total mortalities and mortalities due to HSMI or CMS, which support suggestions that excessive handling related stress can be a risk factor (Garseth et al., 2018; Kongtorp et al., 2006). Contrary to what has been previously hypothesised, freshwater SGR did not have a significant effect on HSMI or CMS related mortalities in our study (Frisk et al., 2020; Garseth et al., 2018). This poses the question of whether smolt size and not freshwater SGR was behind the cardiac morphological changes observed by Frisk et al. (2020).

The significance of production site as a random effect suggests variation in geography and/or production regimes for these mortality causes. This may be due to differences in the spread of infection and the fact that differences in husbandry practices and environmental factors will influence the impact of the pathogen once a cage has been infected (Garseth et al., 2018). It could also reflect the tendency of the sites to receive smolts from a limited number of hatcheries. It has previously been reported that CMS can be transmitted vertically from brood stock to progeny (Bang Jensen et al., 2020).

For both total mortality and mortality ascribed to HSMI or CMS, plots of fitted versus predicted values revealed a considerable spread, especially for higher mortality numbers. We also note that the adjusted R^2 and estimated deviance explained are low, at least for the total mortality model, and much of the explained deviance is probably due to the inclusion of production site as a random effect. While it is interesting to note that there is considerable variation among sites, it is not very useful as an explanation of phenomena. As our models used total mortalities for the whole production cycles, instead of mortalities following specific events, e.g. treatments, a low explanatory capacity of the models is to be expected. In a seawater production cycle lasting up to two years, numerous factors can be expected to affect mortality numbers across individual production cycles and over time, for example infectious diseases and parasitic infestations.

The use of commercial production data has proven a strength to this study, not just because we can use this data to give a realistic picture of actual Atlantic salmon production practices, but also because we have data from more than 800 full seawater production cycles, conducted over many years. Many studies of the effects of freshwater hatchery production parameters upon subsequent seawater growth only follow the fish for a few months after transfer (e.g. Striberny et al., 2021) which, due to the capacity of Atlantic salmon for compensatory growth (Duncan et al., 2002; Rørvik et al., 2018), may give results that are less relevant in a production setting. A drawback with using commercial data, collected by workers for whom data collection is not a priority, is of course that we cannot ascertain their veracity or precision, although the amount of data analysed here compensates for this to some extent. The fact that producers follow an established production strategy and must leverage practical concerns in decision making also means there is a constraint on variation in the dataset for some variables. For example, attempts at modelling interactions between freshwater SGR and smolt size, during the initial exploration of the data, proved unsuccessful due to the relatively high correlation between the two variables, and their stratification due to the strategies of producing 0+ or 1+ smolts. The covariation of phenomena like transfer time, harvest time, photoperiodic and temperature regimes, freshwater growth rate, smolt size, and seawater growth rates makes establishing the direction of causal relationships difficult. There is also a geographical limitation to our study, in that the dataset only covers the north of Norway, and it is not a given that the results can be extrapolated to other regions.

5 Conclusions

This study suggests that the seawater growth performance of farmed Atlantic salmon in Northern Norway is influenced by freshwater growth rate and the timing of seawater transfer. Increased freshwater growth rate negatively impacts seawater growth rate in the commercial dataset we analysed. Total mortality increases with lower freshwater SGR and the presence of mechanical delousing treatments. Mortalities attributed to HSMI or CMS are significantly higher when mechanical treatments have been used but are not significantly affected by bathing treatments. Mortalities due to HSMI or CMS also increase with larger smolt sizes and decrease with higher mean seawater temperatures up until 9 °C. Freshwater growth rate did not have a significant effect on mortalities attributed to HSMI or CMS in our analyses. The results presented in this analysis enhance our understanding of the factors that can affect Atlantic salmon production performance in the studied region and may contribute to the optimisation of production regimes.

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The dynamics and prevalence of ulcerative diseases and mouth rot in Atlantic salmon (*Salmo salar* L.) farms in Northern Norway

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ABSTRACT

Ulcerative diseases linked to the pathogens *Moritella viscosa* and *Tenacibaculum* spp. are a considerable challenge to the health and welfare of numerous farmed salmonids, including Atlantic salmon (*Salmo salar* L.). The pathogens can also be drivers for increased mortality and reduced slaughter quality, and therefore have a direct economic impact upon the industry. In this study, we aim to describe the temporal prevalence of injuries associated with these diseases, specifically ulcers and head/jaw necrosis and erosion (commonly termed mouth rot), using northern Norwegian production records from a large commercial Atlantic salmon farming company. We also model the effect of selected production related variables upon the incidence of these conditions with generalised additive models (GAM). Our analyses show that the incidence of ulcerative disease in our northern Norwegian dataset is predominantly confined to the winter months, peaking in February through April, conforming with what has been referred to as winter ulcer disease. The prevalence of mouth rot is mostly limited to the first 7 months following seawater transfer and is the highest in the first 5 months of the calendar year. We found that 1+ smolts have a higher prevalence of ulcers but a lower prevalence of mouth rot. Smolt supplier may also significantly impact the prevalence of ulcers. The occurrence of mouth rot following seawater transfer seems to be associated with a higher incidence of winter ulcers. Transferring fish to seawater at low (<8 °C) and falling temperatures also increases its incidence. In addition, larger smolts also see increased prevalence of mouth rot.

Key words: Atlantic salmon, aquaculture, mortality, winter ulcer disease, mouth rot, *Moritella viscosa*, *Tenacibaculum* spp., health, welfare

1 Introduction

Skin ulcers and wounds are a severe fish health and welfare problem in aquaculture, and Atlantic salmon (*Salmo salar* L.) farming is no different. Ulcerative diseases have been recorded in Norwegian salmonid aquaculture since the late 1980's and reports indicate that they are a problem in most, if not all, major salmonid producing regions (Bruno et al., 1998; Gudmundsdóttir and Björnsdóttir, 2007; Lillehaug et al., 2003; Lunder et al., 1995; MacKinnon et al., 2019; Salte et al., 1994; Takle et al., 2015). Ulcers predominantly manifest themselves during the winter months in Atlantic salmon farming, when temperatures are below 8-10 °C, and are commonly associated with a condition called winter ulcer disease. The prevalence of this condition ranges from 1 % to 50 % of sampled fish in affected farms. The severity of the ulcers may vary, but in the worst case they can extend beyond the dermis and expose musculature. Cumulative mortality rates associated with ulcers range from below 10 % to over 20 %, reaching over 50 % in some outbreaks (Bruno et al., 1998; Gudmundsdóttir and Björnsdóttir, 2007; Lunder et al., 1995). Surveys have shown that ulcers are recognized as a major welfare problem and a cause for production losses by Atlantic salmon farmers in Norway (Bleie and Skrudland, 2014; Colquhoun and Olsen, 2020).

Skin ulcers are also a driver for quality downgrading at harvest. In northern Norwegian salmon aquaculture, ulcers can be the most common cause for downgrading, affecting from 2 % to over 20 % of the fish in harvest and processing batches during winter (Alvestad et al., in preparation; Takle et al., 2015). Ulcers are therefore responsible for economic losses both through mortalities and reduced product quality in fish surviving until harvest (Vågsholm and Djupvik, 1998).

The epidermis of fish is a delicate organ and skin ulcers can be linked to several causative agents, including dinoflagellate algae, environmental pollutants such as heavy metals and PCB, or environmental stressors, such as extreme temperatures or hypoxia. However, in many cases, the causal mechanisms are poorly understood (Noga, 2000). In Atlantic salmon aquaculture, ulcerative disease has commonly been associated with bacterial infection although the exact nature of this association has recently been disputed. Pathogens that are commonly associated with the manifestation of winter ulcers are *Moritella viscosa*, *Tenacibaculum* spp., and *Aliivibrio wodanis* (Lunder et al., 2000; Takle et al., 2015). Whilst *M. viscosa* has long been thought to be the causative agent behind winter ulcer disease, it remains unclear as to whether it causes ulcers, e.g. through an initial systemic infection, or is merely present in ulcers as an opportunist (Bruno et al., 1998; Grove et al., 2008; Karlsen et al., 2017a). *Tenacibaculum* spp. has in recent years been found to be widely present in samples taken from winter ulcers in Atlantic salmon, some dating back to 1996, and its presence is likely underreported (Olsen et al., 2011). Numerous roles have been proposed for *Tenacibaculum* spp. in relation to this, either as an opportunist, in co-infection with *M. viscosa*, or as a driver behind ulcerative pathogenesis

(Olsen et al., 2011; Småge et al., 2016). *Tenacibaculum maritimum* has recently been implicated as a driver for mortality in a disease outbreak manifesting as mouth rot in post-smolts immediately following seawater transfer in British Columbia and Washington state (Frisch et al., 2018a). Similar outbreaks have been associated with the presence of *Tenacibaculum* spp., including '*Tenacibaculum finnmarkense'*, in Norway (Colquhoun and Olsen, 2020; Småge et al., 2018, 2016). *A. wodanis* has also been observed in co-infection with *M. viscosa* and was proposed to have a role in modulating infection (Karlsen et al., 2014).

Karlsen et al. (2017) found both *M. viscosa* and *Tenacibaculum* spp. in skin mucus of healthy fish and proposed that disruptions to the composition of the host-microbiota may be a driver for disease development, rather than winter ulcers being the result of exposure to a specific pathogen *per se*. Both low temperatures, which inhibit the immune response and wound healing, and handling have been identified as risk factors for disease development (Colquhoun and Olsen, 2020; Jensen et al., 2015; Salte et al., 1994). Furthermore, it is unclear if mortalities are caused by the impacts of the ulcers themselves or whether they are due to systemic infection (with ulcers as a symptom). Atlantic salmon infected with a *M. viscosa* isolate from lumpfish were reported to die without exhibiting external clinical signs of disease (Einarsdottir et al., 2018).

Both proactive and reactive medicinal approaches to combatting winter ulcers have achieved limited success. While several vaccines that protect against winter ulcer disease are available and in use, winter ulcers remain prevalent (Gudmundsdóttir and Björnsdóttir, 2007; Karlsen et al., 2017a; Takle et al., 2015). Antibacterial agents, e.g. florfenicol, oxolinic acid, and oxytetracycline, can also be administered to treat ulcerative infections. However, they have not been demonstrated to be effective in treating skin ulcers or in hindering mortalities during outbreaks in Atlantic salmon aquaculture (Coyne et al., 2006, 2004; MacKinnon et al., 2019). Investigating the production related factors that can affect the dynamics and prevalence of ulcer related mortalities can help improve our understanding of the problem, help mould potential responses and also help control the outcomes of the disease.

In this study we aimed to describe the temporal dynamics and prevalence of outbreaks of ulcers and mouth rot in regional Atlantic salmon aquaculture, using data from 40 seawater production sites in northern Norway. Furthermore, we analysed the possible association of variability in mortality attributed to these conditions with production related variables.

2 Materials and methods

2.1 Data origin and pre-processing

We obtained farm records from the marine phase of Atlantic salmon production from the database of one large, commercial aquaculture company. The data entries in the records are collected daily by the respective farms' employees, for purposes including production monitoring, decision support, and reporting to external actors, including government institutions. For each farm, the individual cage represented the smallest identifiable unit for which data could be retrieved. As such, we chose individual production cycles as our subjects of analysis. A production cycle commences upon stocking, i.e. when a group of salmon post-smolts are stocked in a cage, and terminates when the last fish of that group is harvested (Soares et al., 2011).

Cause specific mortalities in the dataset were recorded by the fish farmers in accordance with standardised company guidelines. According to these guidelines, fish exhibiting ulcers or mouth rot are to be removed and euthanised as soon as possible. The data analysed in this study is therefore only indicative of the prevalence of ulcers or mouth rot, and not indicative of how many fish actually die due to these conditions.

2.2 Data analysis

To describe the prevalence of ulcers and mouth rot in time we visualised the distribution of mortalities attributed to these causes by calendar month and also by production month, i.e. the number of months since seawater transfer, using boxplots. We also report descriptive statistics on the covariates and target variables.

To elucidate the relationships between the prevalence of ulcers or mouth rot and the production related variables, we fit Generalized Additive Models (GAMs) using REML estimation (Wood, 2017). For the GAM analyses, the target variables were i) aggregated mortality rates due to ulcers during the first winter at sea, i.e. during February through April, and ii) aggregated mortality rates due to mouth rot during the first month after seawater transfer. In both cases, a backward selection of variables, by sequentially removing variables found non-significant ($p < 0.01$) upon fitting, was used in combination with Akaike's information criterion (AIC) for model selection. The models were checked for residuals' independence, homogeneity, fit, and normality. Linear dependence among explanatory variables, i.e. concurvity, was also checked, with worst estimates for concurvity variables at or above 0.80 warranting further inspection.

2.2.1 Generalised additive mixed model for the prevalence of ulcers

To model the variation in the prevalence of ulcers, we included only the production cycles that have one or more mortalities recorded within this category (N = 566). The target variable was calculated as the sum of daily ulcer related mortalities occurring during the first winter at sea, specifically the months of February through April. We selected these specific months as they have the highest prevalence of mortalities attributed to ulcers. The target variable was log transformed prior to modelling and a normal distribution and identity link function was assumed. The model specification included production site as a random effect. The full model specification was thus:

$$\boldsymbol{U = \alpha + f_1(SS) + f_2(Temp.31) + f_3(Temp.M) + f_4(Dty) + f_5(ME) + f_6(Stock)}$$
$$\boldsymbol{+\ SSt\ + YC\ +\ SSupp + \epsilon}$$

Where *U* represents the log-transformed mortality rate attributed to ulcers, *SS* is smolt size, *Temp.31* is the mean seawater temperature during the first 31 days in seawater, *Temp.M* is the mean seawater temperature from February through April, *Dty* is the mean production density from February through April, *ME* is the number of mortalities, transformed by fourth root, due to mouth rot during the first 31 days in seawater, *Stock* is the number of fish originally stocked in the cage, *SSt* is the smolt production strategy used, either 0+ or 1+, *YC* is the year class, i.e. the year in which the seawater production cycle commenced, and *SSupp* is the smolt supplier.

