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Growth models for assessing anthropogenic impacts on King scallop, *Pecten maximus* (Bivalvia), at Frøya, Norway

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Abstract

In marine ecosystems, the performance of coastal species is affected by anthropogenic activities such as aquaculture and plastic pollution. These impacts will increase in the future as the human population and consumption grow. It is crucial to obtain the true effects of environmental factors on ecosystems and their organisms for management and scientific purposes. Hence, precise growth models requiring easily recorded data and with biologically relevant and interpretable parameters are needed. King scallop *Pecten maximus* is a commercially harvested filter feeding bivalve species populating large parts of coastal Europe. Here, I used King scallop as a model species to assess the effect of these anthropogenic stressors in an outer coastal region in Trøndelag, Norway. Traditionally, growth of this species has been modelled by the Von Bertalanffy growth equation, known to model early age classes imprecisely, reducing accuracy for assessing environmental impacts. I used the least square criterion to compare this growth model to an alternative model based on the Gompertz growth equation, with equally many parameters. A total of n = 89 specimens were sampled from n = 3 locations, one of which had been exposed to aquaculture since 2014. Results demonstrates the Gompertz growth equation models the growth of this species more accurately than the Von Bertalanffy, producing less outliers, having lower residual variance and less heteroscedasticity. This demonstrates that the G is more suitable for growth analysis than the VB. Generalised linear models and mixed effect models were applied to determine if presence of aquaculture or birth year influences growth by model selection using AIC. Model results show a strong effect of birth year, while only maximum growth rate from the Gompertz function and the maximum shell height from the Von Bertalanffy show significant effects of aquaculture. Parameter estimates from the two growth models produce ambiguous results, highlighting that selecting the most correct growth function is essential to avoid erroneous conclusions. Nevertheless, based on the Gompertz results, aquaculture seems to influence growth performance, although small sample size and few sampling sites strongly advocates cautious interpretation. The apparent effect of birth year could reflect temperature increases in the water masses, large-scale and perhaps delayed effects of aquaculture, or a selection pressure due to increased harvest. Lastly, an attempt to assess effects of plastic content in scallops failed as the method for analysing plastic content proved unsuitable, i.e. hydrogen peroxide did not adequately digest the biogenic material. A series of methodological adjustments suggest using other digestive agents known from literature such as HNO_3 or KOH, together with Nile Red dye for increased visibility and NaCl for density separation in this method of rapid screening of plastic content.

Sammendrag

Menneskelige påvirkningsfaktorer som akvakultur og plastforurensning påvirker marine økosystemer og -arter. Denne påvirkningen vil øke i takt med menneskelig populasjonsvekst og dertil økt forbruk. Det er essensielt for forvaltning og forskning av kystområder og biologiske ressurser å tallfeste den reelle påvirkningen av miljøpåvirkning. Dette demonstrerer behovet for presise vekstmodeller som krever lett tilgjengelig datagrunnlag og med biologisk relevante parametere. Stort kamskjell, *Pecten maximus*, er en kommersielt utnyttet art med utbredelse langs Europas vestkyst. Tradisjonelt har veksten til denne arten blitt modellert med Von Bertalanffys vekstmodell – hvor feilestimater er velkjent for yngre aldersgrupper. Dette reduserer nøyaktigheten av deteksjon av miljøpåvirkning. Jeg har brukt minste kvadraters metode for å sammenligne denne modellens presisjon med en alternativ modell basert på Gompertz' vekstkurve, som har like mange parametere. Totalt 89 individer ble samlet fra tre lokaliteter ved Frøya langs ytre Trøndelagskysten, hvorav én har vært påvirket av akvakultur siden 2014. Resultatene demonstrerer at Gompertz vekstkurve modellerer veksten til stort kamskjell mer presist enn Von Bertalanffy: den gir færre ekstremverdier, lavere residualvarians og lavere heteroskedastisitet. Dette demonstrerer at G er mer egnet til å analysere veksten enn VB. Videre ble GLM og LME brukt sammen med AIC for å undersøke om akvakultur eller fødselsår påvirker veksten. Resultatene viser en sterk effekt av fødselsår, men kun maks vekstrate fra G og maks skallhøyde fra VB er signifikant påvirket av akvakultur. Parametere fra de to vekstmodellene viser sprikende påvirkning av disse faktorene, noe som demonstrerer viktigheten av å bruke den mest presise modellen for å unngå feilaktige konklusjoner. Basert på G er det mulig at det er en påvirkning av akvakultur på veksten, men lite utvalg og få lokaliteter tilsier at man skal være forsiktig med å trekke konklusjoner. Effekten av fødselsår reflekterer sannsynligvis økte vanntemperaturer, storskala påvirkninger av akvakultur eller økt kamskjellfangst med påfølgende økt dødelighet. Forsøket på å undersøke effekten av plast i vevet til kamskjellene mislyktes ettersom hydrogenperoksid ikke brøt ned det organiske vevet tilstrekkelig. Metoden ble forsøkt forbedret, og det anbefales å bruke andre stoffer til nedbrytningen, for eksempel HNO3 eller KOH som er kjent fra litteraturen, sammen med fargestoffet nilrødt for synliggjøring av plastpartikler og NaCl for tetthetsseparasjon.

Key words/nøkkelord: Von Bertalanffy, Gompertz, least square criterion, LSC, Pectinidae, Mollusca, aquaculture/akvakultur, fish farming/fiskeoppdrett, growth model

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Table of content

Abbreviations

AIC — Akaike Information Criterion. Used for model selection between the candidate statistical models for the different parameters from Von Bertalanffy and Gompertz models.

AQ — Exposure to aquaculture activities. A two-level factorial variable in the statistical models for growth model parameters. Individuals of scallops are classified as either exposed or non-exposed to aquaculture activities, depending on their respective exposure time (see 1.2.5).

b — Parameter in the Gompertz growth equation shifting the curve along the x-axis (time axis). Its' biological relevance is limited; however, it can give information on the age of onset of somatic growth predicted by the model.

BY — Birth year of individuals of *Pecten maximus*. Used as a continuous numerical variable in statistical models for growth model parameters.

c — Growth rate parameter in the Gompertz growth model.

CTD — Conductance, Temperature and Density, the common name of an instrument measuring these parameters in the water column as well as the common name of the collected data.

DIC — Dissolved inorganic carbon. Essentially CO_2 and associated equilibrium with HCO_3^- and H_2CO_3 when dissolved in water: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$.

DOC — Dissolved organic carbon, bound in dissolved organic compounds or in particles below 0.45 µm.

DON — Dissolved organic nitrogen, bound in dissolved organic compounds or in organic particles below 0.45 µm.

DOP — Dissolved organic phosphorus, bound in dissolved organic compounds or in organic particles below 0.45 µm.

G — Gompertz growth model.

G'_{max} — The maximum growth rate of the Gompertz growth models. unit: mm/year.

GLM	Generalized linear model. One of the statistical models used for candidate models for analysing the growth model parameters. Allows for different error structures than gaussian. Does not consider nested structure of the data.
H_∞	Asymptotic height, which the two growth models approaches as age (t) goes towards infinity.
HDPE	High Density Polyethylene.
I	Intercept (towards the mean birth year across all individuals) of the statistical models.
IMTA	Integrated Multi Trophic Aquaculture system.
k	Brody growth rate coefficient in Von Bertalanffy (unit: $time^{-1}$). Can be interpreted as the exponential rate of approach to the H_∞, or alternatively, e^{-k} is the fixed fraction by which the annual growth increment is multiplied each year (Schnute and Fournier, 1980).
IMR	Norwegian Institute of Marine Research.
LME	Linear mixed-effect models. One of the statistical models used for candidate models for analysing the growth model parameters. Considers nested structure of the data and gaussian error structure around the groups of the random factor.
MSSQ	Mean Sum of Squares. This is the residual variance of the individually fitted Von Bertalanffy and Gompertz models. used for growth model selection of Von Bertalanffy vs Gompertz. Named as such here to avoid confusion with general us if the term variance.
NR	Nile Red.
POC	Particulate organic carbon, bound in organic particles in the water masses, size above 0.45 µm.
PON	Particulate organic nitrogen, bound in particulate organic compounds above 0.45 µm.
POP	Particulate organic phosphorus, phosphorus bound in particulate organic compounds in the water masses, above 0.45 µm.
t	Time parameter in both growth models, equivalent to age. Unit: years.

t_0	The theoretical age at which H(t) is equal to 0 in Von Bertalanffy growth model.
VB	Von Bertalanffy growth model.
Ø’	Index of overall growth performance based on Von Bertalanffy growth model.

1. Chapter one: Growth and growth models of *Pecten maximus*, and potential effects of exposure to aquaculture activities

1.1. Introduction

1.1.1. Ecology of *Pecten maximus*

The King Scallop, or Great Scallop (*Pecten maximus*, L. 1758), is distributed along the European coast from Gibraltar, Spain to Lofoten in Norway with a few observations also along the Azores and Canary Islands (Moen and Svensen, 1999, Stensås, 2014, GBIF, 2017, FAO, 2017, Brand, 2016). Its' family, Pectinidae (order Pectinida), includes over 100 genera in nine subfamilies, and has a global distribution (Bouchet, 2011). Many species in this family, including *P. maximus*, has been subject to human harvest and consumption (FAO, 2016). Since 2008, global harvest of Pectinidae seems to have stagnated at annual catch of about 800 000 metric tons, of which harvest of *P. maximus* is currently the third most important in terms of weight, with about 4.9 % of global catches of pectinids (FAO, 2016). In Norway, the highest densities of *P. maximus* are believed to be in Hordaland and in outer coast of Trøndelag. Norwegian commercial harvest was initiated in the 90s and has since 2000 fluctuated around 700 tons annually, most of which caught from Trøndelag (appendix, Figure A1.1, Strand et al., 2016). Effort has been made to cultivate this species, but currently this is not economically viable as there are challenges in recruitment and survival of juveniles in rearing (Andersen et al., 2009, Galley et al., 2017, Holbach et al., 2017).