All production cycles in our dataset are assumed to have been vaccinated against *M. viscosa*. We did not have data on the specific vaccine used in all cases and this was therefore not included as an explanatory variable.

2.2.2 Generalised additive model for the prevalence of mouth rot

To model the prevalence of mouth rot, we included all production cycles from sites that had recorded mortalities due to this factor (N = 113), and in the year classes these production cycles belonged to. The target variable was computed as the sum of daily mortalities due to mouth rot for the first 200 days after seawater transfer, due to only a negligible amount of mortalities occurring after this window. Due to the highly variable number of recorded mortalities in this category, with several instances of severe outbreaks, we decided to transform the mortality counts into an ordered categorical variable with the levels: “Low”, including observations with 0-122 mortalities, “Medium”, including observations with 122-3317 mortalities, and “High”, including observations with more than 3317 mortalities. An ordered categorical distribution with an identity link function was therefore assumed. The cut-off values chosen represent the 3rd quartile (122) and the mean (3317) for the number of mortalities attributed to mouth rot.

When we attempted to fit the models with seawater production site, smolt supplier, and/or year class as either factors or random effects, we could not achieve convergence when fitting the model. Various attempts at fitting models with these variables as the sole predictors did not find them significant, and they were therefore dropped upon further modelling. The final model specification, without any random effects, was thus:

$$\boldsymbol{ME200 = \alpha + f_1(SS) + f_2(Temp.31) + f_3(Lice) + f_4(U) + f_5(Stock) + f_6(Temp.Grad) + f_7(Dty200) + SSt + \epsilon}$$

With *ME200* representing the mortality rate due to mouth rot during the first 200 days in seawater, transformed into three categories, *Lice* being the average number of salmon lice, of all stages, counted on a fish during the first 200 days in sea water, and *Temp.Grad* representing the difference between the average temperatures during the fourth and first weeks after transfer, as an indicator of whether the temperature trend was rising or falling during the time of transfer. *Dty200* is the mean production density during the first 200 days at sea. The other variables are the same as for the ulcer prevalence model.

2.3 Software

Data cleansing and pre-processing was done using the Python programming language (version 3.7.3) and the Pandas (McKinney, 2010) library. Analyses were run in R (version 3.6.1) using the package mgcv for the GAM analyses (Wood, 2011) and mgcViz for visualisation.

3 Results

3.1 Descriptive analysis

Boxplots visualising the distributions of mortalities in time, grouped by calendar month and production month, are shown in Figure 1. Overall mortalities, i.e. not cause specific, are included for comparison. There is a clear temporal pattern in the prevalence of ulcers, with February through April seeing the highest levels, and the first winter at sea seems to be the worst, at least for production cycles including 0+ smolts. Mouth rot predominantly occurs during spring and within the first seven months of the seawater production phase.

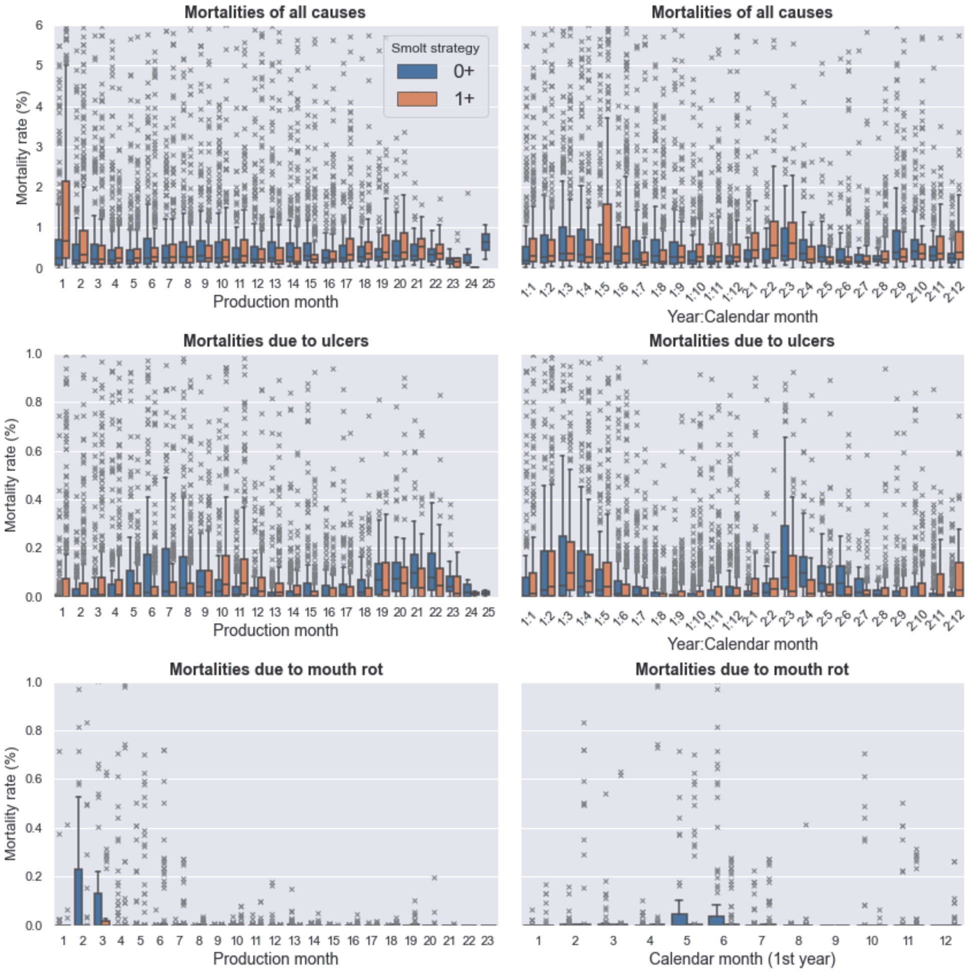

Figure 1: Boxplots showing the monthly distribution of total mortality rates, mortalities due to ulcers, and mortalities due to mouth rot, throughout the throughout the production cycle (left column) and throughout the calendar year each of the first two production years (right column). The plots have been truncated along the y-axis and not all outliers are shown. Mortalities attributed to ulcers and mouth rot include fish culled for these reasons.

3.2 Generalised additive model for ulcer prevalence

The model specifications, their spent degrees of freedom, and AIC values for the generalised additive mixed models with ulcer related mortalities as a response are shown in Table 1. Smolt size was the only non-significant co-variate. Thus, there are only two candidate models, both with equal AIC values. We chose to report on the simpler one, excluding smolt size as a covariate. The deviance explained by this model was 67.8 %.

Table 1: Specifications of the candidate models for the prevalence of mouth rot, including spent degrees of freedom and AIC values. SS: smolt size (g), Temp.31: temperature (°C) during the first 31 days following transfer to seawater cages, Temp.mean: mean temperature (°C) during February through April, Dty: mean density during February through April, ME: the number of mortalities and culled fish attributed to mouth rot during the first 31 days after stocking, Stock: the number of fish originally stocked in the cage, SSt: smolt production strategy (0+ or 1+), YC: year class, and SSupp: smolt supplier.

Model specification	Df	AIC
SS + Temp.31 + Temp.Mean + Dty + ME + Stock + SSt + YC + SSupp	64.1	1776.6
Temp.31 + Temp.Mean + Dty + ME + Stock + SSt + YC + SSupp	64.1	1776.6

The model terms and significances for the chosen model are listed in Table 2. Only the 2017 and 2018 year classes, and the smolt suppliers labelled “J” and “N” were significant ($p < 0.01$). The model diagnostics did not reveal any problems.

Table 2: The estimates and significances for parametric coefficients and approximate significances of smooth terms, including chi-squared values, for the chosen generalised additive model for the prevalence of ulcers

Parametric coefficients	Estimate	Std. Error	Pr(>|t|)
Intercept	5.3368	0.4017	<0.001
Smolt strategy (1+)	0.74	0.1895	<0.001
Year class 2014	-0.53	0.4709	0.258
Year class 2015	0.03	0.2092	0.882
Year class 2016	0.20	0.4797	0.677
Year class 2017	-1.23	0.2905	<0.001
Year class 2018	2.01	0.5670	<0.001
Smolt supplier M	0.76	0.3935	0.054
Smolt supplier L	-0.01	0.3159	0.984
Smolt supplier C	-0.12	0.3044	0.693
Smolt supplier H	0.90	0.2388	<0.001
Smolt supplier E	1.72	1.1518	0.134
Smolt supplier G	-0.16	0.2270	0.496
Smolt supplier J	0.75	0.5587	0.185
Smolt supplier A	-1.29	0.5828	0.035
Smolt supplier O	4.49	0.8601	<0.001
Smolt supplier B	0.07	0.5453	0.893

Smolt supplier K	0.12	0.2570	0.655
Smolt supplier F	0.00	0.2456	0.985
Smolt supplier I	-0.71	0.2823	0.014
Smolt supplier N	-0.17	0.3990	0.671
Smooth terms		**F**	**p-value**
Mean seawater temperature, Feb-April		112.72	<0.001
Mean stocking density		38.03	<0.001
Prevalence of Mouth rot		322.20	<0.001
No. fish stocked		52.94	<0.001
Mean seawater temperature, first 31 days		39.28	0.001
Seawater production site		13.42	<0.001

Plots showing the partial dependencies, represented by smoothing curves, between ulcer prevalence and the chosen models' covariates are shown in Figure 2. The prevalence of ulcers appears to increase with the prevalence of mouth rot, the number of fish stocked in the cage, and mean seawater temperature during the first 31 days at sea. Production cycles comprising of 1+ smolts have a significantly higher prevalence of ulcers than those comprising of 0+ smolts. For year classes, 2017 seems to have a significantly lower prevalence of ulcers. For 2018, a higher prevalence is observed, yet only production cycles that had lasted for 200 days prior to the year's end were included. Thus, this year mostly contained production cycles comprising of 1+ smolts. Notwithstanding the smolt suppliers for which there are few observations, and whose effects are extreme, there are suppliers that are associated with both the higher prevalence of ulcers, e.g. one labelled "H", and lower, e.g. the one labelled "I".

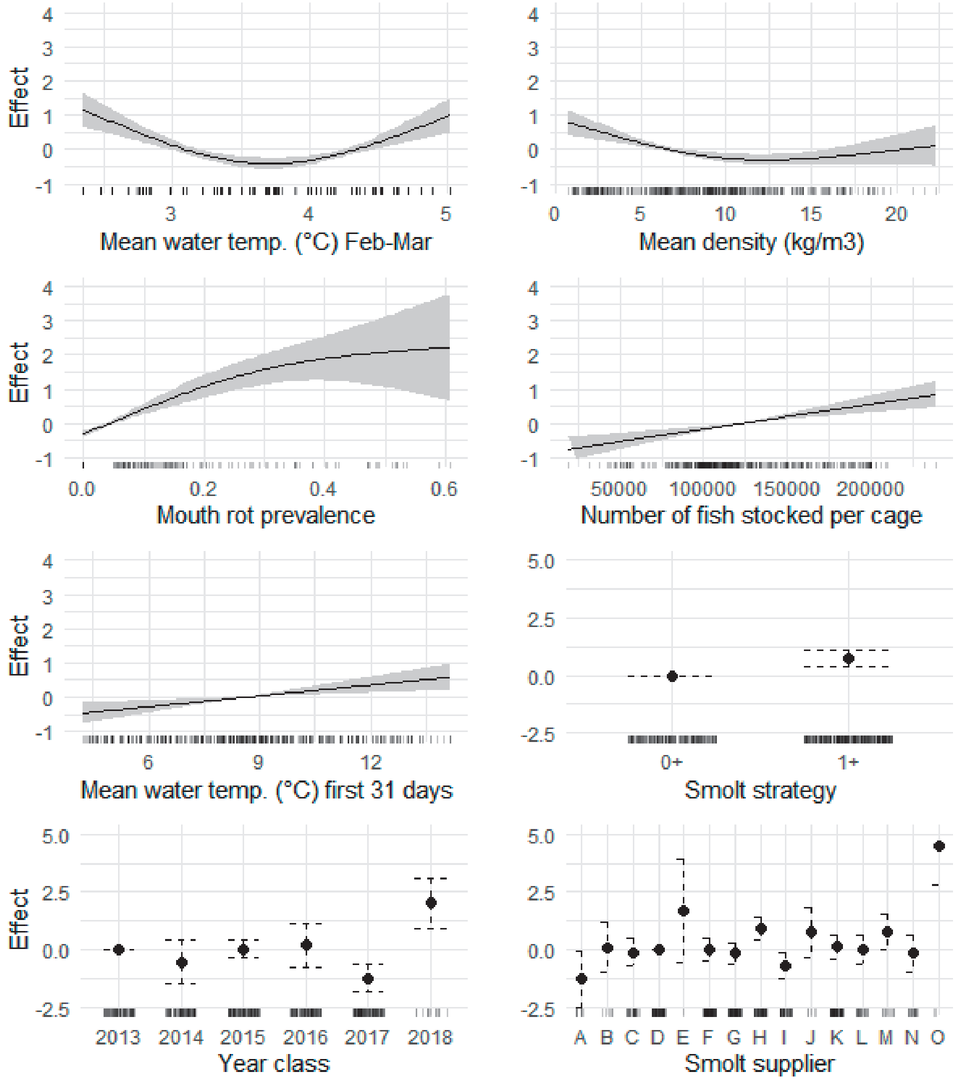

Figure 2: Smoothing curves for the partial dependences of the covariates in the chosen generalized additive mixed model for ulcer prevalence during the first winter (February through April) at sea. 95 % confidence bands are included as shaded areas for the parametric terms and whiskers for the non-parametric terms.

3.3 Generalised additive model for mouth rot prevalence

The specifications for the models including the prevalence of mouth rot as a response are shown in Table 1. The model with the lowest AIC value includes smolt size, mean water temperature during the first 31 days in seawater, mean density during the first 200 days in seawater, the number of fish stocked in the cage, the difference in water temperature between the first and fourth week in seawater, windspeed during seawater transfer, and smolt strategy (0+ or 1+) as explanatory variables. The deviance explained by this model was 56.7 %.

Table 3: Specifications of the candidate models for the prevalence of mouth rot, including spent degrees of freedom and AIC values. SS: smolt size (g), Temp.31: temperature (°C) during the first 31 days following transfer to seawater cages, Lice: average salmon lice count during the first 200 days following transfer to seawater cages, Stock: the number of fish originally stocked in the cage, Temp.Grad: the difference between the average temperature (°C) of the fourth and first weeks following stocking, Wind: wind speed (m/s) during stocking, and SSt: smolt production strategy (0+ or 1+).

Model specification	Df	AIC
SS + Temp.31 + Lice + Dty. + Ulcers + Stock + Temp.Grad + Wind + SSt	17.2	241.6
SS + Temp.31 + Dty. + Ulcers + Stock + Temp.Grad + Wind + SSt	15.9	239.4
SS + Temp.31 + Dty. + Stock + Temp.Grad + Wind + SSt	13.9	238.6
SS + Temp.31 + Dty. + Stock + Temp.Grad + SSt	12.0	238.8
SS + Temp.31 + Dty. + Stock + Temp.Grad	11.0	241.6

The model terms and significances for the chosen model are listed in Table 4. Despite this model configuration having the lowest AIC value, the covariates for wind speed when stocking fish into seawater and the difference between smolt strategies are not significant. The model diagnostics did not reveal any problems.

Table 4: The estimates and significances for parametric coefficients and approximate significances of smooth terms, including chi-squared values, for the chosen generalised additive model for the prevalence of mouth rot.

Parametric coefficients	Estimate	Std. Error	Pr(>|t|)
Intercept	-3.7458	0.6013	<0.001
Smolt strategy (1+)	-1.1656	0.6533	0.074

Smooth terms	Chi.sq	p-value
Smolt weight	52.27	<0.001
Mean seawater temperature, first 31 days	17.50	<0.001
Mean stocking density	44.02	<0.001
No. fish stocked	18.65	<0.001
Temperature difference, week 1 vs. week 4	18.45	<0.001
Wind speed (m/s) at stocking	4.53	0.136

Plots showing the partial dependencies, represented by smoothing curves, between the prevalence of mouth rot and the parsimonious models' covariates are shown in Figure 3. The prevalence of mouth rot seems to increase with increasing smolt size, and the number of fish stocked per cage. It seems to decrease with increasing mean temperature during the first 31 days after transfer and mean density during the first 200 days in seawater. For production cycles where the average temperature during the fourth week after seawater transfer is lower than in the first week, i.e. when the fish is transferred to seawater when the seawater temperature is declining, the prevalence of mouth rot seems to be higher. There is a clear intercept at 0, indicating no change in temperature. Production cycles with 1+ smolts are associated with lower incidence of mouth rot. There is a tendency towards an increasing prevalence of mouth rot with increasing wind speed but, again, this smoother is not significant.

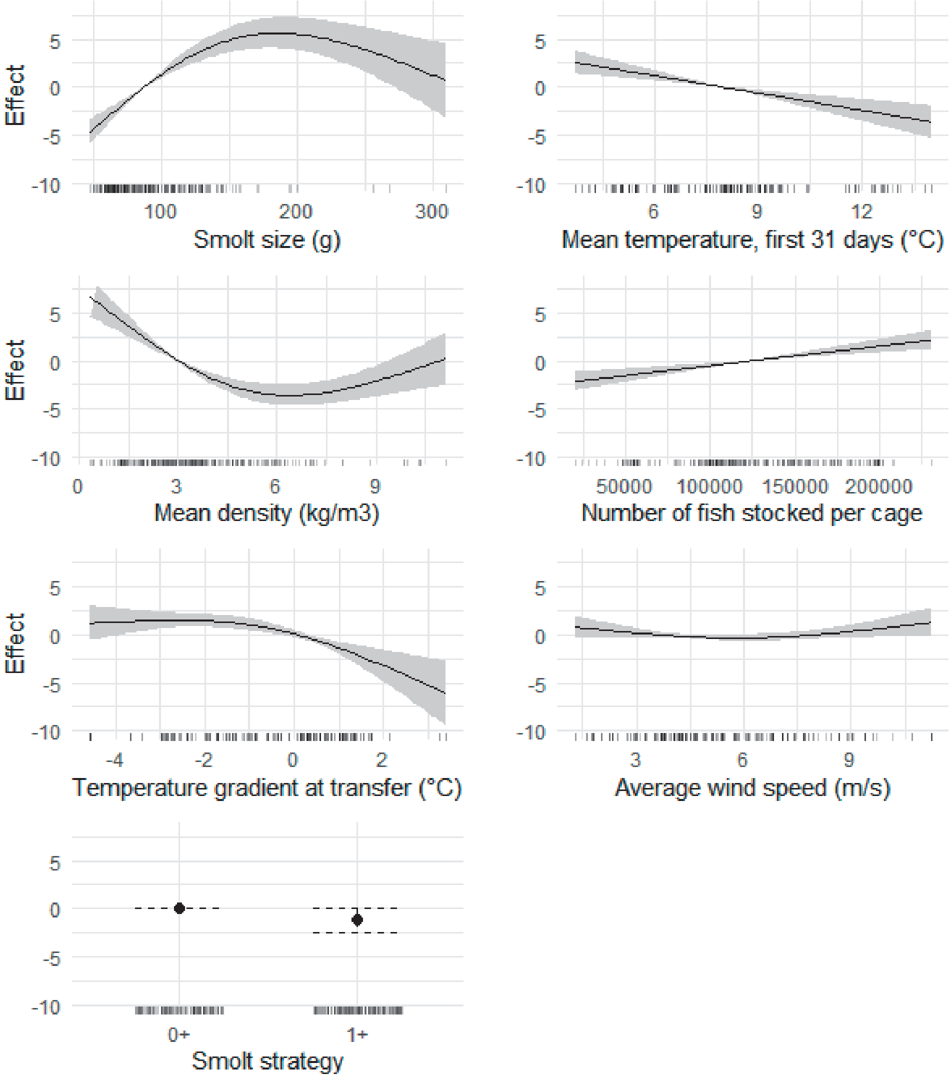

Figure 3: Smoothing curves for the partial dependences of the covariates in the chosen generalized additive mixed model for the prevalence of mouth rot during the first 200 days at sea. 95 % confidence bands are included as shaded areas for the parametric terms and whiskers for the non-parametric terms. The temperature gradient at transfer represents the difference between average seawater temperature during the fourth week after seawater transfer and that of the first week.

4 Discussion

Our analyses of data from production cycles commencing 2013 – 2017 show a relatively high incidence of ulcerative disease and mouth rot in the farms included in the study. The prevalence of ulcerative diseases seems to have been stable in the production cycles commencing from 2013-2016, and significantly lower in 2017. The drivers for this reduction e.g., in relation to improvements in the vaccines used or other preventive or abating measures have not been identified. In general, the fish producer from whom we sourced the dataset uses both vaccination and functional feeds as preventive measures against ulcers, and functional feeds as a mitigating measure. For mouth rot, antibacterial agents are often used as treatments.

The boxplots in Figure 1 clearly show that mortalities attributed to skin ulcers in this dataset predominantly occur in winter, with the highest average mortality rate occurring in February through April. This corresponds with the period of coldest water temperatures in the study region and suggests that the observations conform with what has previously been called winter ulcer disease. For smolts transferred in autumn (0+) we see that the first winter is the most severe, while for smolts transferred in spring through summer (1+) the mortalities are more evenly distributed throughout the production cycles. This may in part be explained by the fact that timing of transfer spans a larger range of possible dates for 1+ smolts and thus the time series used for plotting are poorly aligned with calendar months. The GAM analyses reveal that the production and transfer of 1+ smolts is associated with an increase in the prevalence of ulcers. Smolts of this category are usually transferred to seawater during spring to early summer. As 1+ smolts have been in seawater for a longer period of time before experiencing their first winter than 0+ smolts, their skin barriers should have had more time to regenerate and develop following seawater transfer (Karlsen et al., 2018). However, these smolts may also experience both elevated lice levels and delousing treatments during the summer and are therefore more likely to have experienced handling trauma and stress that may negatively impact upon both skin integrity and immune status. Elevated lice levels can significantly worsen the impact of *M. viscosa* infections in Atlantic salmon, inhibiting the capacity for the ulcers to heal (Carvalho et al., 2020).

The smoothing curve for mean temperature during February through April has a U-shape, indicating a slightly increased prevalence of ulcers at temperatures below 3 °C and above 4.5 °C. The range of average temperatures in our study is below the threshold for an increased risk of ulceration, which has previously been observed to be around 8-10 °C (Greger and

Goodrich, 1999; Gudmundsdóttir and Björnsdóttir, 2007; Løvoll et al., 2009), and it is therefore difficult to see what causes this variation. If we had used the information contained in the time series of daily temperature registrations, instead of using an aggregate value for the whole period for each production cycle, more could possibly have been said about the patterns in ulcer prevalence in relation to temperature. The increase in the prevalence of mortalities associated with higher post-transfer seawater temperatures in our study could be related to the timing of seawater transfer acting as a confounder, with 0+ smolts experiencing the higher post-transfer temperatures in the farms we studied.

A higher production density, i.e. the amount of biomass per available unit of production volume, during the first winter at sea seems to be associated with a lower prevalence of ulcers, albeit not strongly so. Our results also indicate an increasing prevalence of ulcers when more fish are stocked in a cage. Higher densities have been shown to impair wound healing in Atlantic salmon (Sveen et al., 2018) and the smoothing curve for density therefore seems counterintuitive. However, the production densities in our analysis are generally low and Norwegian aquaculture regulations impose a 25 kg/m^3 maximum density restriction in sea cages. In studies where a high rearing density can be detrimental to skin integrity and health, the problematic densities are above 75 kg/m^3 (Calabrese et al., 2017; Sveen et al., 2018). As can be expected, stocking numbers vary according to the dimensions of the cages available at the different farms, with more fish being stocked in cages with larger available volumes. Some authors have noted that winter ulcers are more common in individuals that are smaller than the average (Coyne et al., 2006, 2004), and may be possibly emaciated. Our results show the same relationship to stocking density and the number of fish stocked when modelling the prevalence of mouth rot.

Only two smolt suppliers show significant deviation from the mean, with regards to ulcer prevalence, one producing a higher prevalence and one a lower. It therefore appears as if factors relating to smolt production can have an impact on the prevalence of ulcers after seawater transfer, but that it is normally not the case. Ulcers can also occur in the freshwater stage of Atlantic salmon production and the extent to which hatcheries or smolt production sites has problems with ulcers is highly variable (Takle et al., 2015). While we cannot elaborate on the exact cause for the differing ulcer prevalence in fish from these suppliers, whether it is e.g. related to handling and vaccination practices or water quality, the result is indicative of persistent differences in production characteristics in these farms.

Losses to mouth rot are clearly confined to the first seven months of seawater production in the farms and year classes we analysed. Whilst we did not have access to diagnostic data concerning the specific pathogens involved in these outbreaks, the guidelines the farmworkers abide by in recording cause-specific mortalities equate mouth rot with Tenacibaculosis. The patterns we observe in our study reflect those found to be associated with *Tenacibaculum* spp. related outbreaks elsewhere in Norway and on the west coast of North America and Tasmania (Frisch et al., 2018a, 2018b; Klakegg et al., 2019; van Gelderen et al., 2011). Beyond the first 7 months of seawater production there only seems to be limited cases in our study, and the majority seem to occur within the first 5 months of the calendar year.

We also see from the boxplot that total cause-indiscriminate mortality is higher immediately following seawater transfer, indicating the pressure the various situations associated with this action imposes on the fish's health. Similar patterns of elevated post-transfer mortality has been observed in other studies (Aunsmo et al., 2008; Soares et al., 2013). The transfer of smolts from a freshwater hatchery site to seawater cages can expose them to several stressors through operations such as pumping and well-boat transport (Iversen et al., 2005, 1998). Moreover, smolt quality, e.g. osmoregulatory capacity, may vary between and within groups depending on the smoltification procedure employed by the hatchery (Striberny et al., 2021). It has been observed that skin thickness and immune function is compromised in Atlantic salmon post-smolts following seawater transfer, and whilst it gradually improves over time, the fish are possibly subjected to a post-transfer period where they are more susceptible to infection and disease (Karlsen et al., 2018). An interesting finding in our study is the increased prevalence of mouth rot with increasing smolt size. To what extent this reflects reduced skin barrier or immune function in smolts transferred to seawater at larger sizes is not clear.

Transferring smolts to seawater when the seawater temperature is declining appears to increase the incidence of mouth rot in our study, also supported by the significantly lower incidence of mouth rot in smolts transferred in spring. This has also previously been observed by the industry (Fagerheim and Petersen Onsrud, 2019). It is unclear if this is connected to the prevalence of pathogens being higher when seawater temperatures are falling, or if transferring fish to seawater during this time has an adverse impact on the physiological state of the fish affecting e.g. skin integrity or healing capacity (e.g. Jensen et al., 2015). Our results also show that transferring smolts to seawater when seawater temperatures are low (< 8 °C) is also associated with higher prevalence of mouth rot.