P. maximus is a suspension feeder filtering the epibenthic water masses. It prefers sandy and gravelly sediments but is also sometimes found in finer silt. Thus, the distribution is relatively patchy and highly influenced by environmental conditions such as topography and water currents. Environmental preferences of this scallop coincide to some degree with the environmental conditions in areas where concession for aquaculture is given due to their shared need for sufficient water currents. Thus, it is expected to be present close to many fish farms. Its' depth distribution ranges from sublittoral to about 150-200 m, with the highest densities expected at 5-45 m (Moen and Svensen, 1999, Brand, 2016, Søvik et al., 2010). However, as most knowledge in Norway is based on specimens collected by divers, knowledge is scarce below 30 m and the deeper end of the distribution range is uncertain. It lives partly buried in the sediments, with the flat left shell half parallel to the sediment surface. The anterior opening is oriented towards the general direction of the current, maximizing inflow of waters for feeding (MacDonald et al., 2006). The flat left shell half is usually covered with sand, and often hosting

substratum for algae or serpulid polychaetes (personal observation), while the curved right half is usually clean.

Main diet of *P. maximus* is phytoplankton, detritus and small zooplankton, of which phytoplankton is the most important source of energy. The nutritious quality differs between phytoplankton species, and diatoms are shown to contribute the most to growth in scallops. Dinoflagellates on the other hand, does not seem to contribute significantly to growth (Chauvaud et al., 2001, Chauvaud et al., 2005). Too high abundances of diatoms, or other algae, are believed to lead to clogging of gills. Hence, there is a threshold for maximum feeding rate (Chauvaud et al., 1998). Contribution to growth by detritus is low, probably as microbial degradation reduce nutritional value (Lavaud et al., 2014, Farías and Uriarte, 2006, MacDonald et al., 2006).

Temperature is one of the key factors for growth initiation, although the ultimate cause is probably the planktonic community's performance at different temperatures. Hence, this temperature threshold for growth is dependent on the local planktonic community's temperature preferences (Chauvaud et al., 2005, Heilmayer et al., 2004, Chauvaud et al., 1998). In French scallops, growth initiates at about 10^{o}C (Chauvaud et al., 2005). At which specific temperatures scallops from Trøndelag initiates growth is not known to the authors' knowledge, however the planktonic community differ from French waters and growth might be initiate at lower temperatures in Trøndelag. Scallops from the colder range of the distribution area are known to grow more efficiently, i.e. more per day but has fewer growth days per year. This is probably due to lower energy required for metabolic maintenance in the lower end of the range of temperatures supporting growth (Chauvaud et al., 2005). Norwegian Institute of Marine Research (IMR) has time series of CTD-data at Bud at Nordmøre, 134 km from Sistranda. By visual inspection, these data appear to indicate low or no increase in temperature at 20 m depth. The water masses around Frøya are in the same water mass regime as Bud and can be expected to have similar trends in temperature. Based on this preliminary visual inspection of the temperature data, growth is not expected to be strongly affected by temperature increase. However, these data will be tested statistically here.

Pecten maximus feeds by filtering inflow water between gill filaments, which serve both as gas exchange- and filtering organ. Gills are covered with mucus and cilia, adhering particles to them and slowly transporting them towards the mouth (MacDonald et al., 2006). Lowest particle sizes retained by the gills are approximately 5 µm, thus the diet can possibly include also micro- and nanoplankton such as mycoplankton and even some aggregated

bacterioplankton (Beninger and Le Pennec, 2006). The mouth, located on anterior side, are transporting food items into the stomach. Together, gills and mouth perform a qualitative selection of the food items, rejecting unfavourable material and deposits it as pseudofeces, which is mucus-covered material not processed by digestive organs. However, the controlling mechanisms behind this selective feeding are not fully understood (Beninger et al., 2004, Beninger and Le Pennec, 2006). Hence, the character of this particle selection is contributing to the strength of the effect of aquaculture on food availability. Consumption of fodder from aquaculture has been reported for other pectinid species, and it is reasonable to believe this is possible also for *P. maximus* (Farías and Uriarte, 2006, Seguel et al., 1998). Stensås (2014) found increased growth rate and improved condition index for *P. maximus* in field experiment in Norway, where scallops were placed in metal cages in the water column adjacent to fish farms. To the author's knowledge, this has not previously been tested in natural populations and -environments in Norway.

In southwestern areas in Norway, *Pecten maximus* spawn in July to September whilst populations in Trøndelag usually spawns earlier (Søvik et al., 2010). Further south in Europe spring spawning and sometimes a second spawning in the late summer is not uncommon (Devauchelle and Mingant, 1991, Barber and Blake, 2006, Søvik et al., 2010). Spawning is induced by temperature increase and has been reported in temperatures between 7-16^{o}C (Devauchelle and Mingant, 1991, Barber and Blake, 2006). After hatching, the larvae have a benthic stage before entering metamorphosis and develops into a post-larva at a size of about 200 µm. It then attaches to hard substratum by byssus (Andersen et al., 2009). Usually the byssus is lost before reaching 15 mm, detaching the individual before settling to sediments and completing the metamorphosis (Brand, 2016). It matures at age 2-3 years, however most energy is allocated to growth until the size is larger (Barber and Blake, 2006).

The growth rate in the first years is relatively high, before starting to level off around age of 4-6 years, depending on population. Two relatively distinct strategies were found by Chauvaud et al. (2012); rapid early-life growth in the southern populations and almost stopping at a size around 100 mm, whilst northern populations grow slower initially but seems to keep growing for longer (Figure 1.1-1). Longevity is up to about 20 years, and the maximum height is generally up to 150 mm.

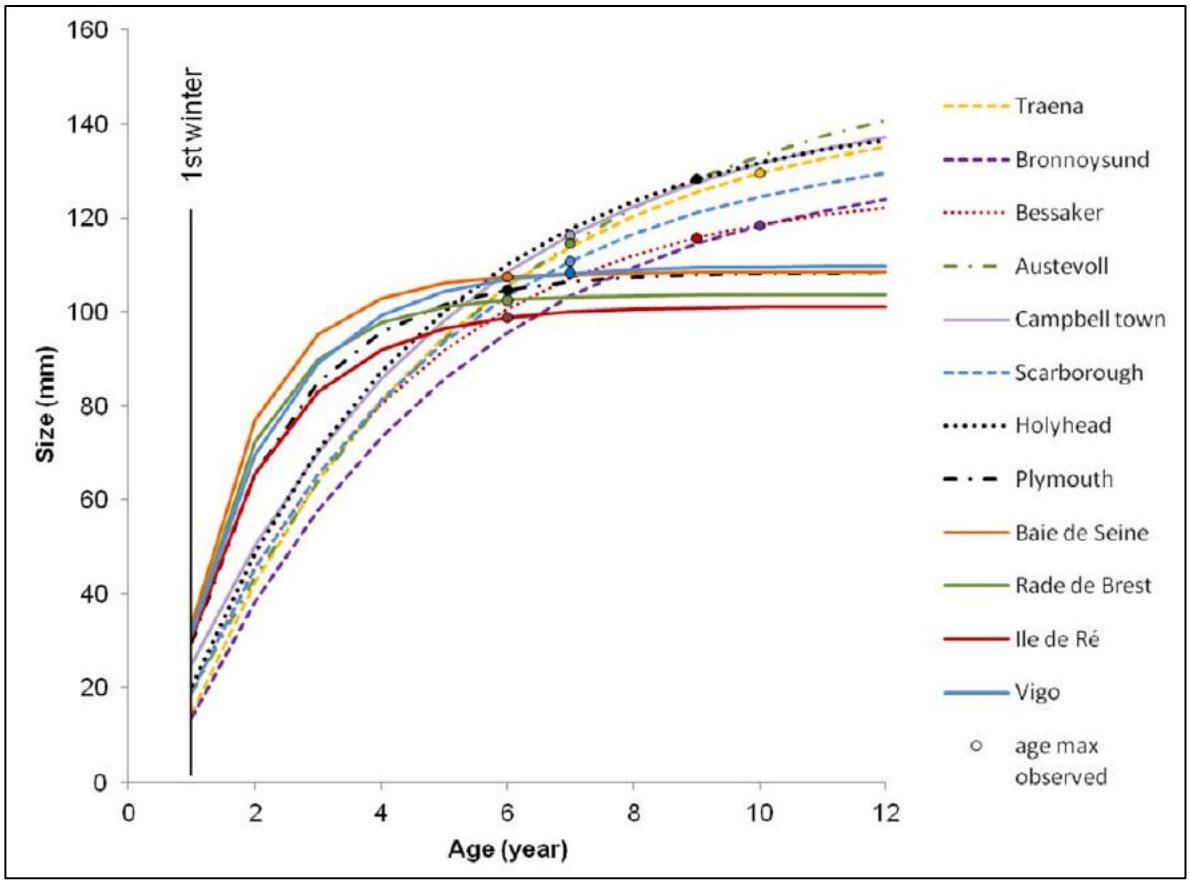

Figure 1.1-1: Shell height predicted by Von Bertalanffy growth model at different ages in European populations of P. maximus. *From Chauvaud et al. (2012).*

During growth, increments of shell calcite is deposited as striae along the outline of the shell, corresponding to a decrease in growth due to a change in metabolism. In pectinids, these are corresponding to daily growth increments (Chauvaud et al. 1998 and references herein). Striae also has distinct lamellae and are clearly visible in most shells. Furthermore, growth has a complete cessation during winter, which together with the subsequent initiation of growth in spring produces a distinct winter ring (Figure 1.1-2). This enables precise determination of age. However, the first growth ring is often undistinguishable and winter rings deposited at old age are - due to the reduced annual growth at these ages - sometimes very close and hard to distinguish from each other. Additionally, some older individuals tend to be eroded, having less distinct winter rings.