The presence of mouth rot following transfer is associated with a higher incidence of ulcers during the first winter at sea in our analysis. It has previously been suggested that *M. viscosa* can be a predisposing factor for the development of *Tenacibaculum* spp. (Olsen et al., 2011), but our results could be interpreted to suggest the reverse. As previously noted, however, we do not have precise diagnostic data and cannot make conclusive inference about infection dynamics based on our analyses. *M. Viscosa* and *Tenacibaculum* spp. are commonly found together in ulcers but their respective roles vis-à-vis each other, or in isolation, with regard to the pathogenesis of ulcerative disease remain unclear (Karlsen et al., 2017b, 2017a; Olsen et al., 2011; Småge et al., 2018). The temporal difference in prevalence of the conditions referred to as ulcers and mouth rot in our dataset is, however, indicative of two different conditions with differing pathogeneses associated with these conditions.

We did not consider spatial variation in the prevalence of either ulcers nor mouth rot, with the exception of the inclusion of production site as a random effect in the ulcer model. This was due to the production sites being spread across several regions, with intermittent production sites belonging to other companies, for which we had no data on ulcer or mouth rot prevalence. MacKinnon et al. (2019) could not find a uniform spatial distribution in an analysis of ulcer outbreaks in Atlantic Canada, and suggested that all farms could have been exposed to a causative agent with only those susceptible, for whatever reason, seeing outbreaks that induce mortality. A later study found that the potential for horizontal transmission of *M. viscosa* is weak (MacKinnon et al., 2020).

A strength of our analysis is that it, to the best of our knowledge, incorporates the largest number of infected farms yet studied, and therefore gives a more complete picture of the prevalence and dynamics of ulcers and mouth rot than previous studies. Despite the data having been collected by the farmers themselves, with the moribund or dead fish only having been crudely diagnosed in many cases, both the amount of data made available to us and the consistent patterns we identified regarding ulcer and mouth rot gives us little reason to doubt the overall veracity of the dataset.

This study demonstrates the potential of using well curated production data in analysing production cycles and finding production related drivers behind the variation in disease and mortality risk. The company who provided the dataset has implemented guidelines for recording cause specific mortalities on-site, as far as this is possible. If this is standardised and paired with diagnostic data from veterinary services, we believe that these datasets could provide valuable insights for improving both farm management and regional fish health governance. As has been noted by other authors (Bang Jensen et al., 2020; Soares et al., 2011), this would be especially potent if data from multiple farming companies could be collated and made available to the research community.

5 Conclusion

Our analyses show that the prevalence of both ulcers and mouth rot follow clear temporal patterns. Ulcers are most prevalent during February through April while mouth rot is most prevalent from February through May. Mouth rot mostly occurs during the first 7 months at sea. A higher prevalence of ulcers is associated with 1+ smolts and the presence of mouth rot. The choice of smolt supplier can also significantly impact the prevalence of ulcers. The prevalence of mouth rot is higher in larger smolts and in smolts transferred to seawater at low (< 8 °C) and falling seawater temperatures. The prevalence of mouth rot is lower in 1+ smolts than in 0+ smolts. These results can be used to guide production management and further epidemiological studies on ulcers and mouth rot in the affected regions. Future studies should consider the link between smolt quality, particularly previous ulcerative disease history and smolt size, and susceptibility to ulcers and mouth rot following seawater transfer.

6 Acknowledgements

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Principal causes for quality downgrading in Norwegian arctic Atlantic salmon (*Salmo salar* L.) farming and their welfare and production related drivers

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ABSTRACT

In Atlantic salmon farming, the downgrading of fish due to quality defects during primary processing is a considerable source of economic losses. In this study, our objectives were to i) identify the most common proximate causes for downgrading at a major Atlantic salmon processing plant in northern Norway, ii) find production and welfare related drivers associated with the proportions of fish in each quality grade, and iii) the proportion of fish downgraded due to the most common causes. We connected the slaughter records to the production records of 24 sea cage sites in the region and modelled the impact of production related drivers on variation in quality traits using transformation based redundancy analysis (tb-RDA) and generalised additive models (GAMs). The four most common causes for quality downgrading were ulcers (39 % of downgraded), dark spots (17 %), deformities (12 %), and early maturation (10 %), with ulcers being the most severe by far. Growth during the seawater stage, the mean seawater temperature and its standard deviation, mortalities due to heart and skeletal muscle inflammation (HSMI) or cardiomyopathy syndrome (CMS), and day of harvest were important explanatory variables behind the variation in quality. The incidence of dark spots strongly increased with increasing mortalities due to HSMI and CMS. A positive correlation between the incidence of dark spots and lice infestation was also observed. Lower mean temperatures and lower temperature standard deviations were associated with an increased incidence of downgrading due to ulcers. Deformities were highest in fish experiencing a mean seawater temperature between 6.5 °C and 7.0 °C. Ulcers were least prevalent in fish harvested from late summer through to autumn, dark spots were least prevalent in fish harvested during summer, mature fish were the least common in late spring and deformities did not vary significantly with harvest date. This dataset increases our understanding of the production and welfare related drivers that influence harvest quality in Atlantic salmon and provides the farmer with an overview of how different drivers affect slaughter quality across different seasons.

Key words: Atlantic salmon, aquaculture, product quality, maturation, dark spots, HSMI, CMS, multivariate analysis

1 Introduction

Producing fish of high quality is of high economic importance for the Atlantic salmon aquaculture industry. Norwegian regulations prohibit the sale of farmed fish with quality defects for human consumption (§17 Forskrift om kvalitet på fisk og fiskevarer, 2013) and fish quality is generally an important determinant for consumer acceptance and willingness to pay (Altintzoglou and Heide, 2016; Gjedrem, 1997; Nguyen et al., 2015). Raw material quality is also a determinant of processing success and efficiency due to the extra time required for certain processing steps, e.g. trimming, to achieve a marketable product (Einen et al., 1999; Mørkøre, 2012). Thus, while severe quality defects result in lost production costs, e.g. related to feed and labour expenditure, and a near total loss of returns, lesser quality defects may yet cause losses due to a reduction in marketability and sales prices, and an increase in processing costs. From a more general sustainability perspective, food production systems need to make headway in relation to the UN Sustainable Development Goals and aquaculture is no different (Stead, 2019), even though farmed Atlantic salmon has one of the lowest carbon-footprints of any farmed animal (Skontorp Hognes et al., 2011). Any defects to product quality can hinder this progress. For example, from an environmental perspective, quality downgrading can be an unacceptable waste of input resources, e.g. feed, thus impacting upon UN SDG 12: “Responsible consumption”, where the reduction of food losses in food production is a central goal. From a fish welfare perspective, numerous quality downgrades may be caused by underlying health issues, injuries or diseases (e.g. ulcers) and certain downgrade drivers such as injuries are also well established Operational Welfare Indicators (OWIs) that are symptomatic of historical or slaughter-induced welfare problems for the fish (Noble et al., 2012). Despite this, the contribution of post-harvest losses, i.e. downgrades and discards, to overall production losses is often neglected (e.g. Iversen et al., 2020; Pincinato et al., 2021).

Quality grading is determined by the specifications of the industry standard NBS 10-01 (Industry Standards for Fish, 1999), which categorises the fish as either Superior, Ordinary, or Production, based predominantly on primary quality characteristics, mostly related to appearance. In practice, grading is usually performed manually by processing plant workers (Misimi et al., 2008) during primary processing. A fish assigned to the Superior category has no faults or defects, such as sores, fin damage, tissue scars, or epidermal damage. Fish assigned the Ordinary category have no muscular tissue damage, open sores, or deformities, but may otherwise have e.g. some damage to the fins or skin, superficial dark spots in the muscle, or minor faults in cutting. Fish that do not satisfy the requirements of the Superior or Ordinary categories, e.g. that have deformities, dark spots, sores, or signs of sexual maturation, are downgraded to the Production category. Fish belonging to this latter category cannot be exported or sold for human consumption within Norway without considerable processing. The proportion of harvested volume classified as Superior was reported to typically vary between 90 % and 97 % in Scotland around the turn of the millennium (Michie, 2001), but more recent reports from commercial producers indicate a typical 8 % incidence of severe dark spots alone in Norway (Färber, 2017).

As numerous injuries can cause downgrading in Atlantic salmon, including deformities, excessive pigment deposits in muscles, and exterior and interior damage such as ulcers/sores, fin damage and scale loss, there are multiple potential welfare (and production) related determinants of fish quality in aquaculture. Drivers for these injuries can be improper handling, e.g. during transport, delousing treatments and harvesting, which can lead to scale loss and wounds. Sores can be caused by the infectious bacterial agents *Moritella viscosa*, *Tenacibaculum* spp., *Aliivibrio (Vibrio) wodanis* and fin damage can also be a result of handling, abrasion and bacterial infections (Ellis et al., 2008; Harmon, 2009; Noble et al., 2018, 2012). From an ethical and product quality perspective, there is also a need for a greater understanding of the consequences of these welfare risks (e.g. Noble et al., 2012).

Most earlier studies on quality variation in farmed Atlantic salmon have focussed on the finer aspects of fillet quality, such as texture and fat content, and these are not covered in detail in the quality grading scheme of NBS 10-01. The proximate composition and texture of fillets has been shown to vary systematically in relation to e.g. temperature, the geographical location of the production unit, and the production company (Ørnholt-Johansson et al., 2017). To the best of our knowledge, there are few, if any, studies that have assessed the overall causes for downgrading during primary processing in Atlantic salmon farming in Norway and have explored the associations between quality downgrading and drivers related to welfare and production.

With this in mind, we sought to answer the following research questions: (i) what are the principal, proximate causes of downgrading, (ii) what are the welfare and production related drivers behind differences in grading, and (iii) what are the drivers behind the principal, proximate causes for downgrading? To answer these questions, we analysed a commercial dataset, covering marine production and post-harvest processing, from a large Atlantic salmon producer with some operations in northern Norway.

2 Materials and Methods

2.1 Data description and pre-processing

For our analysis we used data from the seawater operations of a large Norwegian Atlantic salmon producer in the former Finnmark county (now part of the merged Troms and Finnmark county) and one of their processing plants, all situated between latitudes 70 and 71 °N. The dataset comprises production records from the marine phase of 341 production cycles, completed at 24 sites in the years 2012-2018, and their slaughter quality characteristics as recorded by the processing plant. A production cycle is here defined as commencing upon the stocking of fish in an empty production unit and terminating when the last fish is harvested or removed, i.e. it represents a unique combination of an identifiable production unit and a span of time.

Reports generated during each slaughtering session were the source of the slaughter quality dataset. One seawater production cycle could be slaughtered through several sessions, and the number of sessions used per production cycle was variable. In each session approximately 100 fish were sampled, and external quality traits were assessed and reported by plant workers in accordance with the company's internal procedures, which adhere to the industry standard NBS 10-01. Per fish, only the principal cause of downgrading, e.g. dark spots or sexual maturation, had been recorded. Thus, two possible causes for downgrading may have been present in a sampled fish, but only the most severe was recorded. When several slaughter sessions occurred per production cycle, we added the number of fish belonging to each quality category together, making a summed proportion for each production cycle. The proportion of fish assigned a certain quality category did not vary notably through the slaughtering sessions for each production cycle.

All production related explanatory variables used in the analyses are listed in Table 1, with their units and descriptions. Specific growth rate (SGR) was calculated as $SGR\ (\%) = (\ln W_F - \ln W_I) \times \left({}^{100}/_{\Delta t}\right)$ and the thermal growth coefficient (TGC) was calculated as $TGC = 1000 \times (\sqrt[3]{W_F} - \sqrt[3]{W_I}) \times D^{-1})$, where W_F is the final weight of the fish, W_I is the initial weight of the fish, Δt is the duration, and D is the sum of day degrees (°C) for the production cycle. For the freshwater SGR, as we did not have an accurate initial weight measurement in most cases, we set 0.2 g as a reasonable estimate for the initial weight for all cycles and used smolt weight as the final weight. For the calculation of seawater SGR and TGC, the average smolt weight was used as the initial weight and gross slaughter weight as the final weight. Gross slaughter weights were given as averages per cage per slaughtering session, without data on sample variation. Hence, to avoid the fallacy of averaging averages, we used the slaughter weight from the session with the largest sample size and the number of days from first stocking up until the corresponding day as the duration of the production cycle. Like the quality characteristics, variation in reported slaughter weights among slaughtering sessions was negligible within production cycles.

Cause specific mortality counts were included as explanatory variables to indicate possible adverse health or welfare related conditions that can have an influence on quality. The causes include handling, ulcers, early sexual maturation, O_2 deprivation, and infectious diseases, i.e. infectious pancreatic necrosis (IPN), parvicapsulosis, and heart and skeletal muscle inflammation (HSMI) and cardiomyopathy syndrome (CMS). Counts of mortalities due to HSMI and CMS were combined in the original dataset due to the difficulty of onsite, visual differential diagnosis of the two. Mortality counts due to early maturation and ulcers also included fish that were removed and euthanised due to these characteristics. We divided the cause specific mortality counts by the total number of fish originally stocked in the seawater cage for each production cycle, to get mortalities as proportions. To dampen the effect of extreme values and improve the linearity of the relationship between these variables and the response, we transformed them by taking their fourth roots, $\sqrt[4]{x}$, where x is the cause specific mortality proportion. The numbers of antiparasitic treatments for *Lepeophtheirus salmonis* infestations were transformed to binary values, denoting the absence and presence of each category of treatment, as the values for these in most cases were low.

Table 1: Production variables used for analysis, as recorded for each production cycle, with their units and descriptions, including calculations and transformations if applicable.