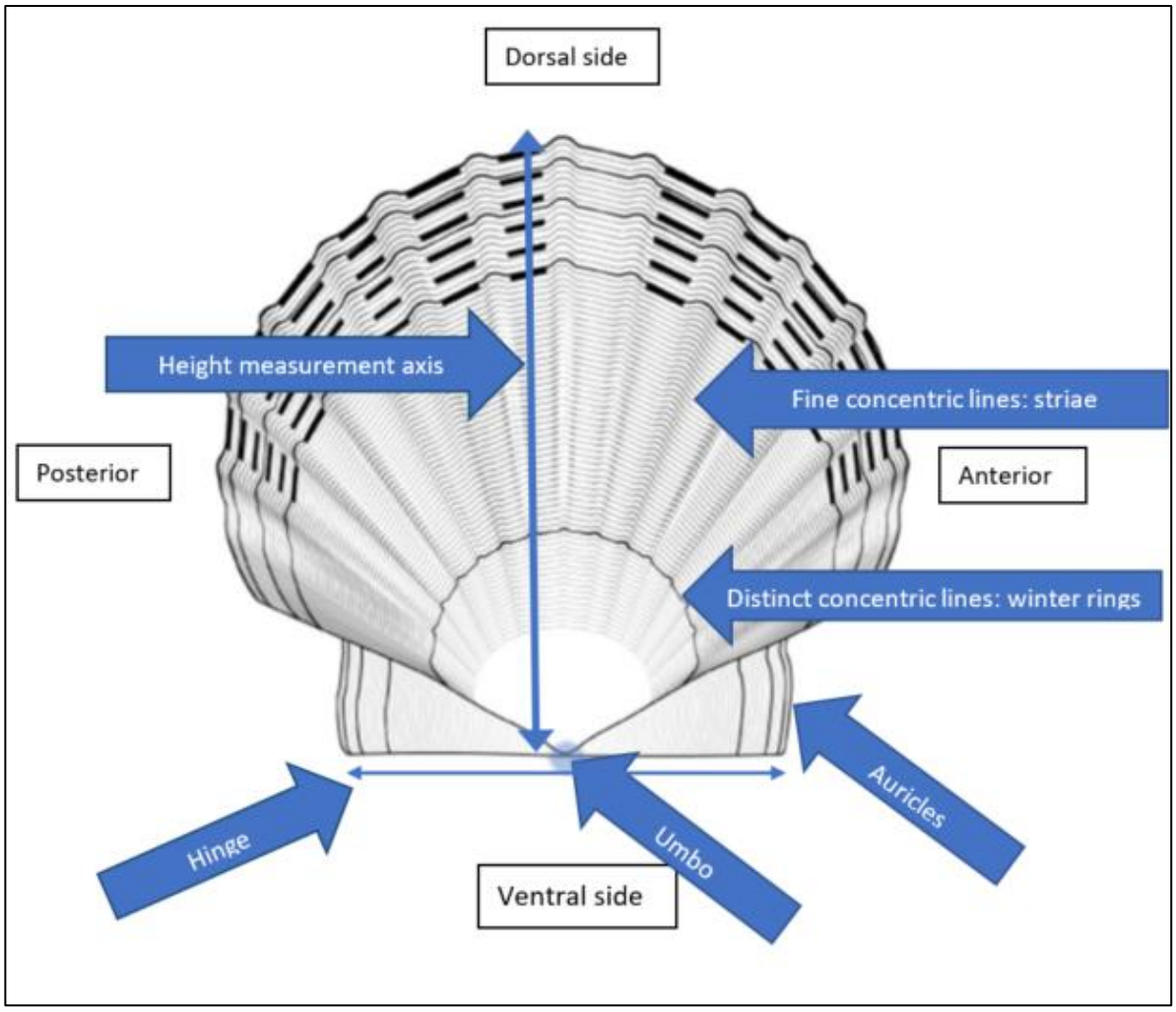

Figure 1.1-2: Schematic left shell of P. maximus. Modified from Gillikin et al. (2017)

Growth in *P. maximus* and other pectinids have traditionally been modelled using Von Bertalanffy growth function (VB) – a common growth function in fisheries ecology (Lloyd-Jones et al., 2014, Chauvaud et al., 2012, Bray, 2008, Gamito, 1998, Laslett et al., 2002, Pedersen, 1994, Ridgway et al., 2010). The VB for length data has theoretical maximum growth at $t = -\infty$, gradually levelling off to zero towards the horizontal asymptote. This implies that growth stagnates from maximum growth early in life towards a horizontal asymptote at older age. The VB is known to have an unprecise fit in younger year classes of *P. maximus*, as initial growth is lower than maximum growth (Chauvaud et al., 2005, Lloyd-Jones et al., 2014). Other growth functions have been suggested for more precise modelling the growth of *P. maximus*. One suggested model is the Gompertz growth equation (G), which in many cases proves to have a better fit than the VB (Natanson et al., 2006, Lloyd-Jones et al., 2014, Laslett et al., 2002). G expresses a sigmoid curve and has the potential to better model the slower growth in younger

year classes than the VB. It is important to select the best fitting growth model when analysing growth patterns to avoid introducing unnecessary variance and biases into the data. The best fitting model will preserve the properties of the data better, hence the effects of environmental parameters are more easily read and determined. "Best fitting model" is generally a trade-off between accuracy and number of parameters. A model with higher number of parameters will be more accurate, however the interpretation becomes more complicated. This is called overfitting. Additionally, every parameter estimate has an uncertainty. Hence, increasing the number of parameters requires the introduced parameters adds more uncertainty to the model than it introduces through its' own uncertainty. Growth models with biologically relevant parameters are also favourable, as interpretation becomes more intuitive.

As the longevity of *P. maximus* allows for back-tracing environmental factors, including promising possibilities for isotope analyses, *P. maximus* has the potential to be used as an indicator species for effects of aquaculture or other environmental factors (Chauvaud et al., 2011, Chauvaud et al., 2005, Jolivet et al., 2015). Its' habitat preference coincides to some degree with areas where concession for aquaculture can be given. And importantly, IMR already have monitoring programmes of this species. Hence, extending the analyses and data collection does not constitute a substantial increase in cost and effort and can potentially give further insight into the effects of aquaculture on the local environment, as well as support coastal management.

1.1.2. Aquaculture

Aquaculture is by FAO defined as "the farming of aquatic organisms such as fish, molluscs, crustaceans, aquatic plants (…)" (FAO, 2002). Norway was in 2014 the sixth biggest producer of cultured marine animals, producing 1.33 million tons round weight in 2014 contributing to 1.8 % of global production (FAO, 2016). Norway, having a long coastline with many fjords, bays and islands, is well suited for aquaculture. Well-established infrastructure along most of the coastline further eases the suitability and support development of this industry. Currently there are 1680 aquaculture localities in Norway, of which 47 are in Frøya Municipality (Norwegian Directorate of Fisheries, 2017). Fish farms are relatively evenly distributed along the coast, although Hordaland, Rogaland, southern coast of Trøndelag and Lofoten are "hotspots" of fish farming localities (Figure 1.1-3). Salmonid farming is by far the most

important in terms of economy and production in Norway. This study investigates areas adjacent to salmon farms in Frøya municipality.

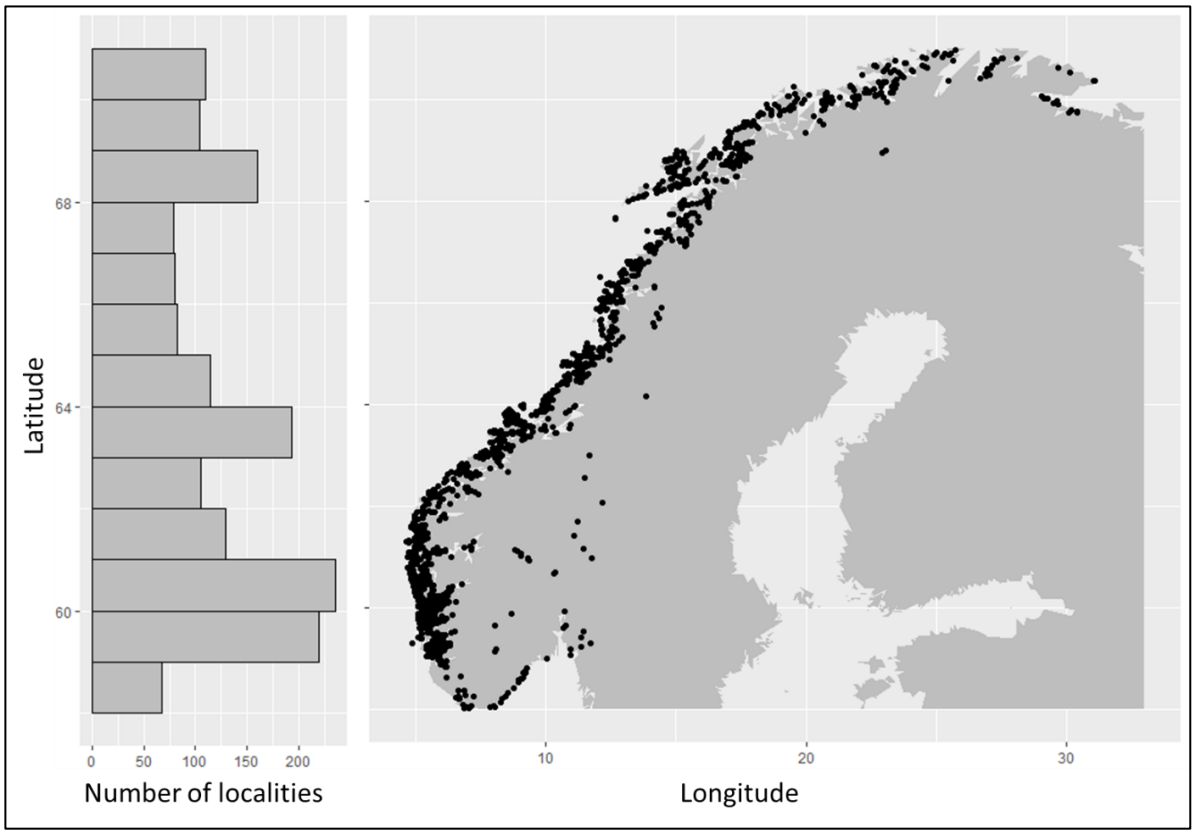

Figure 1.1-3: Current aquaculture localities in Norway (right), including fish farms, hatcheries, kelp farms and blue mussel farms (locations on land are hatcheries and land based Arctic char farms). Left histogram indicates number of localities at each degree of latitude corresponding to the map. Data: Norwegian Directorate of Fisheries (2017) Map: Norwegian Mapping Authority (2017)