Variable	**Unit**	**Description**
Cause specific mortalities		
Handling	-	Mortality rate transformed by fourth root
HSMI or CMS	-	Mortality rate transformed by fourth root
IPN	-	Mortality rate transformed by fourth root
O_2 deprivation	-	Mortality rate transformed by fourth root
Parvicapsulosis	-	Mortality rate transformed by fourth root
Early maturation	-	Mortality rate transformed by fourth root
Ulcers	-	Mortality rate transformed by fourth root
***Caligus elongatus* infestation**		
Total	No.	Max average count from all lice counting sessions
***Lepeophtheirus salmonis* infestation**		
Total	No.	Max average count from all lice counting sessions
Antiparasitic treatments		
Bathing	Presence/absence	Count of instances transformed to binary value
Mechanical	Presence/absence	Count of instances transformed to binary value
Growth metrics		
Freshwater SGR	%	$\ln(SW) - \ln(0.2) \times (100 / DFW)$
Seawater SGR	%	$\ln(GSW) - \ln(SW) \times (100 / DSW)$
Total SGR	%	$\ln(GSW) - \ln(0.2) \times (100 / DT)$
Seawater TGC		$1000 \times (GSW^{1/3} - SW^{1/3}) \times (1/D)$
Stocking parameters		
Stocking density (mean)	kg / m^3	Mean of daily density estimates
Day of seawater transfer	Ordinal day	The first day fish were stocked in the cage
Day of harvest	Ordinal day	The day during which the most fish were harvested
Daylength at transfer	Hours	Estimated daylength based on date and latitude
Daylength at harvest	Hours	Estimated daylength based on date and latitude
Duration of production cycle	Days	Days between seawater transfer and harvest
Temperatures		
Water temperature at transfer	°C	Mean of the first 7 daily temperatures recorded
Water temperature mean	°C	Mean of all daily recorded temperatures
Water temperature SD		Standard deviation of all daily recorded temperatures
Weights		

Smolt weight	grams	Mean weight of all fish originally stocked
Gross slaughter weight	grams	Mean weight recorded for slaughter session with the largest sample size

HSMI: Heart and Skeletal Muscle Inflammation, CMS: Cardiomyopathy Syndrome, IPN Infectious Pancreatic Necrosis, SGR: Specific Growth Rate, TGC: Thermal Growth Coefficient, SW: Smolt Weight, DFW: Duration of the freshwater phase (smolt production), GSW: Gross Slaughter Weight, DSW: Duration of the production cycle (seawater phase), DT: Duration of the freshwater phase plus the duration of the seawater phase, D: Day degree sum for the seawater phase, SD: Standard deviation.

2.2 Data analysis

We calculated and visualised descriptive statistics from the processing plant records to find the principal, proximate causes of downgrading. The relationships between welfare- and production-related variables and slaughter quality were analysed in two steps. First, transformation-based redundancy analysis (tb-RDA) was used as an exploratory tool to get an overview of the multivariate relationships between the different sets of variables, and the corresponding distribution of production cycles. Then, Generalized Additive Models (GAMs) (Wood, 2006) were fitted to individual principal causes for downgrading, to obtain more detailed evaluations of the effects and account for potential non-linearities.

2.2.1 Transformation-based redundancy analysis

We used transformation-based redundancy analysis (tb-RDA) to analyse the effect of production variables on the distribution of slaughtered fish among the different quality grades and among the principal causes of downgrading. tb-RDA is a linear regression method that can accommodate multiple explanatory and response variables in one model, and is well suited for responses representing abundances (Legendre and Gallagher, 2001; Legendre and Legendre, 2012), e.g. the respective amounts of fish classified as Superior, Ordinary, and Production for each production cycle.

Prior to analysis the response variable matrix was transformed using a Hellinger transformation (Legendre and Gallagher, 2001) and the explanatory variables were scaled to unit variance (tb-RDA on a correlation matrix). To assess model adequacy, Monte Carlo permutation tests based on the pseudo-F statistic were conducted to test the global model significance and the significances of the canonical axes, as well as for testing each explanatory variable's explained variance with all others used as covariables, independent of their order in the model. In all cases, 999 permutations were used. Also, the adjusted R^2 was computed for each model as an unbiased measure of explained variation. A combination of a forward and backward stepwise selection of variables was used to find a parsimonious model, with the inclusion criterion being a permutation P-value ≤ 0.05. Variance inflation factors were calculated for the parsimonious models to evaluate multicollinearity, with values > 5 being deemed problematic.

2.2.2 Generalized additive modelling

To better account for any non-linearity between the principal causes for downgrading and the most interesting, based on the outcome of the tb-RDA analyses, potential welfare and production related drivers, we also attempted to fit Generalized Additive Models (GAMs) (Wood, 2006) for each of the principal causes for downgrading. In the GAMs, the number of fish downgraded due to specific causes were treated as proportions of the total number of fish harvested per production cycle. As we also expected some overdispersion in the residuals, we assumed a Tweedie distribution for the response and used a logit link function for all models. Preliminary attempts at including sea cage site or year classes as factor variables in the GAMs did not find them significant. Neither did we find any groupings related to site or year class in the RDA scores. They were therefore excluded upon further modelling. All explanatory variables were fitted using thin plate regression splines, except for those with day of year as their unit, where cyclic cubic regression splines were used. We used stepwise backward selection, eliminating non-significant explanatory variables, to find a parsimonious model.

Additionally, Akaike's information criterion modified for small sample sizes (AICc) was used as an aid in choosing the best model. We validated the models by checking residuals' independence, homogeneity, fit, and normality, and linear dependence among explanatory variables (concurvity).

2.3 Software

All data cleansing and pre-processing was done using the Python programming language (version 3.7.3) and the associated Pandas (McKinney, 2010) and NumPy (van der Walt et al., 2011) libraries. Analyses were run in R (version 3.6.1) using the packages vegan for all aspects of RDA (Oksanen et al., 2019), mgcv for GAM analyses (Wood, 2011), and ade4 for visualisation (Dray and Dufour, 2007, p. 4). RDA triplots were generated using code from Borcard et al. (2011).

3 Results

3.1 Descriptive statistics

The distributions of the proportions of fish downgraded from Superior grade, to either Ordinary or Production, of all sampled fish, are summarised for each proximate cause in Table 2. We see that the dominant cause for downgrading in this dataset is ulcers, affecting on average more than 7 % of the sampled fish, and in 25 % of the harvests affecting more than 10 % of the sampled fish. Dark spots represent the second most frequent cause, affecting on average more than 3.5 % of the sampled fish, and in 25 % of the harvests affecting 5 % of the slaughtered fish. Deformities and maturation are the third and fourth most frequent causes for downgrading, at averages of about 2.4 % and 1.5 % of sampled fish, respectively.

Table 2: The mean, standard deviation, and quartiles of the proportions of fish downgraded, as percentages of the total amount of fish sampled, according to their principal cause for downgrading.

Cause	**Mean**	**St.Dev.**	**1st quartile**	**3rd quartile**
Ulcer	7.43	9.80	1.00	10.62
Dark spot	3.67	4.21	1.00	5.00
Deformity	2.37	4.13	0.00	3.00
Mature	1.53	3.21	0.00	1.80
Erroneous cut	0.77	1.20	0.00	1.00
Snout damage	0.75	1.95	0.00	0.90
Other	0.53	1.31	0.00	0.89
Scale loss	0.47	1.23	0.00	0.89
Head damage	0.45	0.80	0.00	1.00
Bleeding	0.27	0.65	0.00	0.00
Jaw deformity	0.24	1.87	0.00	0.00
Emaciated	0.24	0.94	0.00	0.00
Dead	0.19	2.15	0.00	0.00
Short tail	0.15	0.48	0.00	0.00
Reduced operculum	0.14	0.38	0.00	0.00
Parasite	0.08	0.39	0.00	0.00

Figure 1 shows the proportions of sampled fish downgraded to the Production and Ordinary grades, and both combined, according to the principal cause for downgrading, as percentages of the amount of downgraded fish in the samples. Here we see that while ulcers represent the most common cause for downgrading to Production grade, and overall, dark spots represent the most common cause for downgrading to Ordinary. Dark spots only make a relatively small contribution to downgrades to Production grade. As such, ulcers are not only more common than dark spots as a cause for downgrading, but also much more severe. Matured and deformed fish also contribute more to the Production grade than they do to the Ordinary grade, if only slightly so for deformed fish.

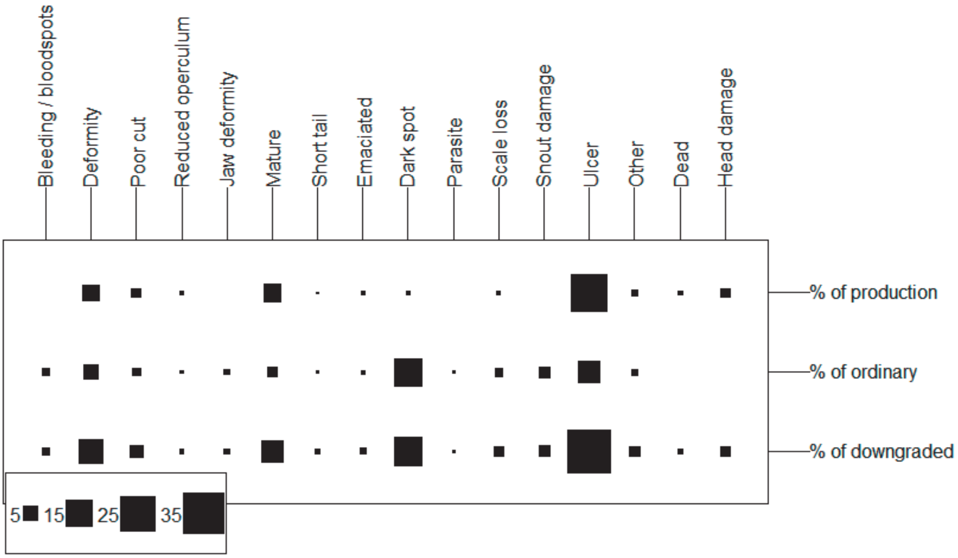


Figure 1: Percentages of fish downgraded according to principal cause of downgrading, for each quality grade and for both, for approximately 500 production cycles processed at the processing plant.

3.2 Transformation based Redundancy Analysis

Subjecting the matrix of quality grade proportions to tb-RDA, with welfare and production related drivers as explanatory variables, resulted in 10 significant ($P \leq 0.01$) variables being found upon variable selection. The adjusted R^2 for this model was 0.37, and both the model and the first two RDA axes were significant ($P \leq 0.001$). The residual degrees of freedom were 332 and the VIF values for all included variables were below 3. The first constrained RDA axis explained 32.7 % of the total variance, and mainly represent the contrast between superior and production grade. The explanatory variables associated with this axis are Day.Harv, Temp.Mean, TGC and Dayl.Tr (driving superior quality) and Mort.Parv, Mort.Ulc and Temp,Trans (driving production quality). The second axis explained 5.6 % of the total variance. It is mainly associated with the ordinary quality grade, for which Mort.Prec ans Mort.HSMI are the main drivers.

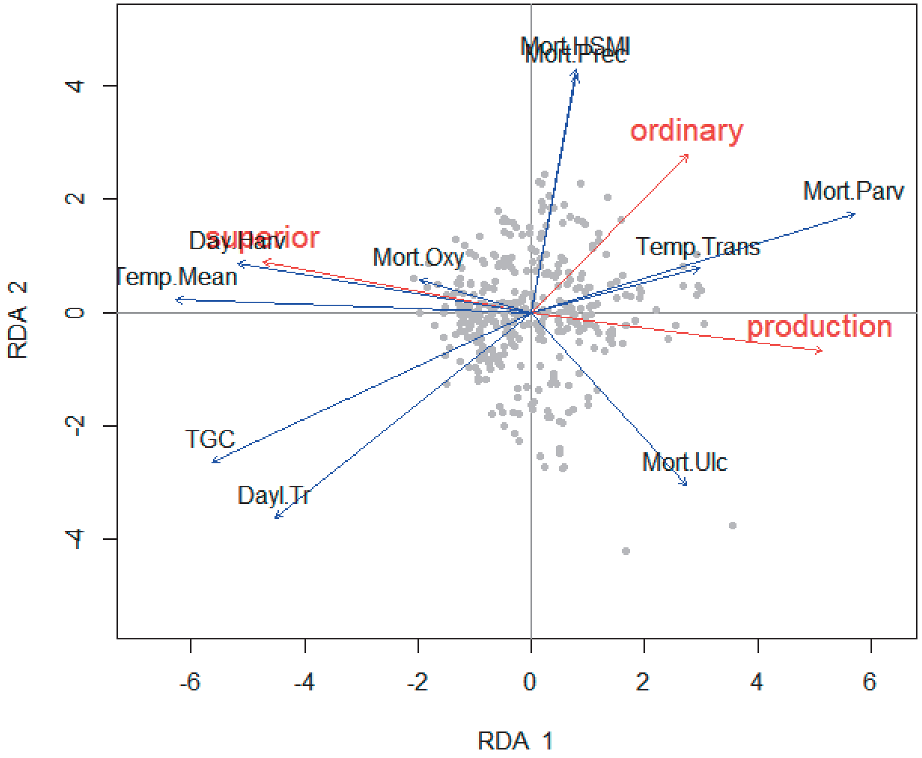
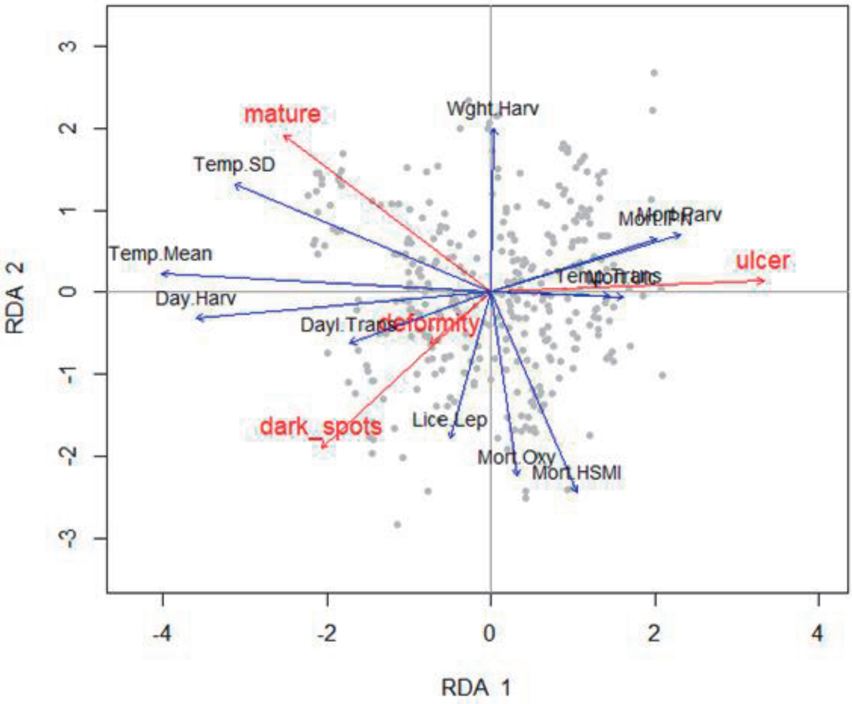


Figure 2: Transformation-based redundancy analysis correlation triplot, using fitted site scores as linear combinations of explanatory variables, with quality grades as response variables and production related drivers as explanatory variables. Day.Harv: Harvest day, Dayl.Tr: Daylength on transfer day, Mort.HSMI: Mortalities due to HSMI or CMS, Mort.Oxy: Mortalities due to oxygen deprivation, Mort.Parv: Mortalities due to parvicapsulosis, Mort.Prec: Mortalities due to early maturation, Mort.Ulc: Mortalities due to ulcers, Temp.Mean: Mean temperature

during the seawater stage, Temp.SD: Temperature standard deviation during the seawater stage, Temp.Transfer: Seawater temperature at stocking of sea cages, TGC: Thermal Growth Coefficient for the seawater stage.

Ulcers, dark spots, deformities, and matures were chosen as response variables for further analysis as these were by far the dominant proximate causes for downgrading, as seen in Figure 1. A tb-RDA conducted with the number of fish downgraded because of these causes in the response matrix, subject to variable selection, resulted in 12 significant ($P \leq 0.01$) explanatory variables, as visualized in the triplots in Figure 3 (RDA axes 1 through 3). The adjusted R^2 for this model was 0.35, and both the model and the first three constrained RDA axes were significant ($P \leq 0.001$). The first constrained axis explained 26 % of the variance in the response and can be interpreted as the contrast between ulcers and mature/dark spots. The second axis explains 8.7 % of the variance, and mainly contrast mature and dark spots. The third axis explains 3.3 % of the variance and is mostly associated with deformities. The residual degrees of freedom were 331 and the VIF values for all included variables were below 3.


mature
Wght.Harv
Temp.SD
Mort.Parv
Mort.IPN
ulcer
Temp.Mean
Temp.Trans
Mort.Ulc
Day.Harv
Dayl.Trans
deformity
dark_spots
Lice.Lep
Mort.Oxy
Mort.HSMI
RDA 2
RDA 1
3
2
1
0
-1
-2
-3
-4
-2
0
2
4

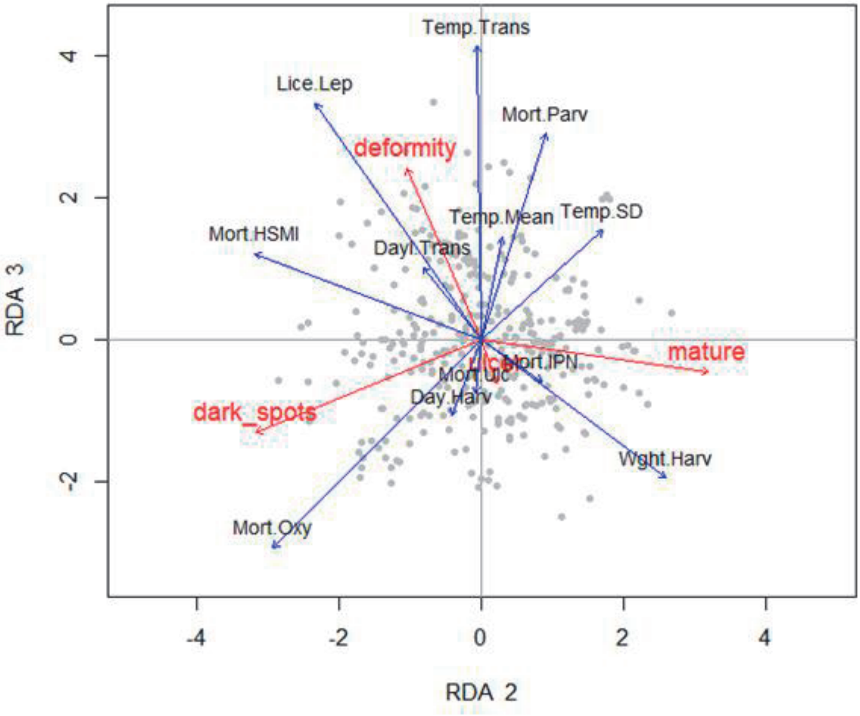

Temp.Trans
Lice.Lep
Mort.Parv
deformity
Temp.Mean
Temp.SD
Mort.HSMI
Dayl.Trans
ulcer
Mort.IPN
mature
Mort.Ulc
Day.Harv
dark_spots
Wght.Harv
Mort.Oxy
RDA 3
RDA 2
4
2
0
-2
-4
-2
0
2
4

Figure 3: Transformation-based redundancy analysis correlation triplot, using fitted site scores as linear combinations of explanatory variables, with quality grades as response variables and welfare and production related drivers as explanatory variables. RDA axes 1 and 2 (top) and 2 and 3 (bottom) are displayed. Day.Harv: Day of harvest, Dayl.Trans: Daylength when stocking into sea cages, Lice.Lep: Maximum number of *Lepeophtheirus salmonis* (all stages) recorded, Mort.HSMI: Mortalities due to HSMI or CMS, Mort.IPN: Mortalities due to IPN, Mort.Oxy: Mortalities due to oxygen deprivation, Mort.Parv: Mortalities due to parvicapsulosis, Mort.Ulc: Mortalities due to ulcers, Temp.Mean: Mean temperature during the seawater stage, Temp.SD: Temperature standard deviation during the seawater stage, Temp.Trans: Seawater temperature at stocking of sea cages, Wght.Harv: Average weight of harvested fish.

3.3 Generalised Additive Models

3.3.1 Dark spots

Modelling the prevalence of dark spots found harvest weight, mean and standard deviation of seawater temperature, daylength upon stocking of sea cages, mortalities due to parvicapsulosis and due to HSMI or CMS, and harvest day to be significant. While the full model specification saw the lowest AICc value, and a model excluding mortalities due to IPN saw the highest adjusted R^2 value, these models had issues with concurvity among variables. A reduced model, with only significant variables included, had the lowest AICc among models without problematic concurvity. The models, with their specifications, spent degrees of freedom, AICc values, and adjusted R^2 values, are listed in Table 3. The reduced model's explained deviation was 37.6 %. Model diagnostics did not reveal any problems.

Table 3: Model specifications, spent degrees of freedom, AICc values, and adjusted R^2, for GAM models ($n = 340$) with the proportion of slaughtered fish downgraded due to dark spots as a response. WH: Average weight of harvested fish, TM: Mean temperature during the seawater stage, TS: Temperature standard deviation during the seawater stage, TT: Seawater temperature at stocking of sea cages, DT: Daylength when stocking into sea cages, MO: Mortalities due to oxygen deprivation, MU: Mortalities due to ulcers, MP: Mortalities due to parvicapsulosis, MH: Mortalities due to HSMI or CMS, MI: Mortalities due to IPN, LL: Maximum number of *Lepeophtheirus salmonis* (all stages) recorded, DH: Day of harvest.

Model specification	**Df**	**AICc**	**Adjusted R^2**
WH + TM + TS + TT + DT + MO + MU + MP + MH + MI + LL + DH	35.6	-1865.03	0.33
WH + TM + TS + TT + DT + MO + MU + MP + MH + LL + DH	38.3	-1855.24	0.36
WH + TM + TS + TT + DT + MO + MU + MP + MH + DH	35.6	-1854.93	0.35
WH + TM + TS + TT + DT + MO + MP + MH + DH	34.0	-1853.34	0.35
WH + TM + TS + TT + DT + MP + MH + DH	34.7	-1850.34	0.34
WH + TM + TS + DT + MP + MH + DH	26.8	-1853.32	0.28

The model smoothers for the reduced model, with the proportion of fish downgraded due to dark spots as a response, are shown in Figure 4.

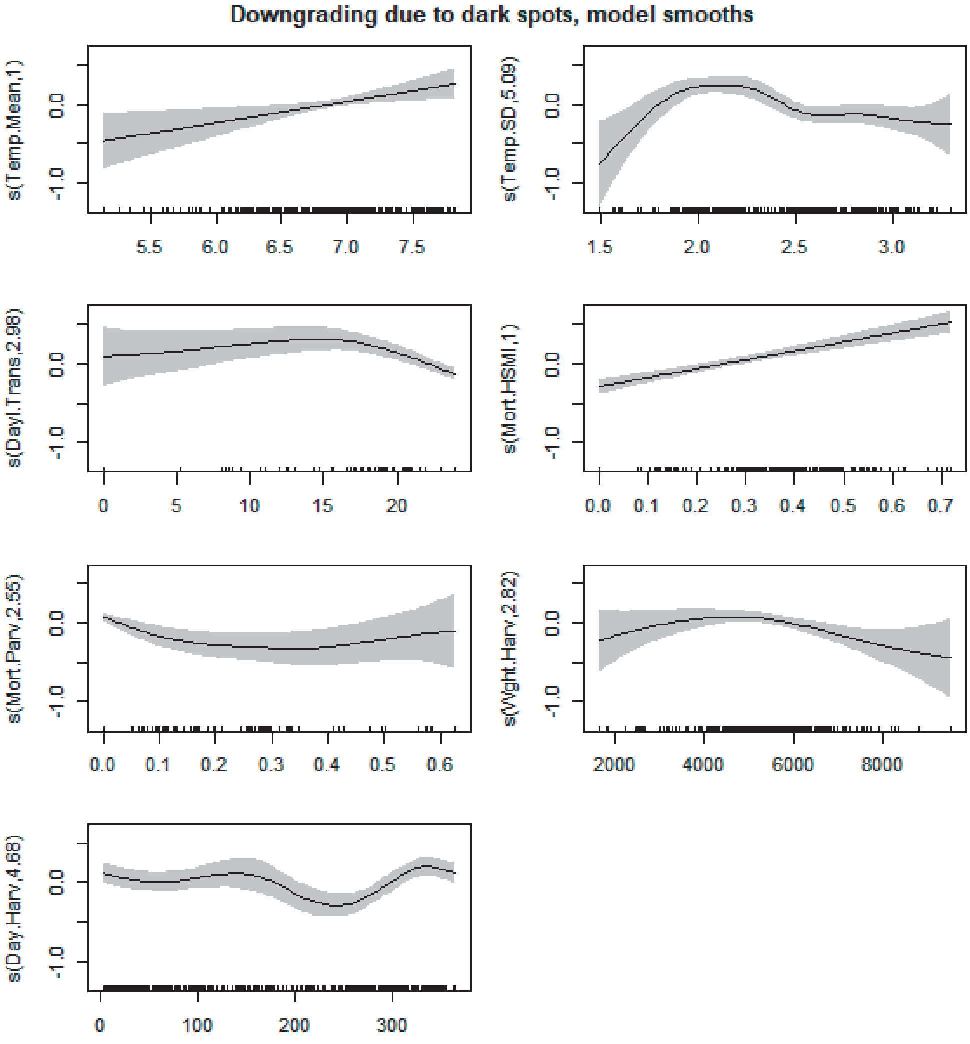


Figure 4: Smoothing curves with 95 % confidence bands for the chosen model for the proportion of fish downgraded due to dark spots. Day.Harv: Day of harvest, Dayl.Trans: Daylength when stocking into sea cages, Mort.HSMI: Mortalities due to HSMI or CMS, Mort.Parv: Mortalities due to parvicapsulosis, Temp.Mean: Mean temperature during the seawater stage, Temp.SD: Temperature standard deviation during the seawater stage, Wght.Harv: Average weight of harvested fish. The marks along the horizontal axis indicate the data points used for model fitting.

3.3.2 Maturation

When modelling the proportion of fish downgraded due to maturation, as a response, we found that a reduced model incorporating harvest weight, mean and standard deviation of seawater temperature, seawater temperature and daylength upon stocking of sea cages, harvest day, *Lepeophtheirus salmonis* counts, and mortalities due to oxygen deprivation, parvicapsulosis, and due to HSMI or CMS as significant variables to be the most parsimonious. This model had the lowest AICc and highest R^2 values. The models, with their specifications, spent degrees of freedom, AICc values, and adjusted R^2 values, are listed in Table 4. For this model, the explained deviation was 71.4 %. Model diagnostics did not reveal any problems.