Aquaculture has grown to become an important industry for Norway, for coastal societies in terms of economic livelihood and labour as well as regional and national economy. There is an expressed goal by the Norwegian government to increase salmon farming by a factor of up to 6 - provided a certain strength of climate change assumed by the authors (Ocean Strategy, 2017). However, this certain strength of climate change is not further expressed. Knowing the impact of this industry on ecosystems and species is crucial for management of fisheries and aquaculture, as well as for the potential necessity of protecting areas, ecosystems or species. Even though aquaculture is by many regarded as a promising food source for a sustainable future, there are challenges regarding environmental impacts of the activities. In brief, the most important are:

1) The source of the fodder, which can be e.g. unsustainable harvest of fish for feed use such as anchoveta outside of Chile, or intensive terrestrial production of soy proteins (Naylor et al., 2009). This is not relevant for the scallops and local environment of this study and will not be investigated further here.

2) The high densities of the cultured species facilitate transfer of pathogens between individuals, and the pathogens are not restricted to stay within the cages. This is very much a concern to media and public in Norway, as the parasite salmon louse's (*Lepeophtheirus salmonis*) abundance and densities are very much affected by the fish farming activities and has a big impact on wild salmon populations (Torrissen et al., 2013). Generally, pathogens are relatively species-specific in terms of their host selection, and it is not likely that diseases and parasites from fish farms per se influences scallops. Thus, this will not be considered more in this study.

3) Pollution of chemicals is potentially very important locally. Chemicals are generally used for disease- and parasite control and limiting epigrowth on the cages to avoid unnecessary wear and damage to equipment and reduce chance of cage failure and consecutive escape events. According to Norwegian Environmental Agency (2016) such chemicals can be e.g. antibiotics, copper ions, hydrogen peroxide and a range of delousing agents. In later years, this has received more media attention, as well as local fishermen are accusing fish farmers of degrading fishery banks and -areas. Even though some of these chemicals can influence scallops, it is not feasible within this study to quantify to what extent scallops have been exposed to such compounds. Thus, this is assumed to have no effect on the individuals measured in this study.

4) Loss of fodder from feeding can also be locally important. During feeding, approximately 5-8 % of fodder fall through the cage uneaten in Norwegian fish farms (Torrissen et al., 2016, Svåsand et al., 2016). Some of which is referred to as dust and some is pellets. Larger dust particles and pellets sinks relatively quickly, increasing food availability for deposit-, suspension- and filter feeders. The smaller dust is more susceptible to being remineralized in the water column by bacterial degradation. Fodder has a high concentration of lipids and proteins, and thus including particulate organic phosphorus and nitrogen as well as carbon, (POP, PON and POC) and making it high quality food source for fish and invertebrates

(Torrissen et al., 2016). *P. maximus* in particular is selective in feeding, and to what degree fodder is selected and consumed is uncertain (Farías and Uriarte, 2006).

5) Enhanced nutrient availability in the water masses can affect local environment. Aquaculture activities can contribute through excrements, increasing carbon (POC, DOC and DIC), nitrogen (PON, DON) and phosphorus (POP, DOP). Carbon concentrations can be enhanced directly by gas exchange (dissolved inorganic carbon, DIC, effectively $CO_{2(aq)}$) in the fish or from faeces (particulate- and dissolved organic carbon, POC and DOC respectively) as well as dissolved- and particulate organic nitrogen and phosphorus (DON, PON, DOP, POP). In 2015, about 26 300 metric tons of organic nitrogen and 3940 metric tons of organic phosphorus was released from Norwegian fish farms (Svåsand et al., 2016). Primary production can be expected to increase in the local waters around fish farms as they are fertilized by these enhanced concentrations of nitrogen and phosphorus. This fertilization can be expected to favour certain taxa of algae, depending on concentrations and N:P ratio (Elser et al., 2007). As there were no current farming activities at the time of this study, this effect cannot be measured and water samples for nutrient- and species composition were not taken.

Points 4) and 5) highlights the increased availability of nutrients and energy from fish farming activities into the local environments. However, the characteristics of the nutrients and food made available can be expected to differ between 4) and 5); The fodder is made out of lipids and proteins which is readily consumable, unlike increased nutrients which lead to blooms and growth of planktonic and benthic algae which naturally have texture and surface like algal cells as well as making a delay between release and reaching a consumable state for scallops (Torrissen et al., 2016). These structural differences are likely to affect whether such particles are consumed by scallops, mediated by the scallops' selective feeding behaviour. As seen in Figure 1.1-4, the discharge of phosphorus and nitrogen have changed since 1990 in Norway, almost entirely due to increase in discharge from aquaculture. In Trøndelag, an increase in primary production of about 2-2.5% due to emissions from fish farming was estimated in 2012 (Taranger et al., 2014). N and P are both normally limiting nutrients for algal growth, and different algal species are adapted to different levels of these nutrients (Elser et al., 2007). Diatoms are also limited by silica (Si) concentrations, which is not expected to be equally affected by aquaculture. Hence, competition and relative fitness of the different groups of algae is expected to change and altered species composition in plankton and benthos relative to the

pre-aquaculture situation can be expected. The strength of this change is likely to differ between sites, due to different physical regime in the area affecting competition, such as light, currents and temperature. Essentially, points 4) and 5) lead to increased eutrophication and increased oxygen consumption. However, this is also dependent of the regime of currents and stratification of the water masses in the area. Generally, concessions to establish aquaculture sites in Norway are only given in areas not in risk of strong eutrophication; i.e. areas with sufficient currents to exchange water masses and dilute pollution of chemicals and nutrients.

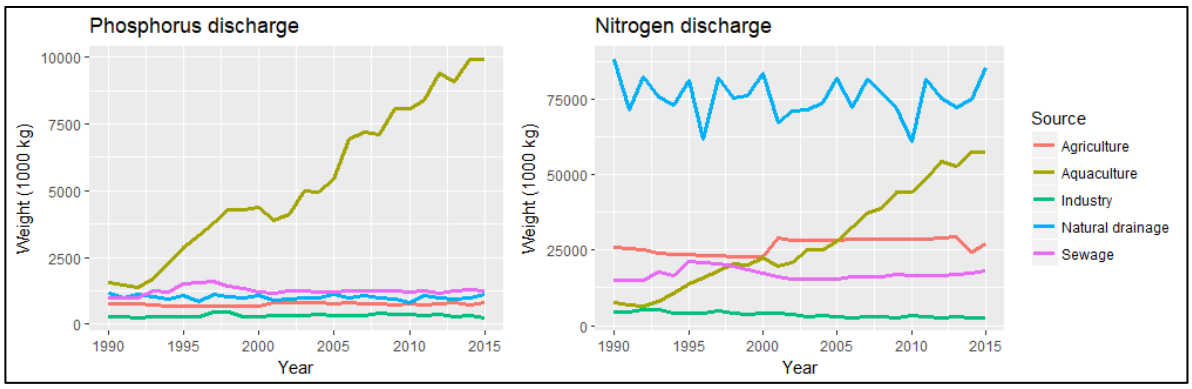

Figure 1.1-4: Discharge of phosphorus and nitrogen by source in Norway. Note the difference in scale between P and N. Data: Norwegian Environmental Agency (2017)

Loading of fodder and nutrients have also been shown to affect physical properties of the sediments. Brown et al. (1987) found reduced redox potential and oxygen levels as well as increased carbon content in the sediments within 15 m from the cages, and faunal composition had a strong succession with distance up to 25 meters from the cages on Scottish west coast and up to 50 m from cages in British Columbia (Brooks et al., 2003). Studies from Norway indicate increased organic sedimentation at 250 meters from cages in fjord systems, and strong increase in sedimentary secondary production, biomass and abundance at sites 227 m from cages. Furthermore, the associated species composition was found to be dominated by certain polychaete and bivalve species, exhibiting up to 50 times higher productivity at within 250 m than 550-3000 m away from fish farms. (Kutti et al., 2008). Nickell et al. (2003) found high abundance and very strong domination by small polychaetes close to farms, whilst further from farms dominance decreased and mean organism size increased.

1.1.3. Aims

This study aims to evaluate the usability of two common growth models to determine which is the most applicable for analysing growth of *Pecten maximus*. Such knowledge is crucial for future assessments, monitoring surveys and management of this commercially harvested species. Secondly, this study aims to disentangle the factors determining growth performance of *P. maximus*, in particular the potential effect of aquaculture activities. This knowledge will help determining the potential value of this species for use as an indicator species of environmental and anthropogenic effects. Also, this can help understanding the effect of aquaculture on the local environment.

1.2. Materials and methods

1.2.1. Study site

This study is conducted in Norway at the outer coast of Trøndelag, outside of Sistranda on the east facing part of the island Frøya (Figure, 1.2-1). Frøya has been subject to the development of aquaculture industry since 1970, however the initial activities were small (Foss and Hamer, 1997). The first localities established within the area of this study were at Lamøya and Bukkholmen. Currently, these have a capacity of 3120 and 3900 metric ton round weight respectively. It is not known for certain whether the capacity differed from this pre-2012 due to change in ownership. Kamholmen was established in 2010, and Seiballskjæret in 2012- with a capacity of 2860 and 2340 tons respectively. Hofsøya was established in 2014 and is the single biggest fish farm in the study area with 4680 tons capacity (Norwegian Directorate of Fisheries, 2017).

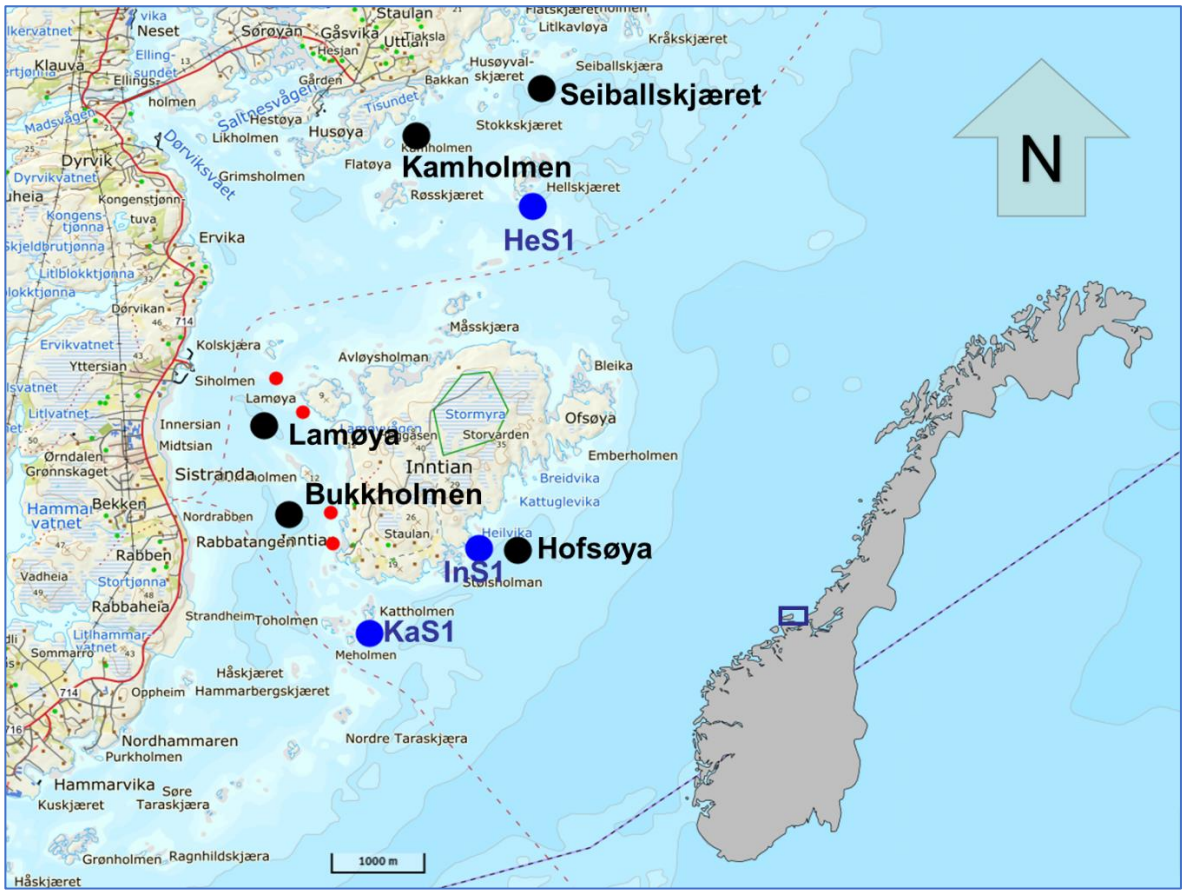

Figure 1.2-1: The study area of this thesis. Black dots are aquaculture localities. Blue dots are sampling stations. Red dots are investigated with camera data and not sampled (Map from Norwegian Mapping Authorities 2018).

Sistranda is the community centre, with about 900 out of total 5000 inhabitants in Frøya municipality. The town is well-developed for aquaculture activities, hosting supporting industrial structures as well as the natural environmental conditions being advantageous for aquaculture. There is relatively high exchange of water masses by tidal- and current action, as well as being relatively sheltered from rough weather and wave action.

1.2.2. Sampling

The sampling stations were chosen based on known occurrences of scallops and their respective proximities to fish farms. Additional potential sampling stations close to Lamøya and Bukkholmen were investigated using a camera rig and film recordings from previous dives to evaluate the possibility to sample the area – however it was decided not to put effort in diving as scallops appeared to be absent.

Samples were collected in collaboration with local sports divers in autumn 2017 (station information in table 1.2-1). Additionally, samples collected at KaS1 in November 2014 were included. At sampling localities, divers collected all visible shells from about 10 to 30 m depth along a transect of about 10-20 m width. For safety reasons, descent and ascent during diving must be done slow and controlled, and time at different depths needs to be carefully monitored and is determining duration at depths. Thus, to avoid putting pressure on performance of the divers, transects were not standardized and duration was defined by the safety precautions. Furthermore, locations were chosen based on known occurrences of scallops and thereby biased towards high densities and to some degree harvested populations. This has implications on which population characteristics that can be performed, such as population density measurements and other quantitative data.

Due to outbreak of infectious salmon anaemia, the area has been put on quarantine and cages were empty at time of sampling (Aquaculture, 2016 , BarentsWatch, 2017). Therefore, water nutrient content would not be affected by aquaculture at time of sampling, and it was decided not to conduct any nutrient sampling.

Table 1.2-1: Metadata on sampling stations, distance to nearest fish farm indicated in parentheses at the Aquaculture exposure-column.

Station name	Station ID	Latitude	Longitude	Sampling date	Depth	Aquaculture exposure
Inntian south, Stølsholman	InS1	63.72097	08.90252	11.09.2017	12-27 m	Exposed since 2014 (~250 m)
Hellskjæret south	HeS1	63.75658	08.90590	12.09.2017	21-30 m	No (~1500 m)
Kattholman south	KaS1	63.71339	08.88273	14.09.2017 and November 2014	17-23 m	No (~1400 m)

1.2.3. Growth measurements and age determination

Age of each individual was determined by counting number of winter rings on the left shell, including the outer edge (Figure 1.1-2). Thus, the age reflects number of summers experienced as the last winter ring has not been produced yet. Year of birth was calculated from the age. Shell height was measured at the left shell at each winter ring between 1 to 10, as well as maximum height, using a digital Cochraft Vernier Caliper 0-150mm. Height measurements were taken from the umbo to the highest point on the respective winter ring, i.e. perpendicular to the hinge as indicated on Figure 1.1-2. In many cases, the first winter ring was not visible as it was worn down and indistinguishable. To determine whether the first visible ring was first- or second winter ring on such individuals and to detect 2-year olds erroneously determined as yearlings, visual inspection was done on the histograms for height distributions of yearlings and 2-year-olds; each showing a normal distribution and fitting well with selecting 24 mm shell height as the separating size (Appendix 2, Figure A2.1).

1.2.4. Modelling of growth and growth parameters

Height-at-age measurements were, as traditionally in fisheries ecology, fitted with a growth model on the design of a typical Von Bertalanffy model for length data as performed by Chauvaud, Patry et al. (2012), using non-linear least square method;

$$H(t) = H_{\infty}(1 - e^{-k(t-t_0)}) \quad [1]$$

In [1], H(t) is the height (mm) at age t, H_{∞} is the asymptotic height (mm) to which the individual is predicted to reach as t increases, k is the Brody growth rate coefficient (unit: $time^{-1}$) which can be interpreted as the exponential rate of approach to the asymptotic height (alternatively, e^{-k} is the fixed fraction by which the annual growth increment is multiplied each year), and t_0 is the theoretical age at which height = 0. The height-at-age-data was also fitted with a model on the structure of Gompertz model (Natanson et al., 2006, Lloyd-Jones et al., 2014):

$$G(t) = H_{\infty}e^{-be^{-ct}} \quad [2]$$

In [2], G(t) is the height at age t, H_{∞} is asymptotic height (mm) similar to that of the VB, b is a displacement parameter (shifting the curve along the x-axis, no unit) and c is a growth rate parameter (unit: $time^{-1}$, not equal to k as the structure of models are different). In both [1] and [2], t is age (years).

The structure of the dataset in this study implies strong autocorrelation within individuals as size at age t is very determining for size at age t+1. Therefore, both models were fitted to the individuals separately, making one growth curve of each VB and G per individual. Parameter estimates were then obtained from these individual models. Thereby the problem of autocorrelation is solved (Laslett et al., 2002). In addition to consider the within-individual dependency, this also preserves the between-individual variation in growth well, facilitating further analysis of the growth.

From the VB, the index of overall growth performance Ø' (unit: $log(mm^2/year)$, however the interpretation of this unit is normally not considered) was calculated according to Chauvaud et al. (2012):

$$Ø' = \log_{10}(k) + 2\log_{10}(0.1 * H_{\infty}) \quad [3]$$

Ø' is empirically derived from the relation between k and H_{∞} in VB, and is taxon-specific (Bray, 2008, Munro and Pauly, 1983). It is a suitable index for expressing overall growth performance and can be used for statistical comparison (Moreau et al., 1986). However, it is not the growth

at the inflexion point as stated by Chauvaud et al. (2012), as the VB on this structure (based on length data) does not express such point.