Table 4: Model specifications, spent degrees of freedom, AICc values, and adjusted R^2, for GAM models ($n = 338$) with the proportion of slaughtered fish downgraded due to maturation as a response. WH: Average weight of harvested fish, TM: Mean temperature during the seawater stage, TS: Temperature standard deviation during the seawater stage, TT: Seawater temperature at stocking of sea cages, DT: Daylength when stocking into sea cages, MO: Mortalities due to oxygen deprivation, MU: Mortalities due to ulcers, MP: Mortalities due to parvicapsulosis, MH: Mortalities due to HSMI or CMS, MI: Mortalities due to IPN, LL: Maximum number of *Lepeophtheirus salmonis* (all stages) recorded, DH: Day of harvest.

Model specification	**Df**	**AICc**	**Adjusted R^2**
WH + TM + TS + TT + DT + MO + MU + MP + MH + MI + LL + DH	51.6	-2188.10	0.65
WH + TM + TS + TT + DT + MO + MU + MP + MH + LL + DH	50.3	-2188.52	0.65
WH + TM + TS + TT + DT + MO + MP + MH + LL + DH	49.7	-2190.77	0.66

The model smoothers for the partial effects of the reduced model, with the proportion of fish downgraded due to maturation as a response, are shown in Figure 5.

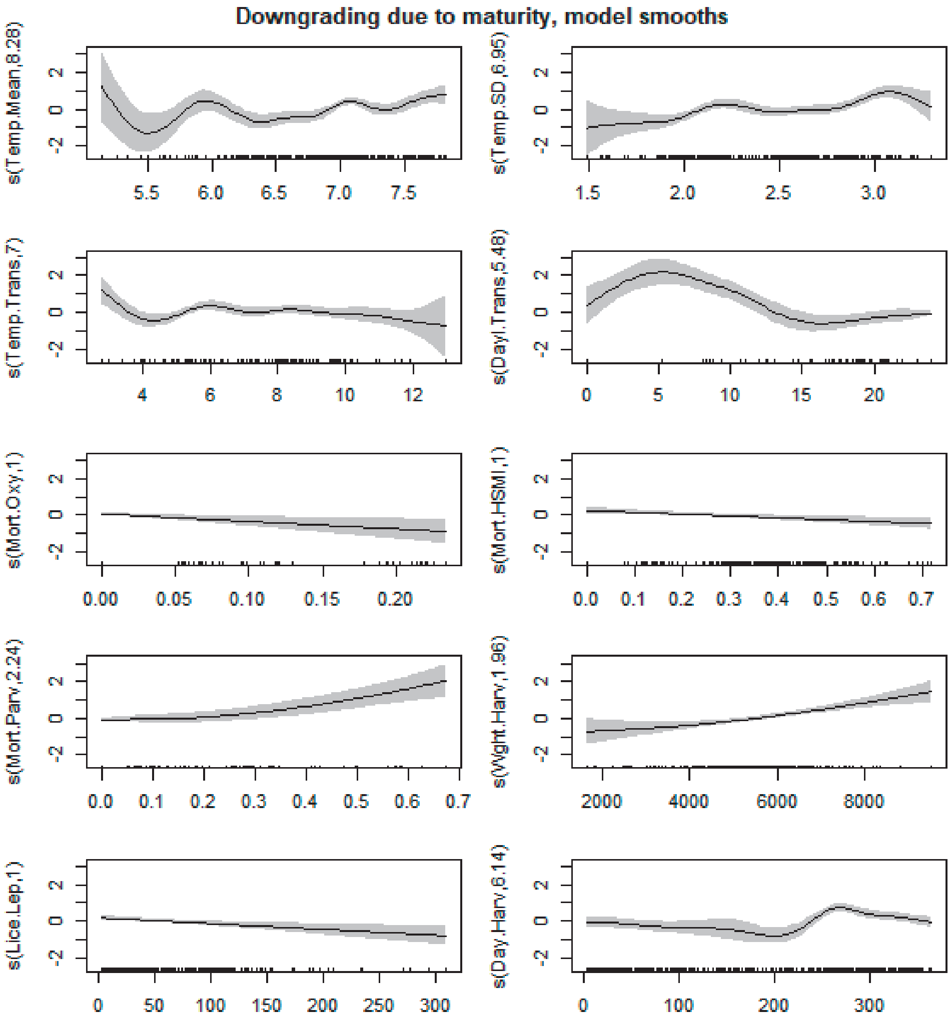


Figure 5: Smoothing curves with 95 % confidence bands for the chosen model for the proportion of fish downgraded due to maturation. Day.Harv: Day of harvest, Dayl.Trans: Daylength when stocking into sea cages, Lice.Lep: Maximum number of *Lepeophtheirus salmonis* (all stages) recorded, Mort.HSMI: Mortalities due to HSMI or CMS, Mort.Parv: Mortalities due to parvicapsulosis, Temp.Mean: Mean temperature during the seawater stage, Temp.SD: Temperature standard deviation during the seawater stage, Temp.Trans: Seawater temperature at stocking of sea cages, Wght.Harv: Average weight of harvested fish. The marks along the horizontal axis indicate the data points used for model fitting.

3.3.3 Ulcers

When modelling the proportion of fish downgraded due to ulcers we found harvest weight, mean and standard deviation of seawater temperature, harvest day, *Lepeophtheirus salmonis* counts, and mortalities due to oxygen deprivation, IPN, ulcers, and due to HSMI or CMS to be significant. AICc values, however, favour the full model, although only slightly. Given problems with concurvity among the explanatory variables, we chose the second most reduced model, which includes mortalities due to parvicapsulosis as an explanatory variable. The models, with their specifications, spent degrees of freedom, AICc values, and adjusted R^2 values, are listed in Table 5. The explained deviation for the chosen model was 53.9 %. Model diagnostics were acceptable.

Table 5: Model specifications, spent degrees of freedom, AICc values, and adjusted R^2, for GAM models ($n = 342$) with the proportion of slaughtered fish downgraded due to ulcers as a response. WH: Average weight of harvested fish, TM: Mean temperature during the seawater stage, TS: Temperature standard deviation during the seawater stage, TT: Seawater temperature at stocking of sea cages, DT: Daylength when stocking into sea cages, MO: Mortalities due to oxygen deprivation, MU: Mortalities due to ulcers, MP: Mortalities due to parvicapsulosis, MH: Mortalities due to HSMI or CMS, MI: Mortalities due to IPN, LL: Maximum number of *Lepeophtheirus salmonis* (all stages) recorded, DH: Day of harvest.

Model specification	**Df**	**AICc**	**Adjusted R^2**
WH + TM + TS + TT + DT + MO + MU + MP + MH + MI + LL + DH	46.9	-1300.70	0.52
WH + TM + TS + DT + MO + MU + MP + MH + MI + LL + DH	47.4	-1297.74	0.55
WH + TM + TS + MO + MU + MP + MH + MI + LL + DH	41.5	-1293.27	0.54
WH + TM + TS + MO + MU + MH + MI + LL + DH	30.4	-1291.55	0.48

The model smoothers for the partial effects of the chosen model, with the proportion of fish downgraded due to ulcers as a response, are show in Figure 6.

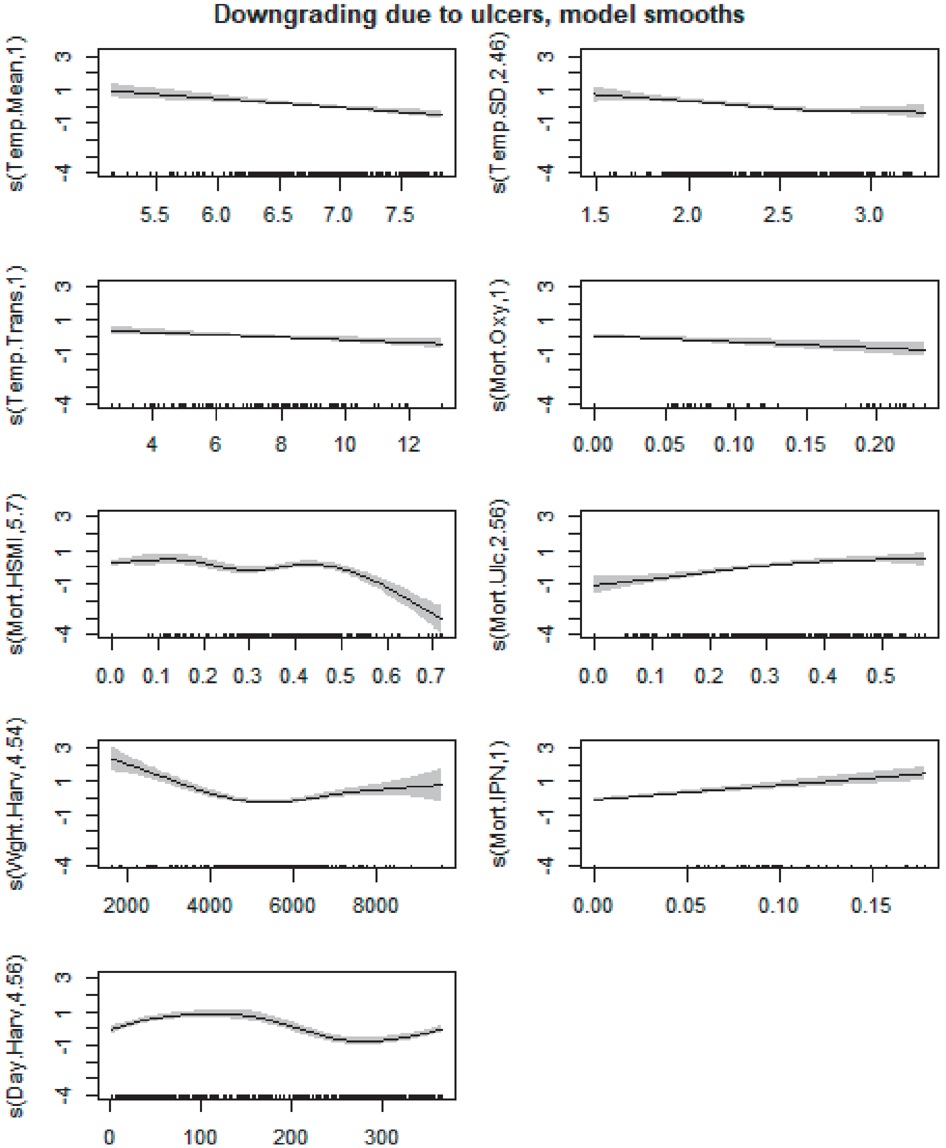


Figure 6: Smoothing curves with 95 % confidence bands for the chosen model for the proportion of fish downgraded due to ulcers. Day.Harv: Day of harvest, Mort.HSMI: Mortalities due to HSMI or CMS, Mort.IPN: Mortalities due to IPN, Mort.Oxy: Mortalities due to oxygen deprivation, Mort.Ulc: Mortalities due to ulcers, Temp.Mean: Mean temperature during the seawater stage, Temp.SD: Temperature standard deviation during the seawater stage, Temp.Trans: Seawater temperature at stocking of sea cages, Wght.Harv: Average weight of harvested fish.

3.3.4 Deformities

The GAM model with the proportion of fish downgraded due to deformities included mean and standard deviation of seawater temperature, seawater temperature and daylength upon stocking of sea cages, *Lepeophtheirus salmonis* counts, and mortalities due to ulcers and parvicapsulosis, as significant variables. The reduced model including only the significant variables was also the model with the lowest AICc and highest R^2 values among models not having concurvity issues. The models, with their specifications, spent degrees of freedom, AICc values, and adjusted R^2 values, are listed in Table 6. The explained deviation for the chosen model was 68.6 %. Model diagnostics did not reveal any problems.

Table 6: Model specifications, spent degrees of freedom, AICc values, and adjusted R^2, for GAM models ($n = 342$) with the proportion of slaughtered fish downgraded due to deformities as a response. WH: Average weight of harvested fish, TM: Mean temperature during the seawater stage, TS: Temperature standard deviation during the seawater stage, TT: Seawater temperature at stocking of sea cages, DT: Daylength when stocking into sea cages, MO: Mortalities due to oxygen deprivation, MU: Mortalities due to ulcers, MP: Mortalities due to parvicapsulosis, MH: Mortalities due to HSMI or CMS, MI: Mortalities due to IPN, LL: Maximum number of *Lepeophtheirus salmonis* (all stages) recorded, DH: Day of harvest.

Model specification	**Df**	**AICc**	**Adjusted R^2**
WH + TM + TS + TT + DT + MO + MU + MP + MH + MI + LL + DH	57.8	-1971.66	0.75
WH + TM + TS + TT + DT + MO + MU + MP + MH + MI + LL	57.8	-1971.66	0.75
TM + TS + TT + DT + MO + MU + MP + MH + MI + LL	55.0	-1973.73	0.74
TM + TS + TT + DT + MO + MU + MP + MI + LL	53.1	-1955.21	0.81
TM + TS + TT + DT + MU + MP + MI + LL	52.0	-1961.94	0.81
TM + TS + TT + DT + MU + MP + LL	49.8	-1965.19	0.81

The model smoothers for the partial effects of the chosen model, with the proportion of fish downgraded due to deformities as a response, are show in Figure 7.