The maximum growth rate of the G (G'_{max}), i.e. growth rate at the inflexion point and the age at which it occurs was calculated by the first and second derivative of G(t):

$$\frac{d}{dt}G(t) = H_{\infty}bc * e^{-be^{-ct}-ct} \quad [4]$$

$$\frac{d^2}{dt^2}G(t) = -H_{\infty}bc^2 * e^{-be^{-ct}-2ct}(e^{ct} - b) \quad [5]$$

Maximum growth occurs at age defined by function [5]:

$$\frac{d^2}{dt^2}G(t) = 0 \Rightarrow t = \frac{\ln(b)}{c} \quad [6]$$

Fitting this t into function [4] gives the maximum growth rate (unit: mm/year) from G:

$$\frac{d}{dt}G\left(\frac{\ln(b)}{c}\right) = H_{\infty}ce^{-1} \quad [7]$$

The maximum growth rate is the growth at the inflexion point and is suitable for statistical comparisons between populations and individuals (Moreau et al., 1986). As the VB has no inflexion point, G'_{max} is not perfectly suited for comparison to Ø and thus growth performance between VB and G. However, as both are parameters of overall growth performance, it is interesting to test whether effects of aquaculture are detectable in either of the models to assess the models' relative sensitivities to detect environmental factors. Next, a comparable parameter between the models is the predicted age at which 50% of the asymptotic height is reached; T_{VB50} [8] and T_{G50} [9] for the VB and the G respectively. These parameters can serve as proxy for maturation or a life-history-strategy, as this size is expected to be around the age of maturation (Barber and Blake, 2006, Chauvaud et al., 2012). These were calculated as following:

$$T_{VB50} = \frac{k\,t0 - \ln(1-0.50))}{k} \quad [8]$$

$$T_{G50} = \frac{\ln\left(\ln\left(\frac{100}{50}\right)\right) - \ln(b)}{-c} \quad [9]$$

1.2.5. Classification of exposure to aquaculture

The station InS1 is situated close to a fish farm (about 250 m, c.f. section 1.1.2) which have been active since 2014. Individuals from InS1 at age of 6 or younger at time of sampling were

categorized as exposed to aquaculture, as they have been exposed since the age of 2, 1 or their full life and most of a scallop's growth is in the first 5-6 years. Older individuals from InS1 and individuals from HeS1 and KaS1 were categorized as non-exposed. This classification is the best available due to the data collected, although where to set the limit between exposed and non-exposed can be up for debate. Shifting to classify one more age class as exposed only gives one more exposed individual and reducing to one less age class gives one less individual in this data set. In any case, sample size of exposed individuals is low and conclusions on this effect should be drawn and interpreted with care.

1.2.6. Statistical analyses

All modelling and statistics has been performed using R version 3.4.3 for Windows (R Core Team, 2017), including the default packages as well as `ggplot2` (Wickham, 2009) and `gridExtra` (Auguie, 2017) for additional graphics, `nlstools` (Baty et al., 2015), `nlme` (Pinheiro et al., 2018), `AICcmodavg` (Mazerolle, 2017) and `MuMIn` (Barton, 2018) for analyses of the constructed models and parameters. The package `lubridate` (Grolemund and Wickham, 2011) was used for transforming date formats from oceanographic time series from IMR.

Temperature data from Bud was analysed by linear mixed effect model, using month as random factor to account for seasonal variation. These data cannot be directly linked to the models for growth model parameters here, as the distance between the sampling stations of this study and the monitoring station which the temperature data is collected is too long for statistical correlation. However, results can give insight in mechanisms and are good starting point for discussion.

1.2.6.1. Growth model selection

Model selection was performed on VB and G using least square criterion. This criterion is generally used to determine which growth model has the least unexplained variance. It is purely analysing the residual variance towards the fitted curve by summing the squared errors of the model, and the model with the lowest error is the preferred one. This criterion will always select the model with the highest number of parameters and hence favour overfitting. This limits the possibilities to compare models of different complexity as the more complex ones will be

favoured. In this study, both candidate models have equally many parameters, thus avoiding the problem of overfitting using this criterion. Residual plots for the models were investigated to identify possible patterns of heteroscedasticity. Heteroscedasticity indicate misfit of the model at parts of the range of the data, and if present one should consider another growth function or transforming the data. Further analysis was performed by evaluating the distribution of the mean and the variance of the MSSQ (mean square residual value for the individual models, i.e. the variance of each individual model). Preferably, a well-fitting growth function will have a low mean value of the MSSQ. When the MSSQ is low, the variance introduced by the growth model is lower, preserving the properties of the original data better than if the MSSQ is higher. Furthermore, low variance of the MSSQ indicate that the growth model fits individual relatively equally and would be preferable for further analysis of growth patterns as the introduced stochastic variance is more constant across models.

1.2.6.2. Statistical analysis of growth model parameters

Both Generalized Linear Model (GLM, family: gaussian) and Linear Mixed-effect model (LME, using maximum likelihood method) were fitted to test the effect of aquaculture exposure (AQ, a two-level factorial parameter in the models, individuals are either exposed- or non-exposed to aquaculture activities) and birth year of the individuals (BY) to the growth model parameters asymptotic heights (H_∞), Ø' and G'_{max} and age of reaching 50% of H_∞ (T_{VB50} and T_{G50}), as well as whether the random effect of sampling station (i.e. local variation) have a significant effect. BY was centred to the mean BY across all individuals to get biologically meaningful intercepts from the models. The distributions of the growth model parameters were checked to verify the suitability of gaussian distribution family for the GLMs. The distribution of Ø', G'_{max}, H_∞ (VB and G) and T_{50} (VB and G) were all found to exhibit a gaussian distribution, hence the gaussian family was selected when fitting GLMs, and there appear to be no need to transform the data to fit LMEs.

Model selection was then performed on the set of candidate models (table 1.2-1) using Akaike Information Criterion (AIC), discarding models with ΔAIC (difference from lowest AIC within the set of candidate models for the particular response parameter) > 2. Models with ΔAIC < 2 were considered to have equal evidential support in the data and were further assessed.

Table 1.2-1: The general structure of the predefined set of candidate models fitted to parameters from individual growth functions. The model class names from this model is used to refer to this model and the respective model structure in the result section 1.3.2.

Model class and internal name reference	**Model structure (fixed effects)**	**Random effect/nested structure**
LME1	Response ~ BY + AQ + BY:AQ	1|Station
LME2	Response ~ BY + AQ	1|Station
LME3	Response ~ BY	1|Station
LME4	Response ~ AQ	1|Station
GLM1	Response ~ BY + AQ + BY:AQ	-
GLM2	Response ~ BY + AQ	-
GLM3	Response ~ BY	-
GLM4	Response ~ AQ	-

AIC considers the increase explanatory value of adding new parameters, as well as punishing unnecessary ones, hence avoiding overfitting. When selected models included mixed effect models, those were refitted using restricted maximum likelihood (REML) prior to obtaining parameter estimates. Models were verified by inspection of potential heteroscedasticity using `plot(model)` to detect potential erroneous models. Parameter estimates and their respective SE, p-values and conditional R^2 (proportion of variance explained by entire model including both fixed and random factors) were obtained from selected models using `summary(model)` and `r.squaredGLMM(model)`. P-values of the model parameters were evaluated, and if a parameter in a model selected by the AIC is significant (significance level set to $\alpha = 0.05$) the effect of the parameter was considered significant.

1.3. Results

Video recordings and pictures of the potential locations around Bukkholmen and Lamøya showed sandy and rocky sediments but no presence of scallops. These areas were highly dominated by dark brittle stars (Ophiurida), probably *Ophiocomina nigra*, many of which extending their arms up into the epibenthic water, as well as numerous sea urchins *Echinus esculentus* (Figure .1.3-1 and -2).

Figure 1.3-1: Typical view close to Bukkholmen. Photo: Antti-Jussi Olavi Evertsen

Figure 1.3-2: Typical view close to Lamøya. Photo: Antti-Jussi Olavi Evertsen

In total 91 individuals were collected, of which two were completely unreadable due to epigrowth of calcareous polychaetes and calcareous red algae. Those two were not analysed further in this study. Of the 89 individuals analysed, 19 were collected at InS1, 53 at KaS1 and 17 at HeS1. Of the 19 collected at InS1, 5 were classified as exposed to aquaculture according to section 1.2.5.

The temperature at 20 m depth at Bud, Nordmøre show no significant increase between 1998 and 2016 (temperature increasing 0.0024^{o}C/year, p=0.86, data: IMR 2018), which is the timespan of the longevity of individuals analysed in this study (appendix 1, Figure A1.2).

1.3.1. Growth models

The VB was successfully fitted to 87 of 89 individuals. the two unsuccessful individuals had 3 and 4 height-at-age data points respectively, making the model exceed 50 iterations to identify the best estimates for these models non-linear least square method. Of the 87, two are exhibiting extreme values of H_{∞} estimates, which is further covered in section 1.3.2. The G was successfully fitted to 88 of 89 individuals, the individual not successfully modelled had only 3 data points, also exceeding 50 iterations. No parameter estimates appear to be extreme. As seen in Figure 1.3-3 and 1.3-4, the plot of the individual G(t) curves follows the pattern of the data more closely than the H(t) curves. Furthermore, it appears that individuals born in late 1990s and early 2000s grow slower than individuals born in later years. The H(t) exhibits negative height values at age 0 to about 1.

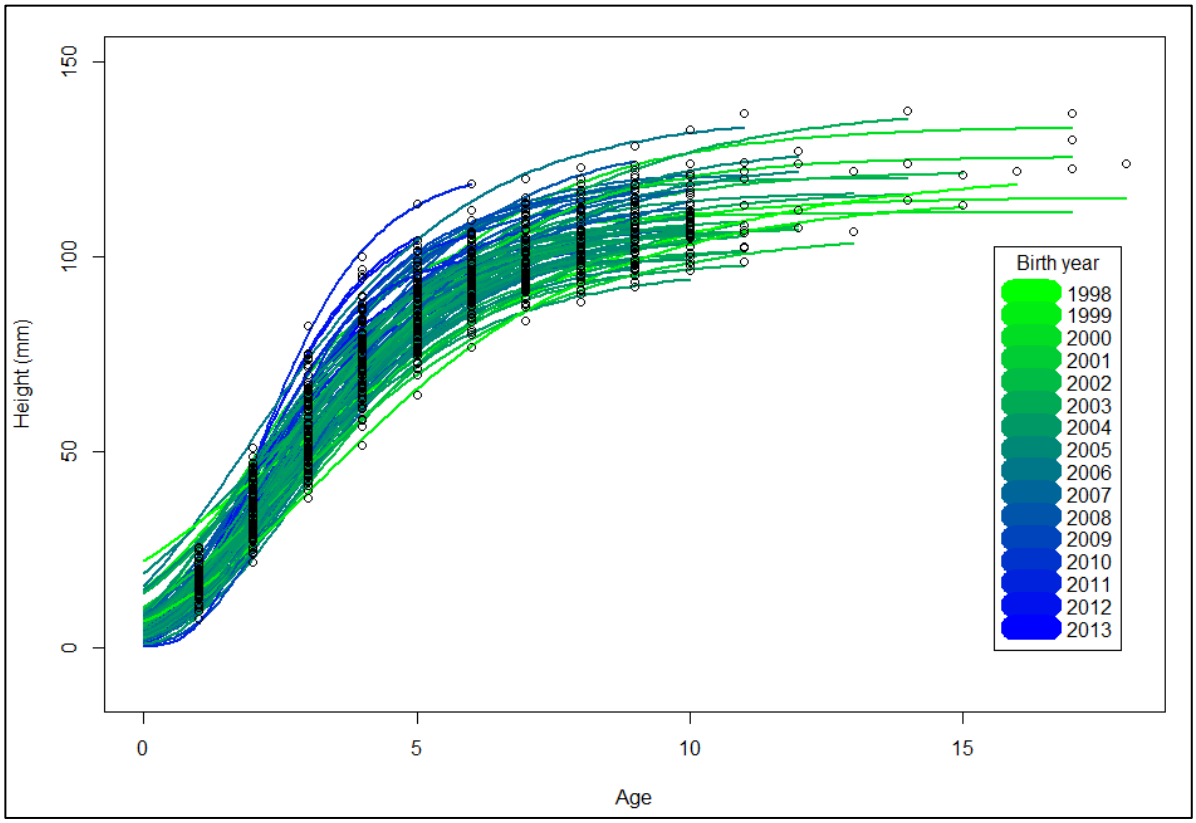

Figure 1.3-3: Individual growth curves fitted by G(t). Black circles are size-at-age data points.

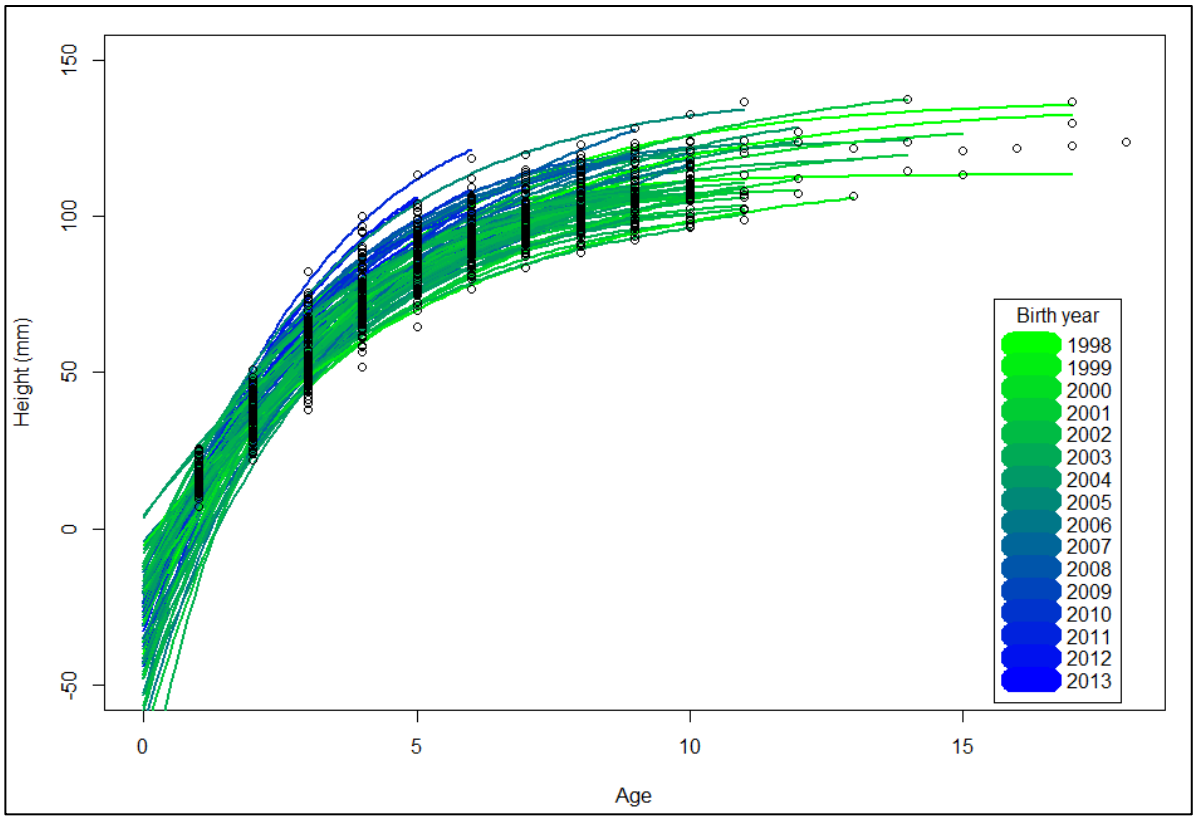

Figure 1.3-4: Individual growth curves fitted by H(t). Black circles are size-at-age data points.

Residual plot of the VB model showed a pattern of heteroscedasticity; residual values at age 1 tend to be positive, residual values at age 2 exhibit a small tendency of being negative, indicating the model has a bias towards lower size at age 1 and towards higher sizes at age 2. G seems to have the residuals at ages 1 and 2 balanced around 0. Both models seem to have slight patterns in the residuals at older ages, at which the G seems to have positive residual values whilst the VB seems to have balanced around 0 or slight negative values (Figure 1.3-5).

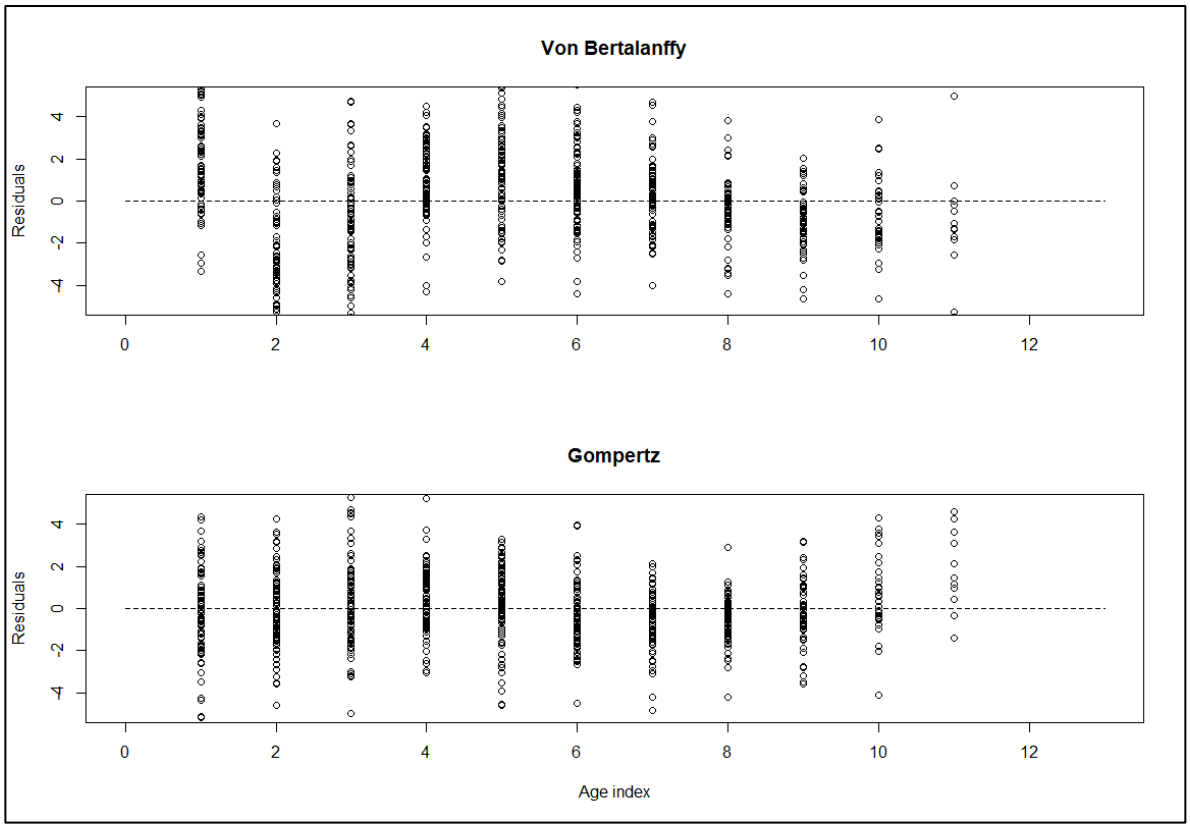

Figure 1.3-5: Residual plots of the VB and G growth models. Dotted line indicates zero. X-axis indicates age index, which corresponds to age at values from 1-10, age index 11 corresponds to maximum age for individuals older than 10 years old.

The distribution of the MSSQ show higher variance and higher mean in the VB model than the G (Figure 1.3-6). The MSSQ for the G model had a mean of 5.27 (var = 41.52) and for the VB the mean was 10.70 (var = 116.81). The distribution of MSSQ from VB also has a longer tail, with higher number of individual models that with higher MSSQ. Hence, the G predicts a growth curve closer to the observed data than that predicted from the VB.