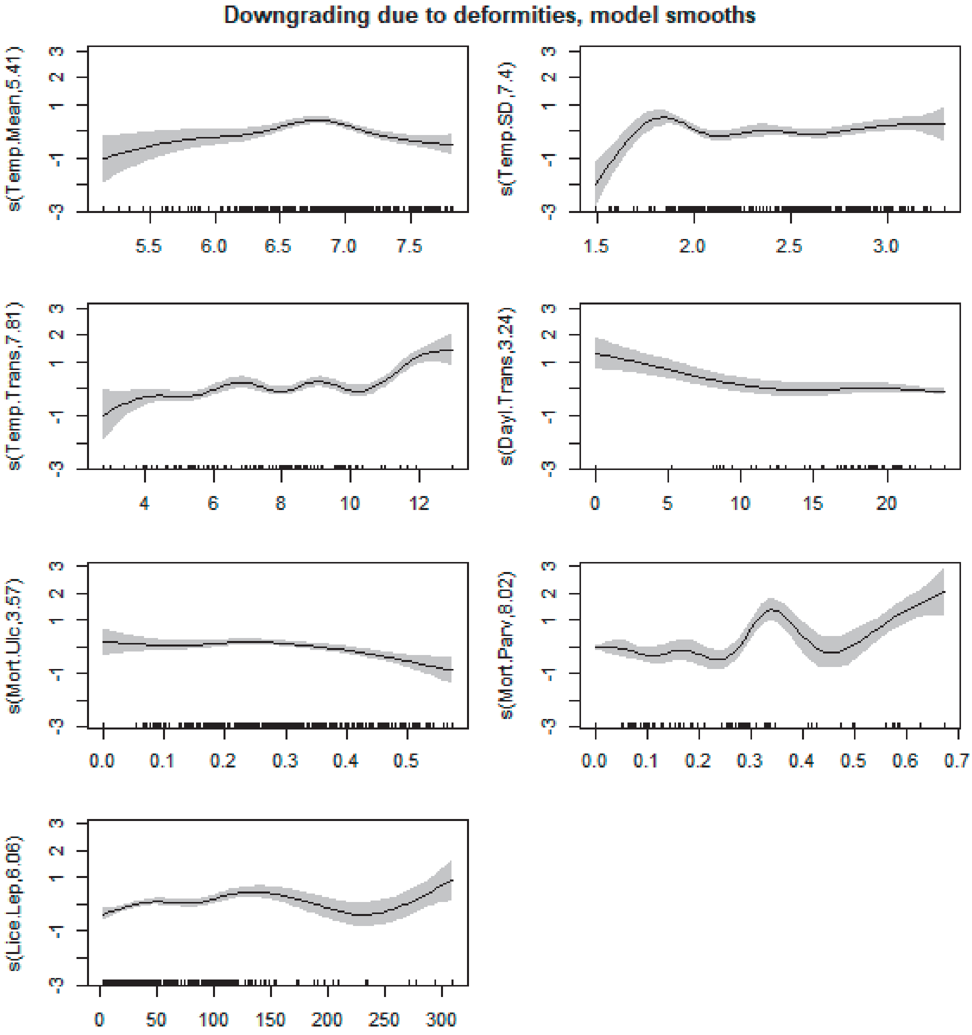


Figure 7: Smoothing curves with 95 % confidence bands for the chosen model for the proportion of fish downgraded due to deformities. Dayl.Trans: Daylength when stocking into sea cages, Lice.Lep: Maximum number of *Lepeophtheirus salmonis* (all stages) recorded, Mort.Parv: Mortalities due to parvicapsulosis, Mort.Ulc: Mortalities due to ulcers, Temp.Mean: Mean temperature during the seawater stage, Temp.SD: Temperature standard deviation during the seawater stage, Temp.Trans: Seawater temperature at stocking of sea cages. The marks along the horizontal axis indicate the data points used for model fitting.

4 Discussion

Our results show that ulcers, dark spots, deformities, and maturation are by far the dominant proximate causes for downgrading during primary processing in the northern Norwegian dataset utilised in our study. The presence of ulcers is the most common reason for downgrading to the Production grade, and the second most common reason for downgrading to the Ordinary grade, accounting for nearly 39 % of all downgrades. While dark spots represent the most common reason for downgrading to the Ordinary grade, negligible amounts of fish were downgraded to the Production category due to dark spots, with this category accounting for 17 % of all downgrades. Thus, ulcers are by far the most serious cause of primary processing losses in our dataset. Downgrading due to maturation and deformities also contributed considerably to the Production grade and overall, accounting for 10 % and 12 % of all downgrades, respectively. Michie (2001) reported matures (25.2 %), other (22.5 %), processing damage (19.8 %), misshape (19.3 %) and sea lice (13.1 %) as the most common causes of downgrading during primary processing in the Scottish industry in 1998. Misshape seems to be synonymous with spinal deformity while sea lice seem to refer to the effects of sea lice infestations, e.g. lesions, abrasions, and scale loss, and not the immediate presence of lice on the fish. Blood spotting, which seems to include dark spots, is only addressed as a cause for downgrading during secondary processing, accounting for 18.6 % of the fish downgraded at that stage. Different quality assurance schemes notwithstanding, the difference from our results may be due to advances in production and processing practices, but also differences climatic conditions associated with the different locations considered.

The prevalence of ulcers as a cause for downgrading in our analyses, especially given the northerly location of the studied sites, is likely related to the general phenomenon of ulcers affecting Atlantic salmon at low water temperatures, i.e. below 8 °C, commonly referred to as winter ulcers. The impact of mean temperature in the tb-RDA analyses, its negative correlation with ulcers in the triplot (Figure 3), and the patterns revealed by the GAM smothers (Figure 6) for mean temperature, temperature variation, and harvest day, all support this conclusion. Winter ulcers have been associated with bacterial infections, notably by *Moritella viscosa* and *Tenacibaculum* spp, although the full extent to which these cause the development of ulcers or infect already formed ulcers as opportunists, potentially aggravating the condition, is unclear (Coyne et al., 2006; Olsen et al., 2011). Seemingly, no remedies are available although vaccination shows promise as a protective strategy (Karlsen et al., 2017). Skin damage leading to abrasions and ulcers have also been associated with sea lice infestation and handling operations, e.g. mechanical or bathing treatments against sea lice. Lice are, however, more abundant during summer in the area we studied. Given the higher proportions of ulcers during winter in our study, and the lack of a demonstrable relationship in the RDA analysis, the impact of lice or delousing treatments is seemingly not relevant here.

Dark spots, or local areas of hyperpigmentation in fillets constitute a special quality related concern in Atlantic salmon aquaculture. The prevalence of dark spots in fillets increased rapidly from 2003 onwards and reached 19 % on average in Norway in 2014, causing economic losses due to costs of manual removal or downgrading (Mørkøre et al., 2015). The mechanism behind their formation has been poorly understood, but recent evidence suggests trauma induced inflammation and an associated immune response linked to the pigmentary system (Krasnov et al., 2016; Larsen et al., 2012). Production related events that have been demonstrated as causes for such inflammation in the literature include piscine orthoreovirus (PRV) and salmon alphavirus3 (SAV3) infections and vaccination with oil-adjuvanted vaccines (Bjørgen et al., 2015; Koppang et al., 2005; Lerfall et al., 2012). Associations with handling, feed composition, and adverse environmental conditions, e.g. reduced oxygen availability, have also been observed (Mørkøre, 2017). Both the tb-RDA analysis and the GAMs clearly suggest that the prevalence of HSMI, for which PRV is a causative agent, or CMS related mortalities increases the prevalence of dark spots. Administering protein rich feed, or feeds containing elevated levels of certain vitamins and minerals, in conjunction with infections have been shown to somewhat reduce the presence of dark spots in PRV-infected fish (Mørkøre et al., 2018, 2016).

As shown in the GAM plot, there is a weak but linear effect whereby increasing mean temperature results in a higher incidence of dark spots. To our knowledge no such association has been made before and it is not clear what it is indicative of. The effect of temperature standard deviation on dark spots in our results is likely caused by the few observations with low standard deviation having an undue effect. The association between the day of harvest on dark spots, showing lower incidence during late summer and early autumn, is interesting, although difficult to explain. In northern Norway, water temperatures are at their highest during this time of the year, but it is also a period with elevated lice infestation pressure, delousing treatments, and thus also handling. While overall maximum lice count does not seem to influence the prevalence of dark spots in our analyses, this does not necessarily preclude an effect of prolonged infestation, for example. Delousing treatments were not significant in our tb-RDA analyses and were therefore not inspected further. However, our dataset included relatively few observations in which mechanical treatments were used, so an effect of these may have been present but not sufficiently strong.

Sexual maturation is a product quality problem because it adversely affects the taste of the fillet and it is therefore also a problem that, if severe, cannot be alleviated during processing. Elevated temperatures, photoperiodic variation, and growth, or nutritional status, are known to stimulate early maturation of Atlantic salmon (Good and Davidson, 2016; Oppedal et al., 1999). Our analyses show a clear association between water temperature mean and standard deviation, in which a higher mean and deviation leads to a higher prevalence of mature fish upon slaughter. This effect is not entirely linear and for both mean and standard deviation two bumps can be observed in the smoothers. This may be a result of temperature regimes being heavily influenced by the timing of transfer and harvest. Patterns of temporal variation throughout the production cycle, in combination with changes in photoperiod and nutritional status, are likely better predictors of maturation status (Imsland et al., 2014; Vikingstad et al., 2016). This is corroborated by the incidence of maturation rising sharply during the late summer, concurrent with the natural spawning time of Atlantic salmon in the wild.

Water temperature is a known risk factor for the development of spinal deformities in seawater, although the effect has not been as extensively studied in post-smolts as in pre-smolts (Fjelldal et al., 2012; Grini et al., 2011). In our analysis, deformities were the highest for fish groups experiencing mean seawater temperatures between 6.5 °C and 7.0 °C, with prevalence decreasing as temperatures increase above this range. However, this may not preclude increasing temperatures resulting in more deformities throughout the temperature range, as a mean value does not reflect length of exposure or the span of temperature variation. The association between temperature standard deviation and deformities seems to be influenced by a few observations in the lower range of standard deviations, otherwise not being strong. Neither smolt weight nor growth during seawater rearing, previously associated with deformities (Vågsholm and Djupvik, 1998a), were found to be significant explanatory variables in our tb-RDA analysis, although transfer related variables that may correlate somewhat with smolt size, namely temperature and daylength at transfer, were. However, the prevalence of deformities seems to increase with increasing transfer temperature and decrease with increasing daylength. As the temperatures are highest and the days longest during summer, the association between these variables and deformities may be detached from the influence of transfer time.

We found increasing lice counts and mortalities due to parvicapsulosis to be associated with an increasing prevalence of deformities. This may indicate that deformed fish more easily suffer infestation, rather than infestation causing deformities, but inflammation caused by myxozoa has previously been found to affect the prevalence of spinal deformities in cyprinid fishes (Kent et al., 2004). Other known risk factors for spinal deformities are salinity, age at vaccination, as well as factors related to genetics, early life phase rearing conditions, and nutrition (Berg et al., 2006; Gil-Martens, 2010; Vågsholm and Djupvik, 1998a), for which we did not have any relevant data.

A high number of downgrading criteria are also morphological OWIs and the production and slaughter data in this study can be used to shed light on the recent and/or artefactual welfare history of the fish. Whilst it is difficult with some OWIs such as opercular erosion or scale loss to ascertain whether they are an acute or historical injuries unless they are accompanied by e.g. haemorrhaging, an indicator of potentially active injuries (Noble et al., 2018), this study demonstrates that farmers can reduce losses to product quality downgrades by reducing the occurrence and frequency of injuries to their fish. The study also supports the links between improved welfare and improved product quality (Michie, 2001; Vågsholm and Djupvik, 1998b). Our study is, to the best of our knowledge, the first to address primary quality characteristics and quantify causes for downgrading through the systematic analysis of commercial production data. It demonstrates the utility and promise of using production data to establish factors contributing to determining end product quality in commercial fish farming and should serve as an impetus for fish farmers to systematically collect, store (digitally), and review product data, and to ensure traceability throughout the value chain. This study also highlights the ongoing use of OWIs as benchmarks for defining product quality and underlines the impacts of potential welfare issues as drivers for end product quality downgrading. Our analyses show that the timing of harvest and slaughter over a year has clear implications for slaughter quality, significantly affecting the incidence of dark spots, maturation, ulcers, and deformities, and demonstrates a potential for including quality aspects in production planning. While there is seemingly no optimal harvest time, for which the incidence of these four causes for downgrading are simultaneously at their lowest, performing similar analyses on particular sites, or groups of adjacent sites, could aid in finding beneficial harvest dates. Prediction models for quality could, furthermore, help refine price forecasting models and increase farmers' profits (e.g. Forsberg and Guttormsen, 2006).

As our analyses rely on commercial data, which are limited in scope, quality, and geographical origin according to the needs of the producer, the results presented here must be considered indicative. Commercial data do, however, offer a realistic picture of the problems facing aquaculture producers. Automating data recording during processing, e.g. using machine vision and automatic weighing, could improve the future reliability of datasets, e.g. through analysing all slaughtered fish instead of limited samples, and its coverage of relevant quality attributes, and thus also allow for more refined and relevant analyses (Dowlati et al., 2012; Saberioon et al., 2017; Sture et al., 2016).

5 Conclusion

This study presents a summary of the causes for downgrading of Atlantic salmon upon primary processing at a processing plant in northern Norway and attempts to connect the most common causes to welfare and production related drivers. The four most common causes for downgrading were ulcers, dark spots, early maturation, and deformities, with ulcers being the most severe. Growth during the seawater stage, seawater temperature standard deviation and mean, mortalities due to HSMI or CMS, and day of harvest were important explanatory variables behind overall variation in quality. Mortalities due to HSMI and CMS were associated with an increased incidence of dark spots. Lower mean temperatures and lower temperature variance were associated with an increased incidence of downgrading due to ulcers. This study highlights an ongoing need for finding husbandry and management solutions to welfare and quality related issues such as dark spots, early maturation, ulcers, and deformities. Beyond remedies for disease specific causes, quality-oriented production planning is a feasible solution to reduce the incidence of adverse quality traits in harvested Atlantic salmon.

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