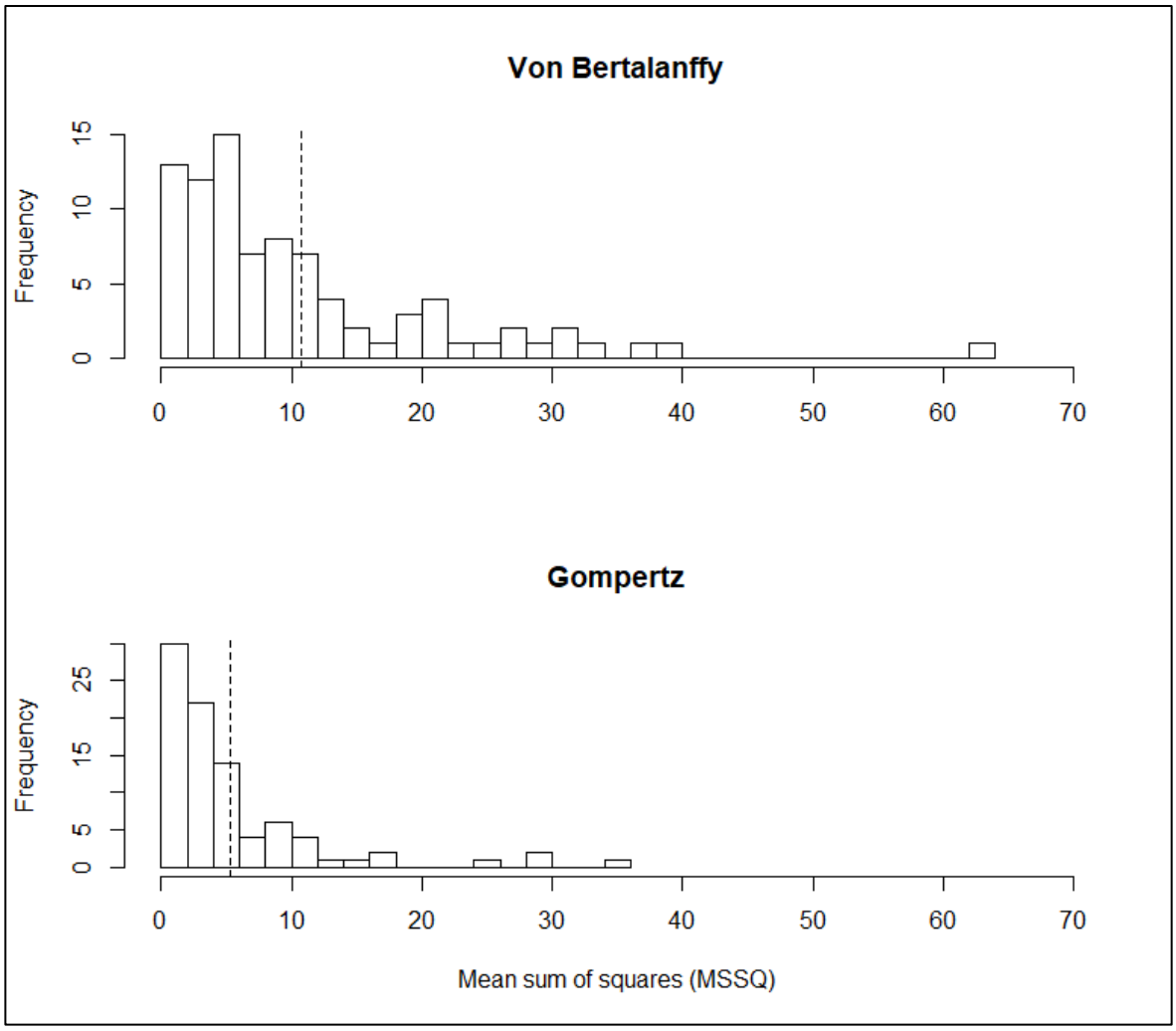

Figure 1.3-6: Histograms showing the distribution of the MSSQ values from the individual growth model of both VB (top) and G (bottom). Dashed lines indicate the mean MSSQ between individual models.

1.3.2. Growth model parameters

All models selected by AIC and their respective parameter estimates are listed in table A2.1 in appendix 2, together with the plots from the model verification analyses (Figures A2.2-A2.10). In general, parameters have a positive correlation to BY except asymptotic height from G and age of reaching 50% of H_∞ for both growth models. Exposure to aquaculture is also indicated to be positively correlated to G'_{max} and $H_{\infty\,(VB)}$.

The best fit based on AIC for Ø' was GLM3 (see table 1.2-1 for model name and structure, goes for all model names) with intercept (I) 4.52 (SE = 0.039, $p < 0.001$) and BY (0.04314 $year^{-1}$, SE = 0.0123, $p < 0.001$) ($n = 87$, $R^2 = 0.126$, Figure 1.3-7a). All other models for Ø' had ΔAIC>2, indicating aquaculture has no significant effect on growth performance. Ø' have two

values which appear to be outliers which has a high impact on the models (Appendix 2, Figure A2.1). Thus, model selection was also tested on the data without those, selecting LME3 with intercept 4.53 (SE = 0.069, $p < 0.001$) and BY (0.019 /year, SE =0.0057, $p > 0.001$) as predictor variable (n = 85, $R^2 = 0.520$, Figure 1.3-7a).

For the growth performance of G models, G'_{max}, there were two models favoured by the AIC (within $\Delta AIC<2$), however only one had significant parameters; GLM2 (n = 87, $R^2 = 0.501$, Figure 1.3-7b) with BY (0.87 mm/year^2, SE = 0.117, $p < 0.001$) and AQ as ($p = 0.0444$) as predictor variables; intercepts are 24.56 mm/year (SE = 1.59) and 21.22 mm/year (SE = 0.36) for exposed- and non-exposed individuals respectively.

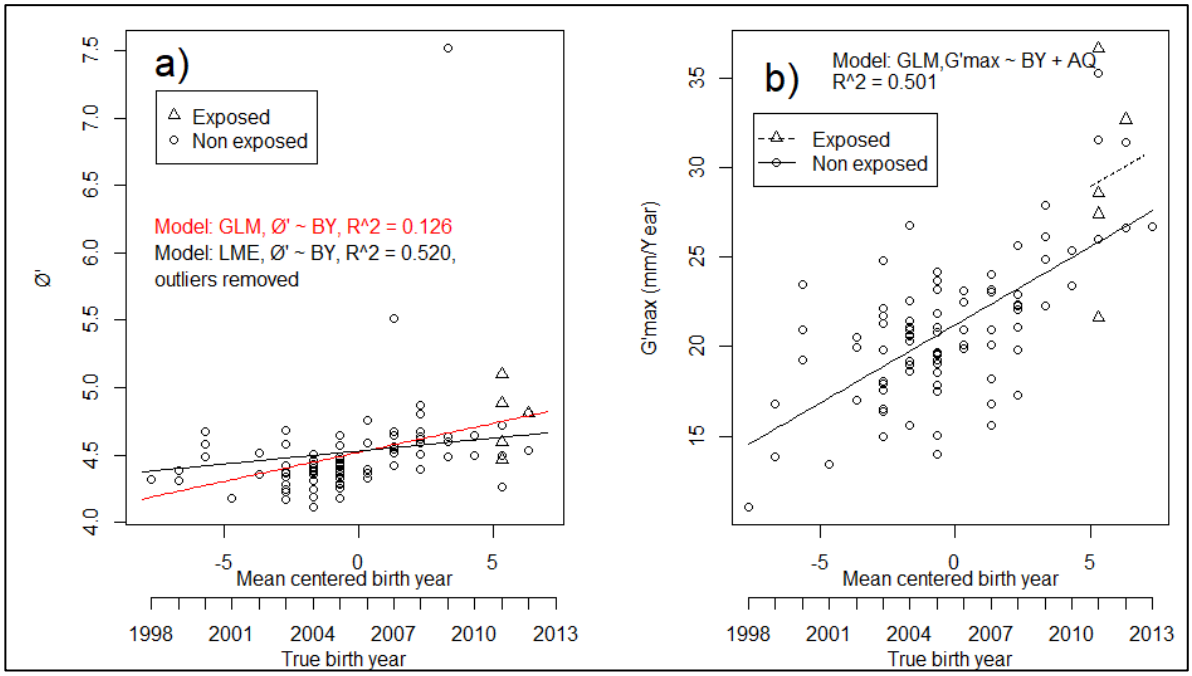

Figure 1.3-7: a) Performance index Ø' from VB and b) maximum growth rate G'_{max} from G, plotted against BY. Fitted lines are the best fitted models. In a) red curve is the best fit including all data points (n=87), black is the best fit after removing two extreme values (n=85). All data points included in b).

Asymptotic height estimated by VB (Figure 1.3-8a) had two values much higher than the others; 989.55 mm and 279.59 mm shell height. When including all values for H_∞, the best model was GLM3 (n=87, $R^2 = 0.036$) with intercept 137.39 mm (SE = 10.10, $p < 0.001$) and BY (5.409 mm/year, SE = 3.162, $p = 0.091$) as the only explanatory. When excluding those two extreme values, three LME models were selected by AIC; 1) LME3 (n = 85, $R^2 = 0.292$) with intercept

128.51 mm (SE = 4.69, p = 0.0303) and BY as the only fixed factor (1.1444 mm/year, SE = 0.049, p = 0.022); 2) LME4 (n = 85, R^2 = 0.339) with only AQ as fixed factor (p = 0.014); intercepts were144.6 mm (SE = 8.03) and 127.7 mm (SE = 5.12) for exposed- and non-exposed individuals respectively; 3) LME2 (n = 85, R^2 = 0.309) with both BY (0.7283 mm/year, SE = 0.5499, p = 0.18) and AQ (p = 0.111) as fixed factors, intercepts were 139.92 mm (SE = 8.45) and 127.66 mm (SE = 4.65) for exposed- and non-exposed individuals respectively. Models LME3 and LME4 has equal support in this study, LME2 has insignificant parameters but are mentioned here for discussion purposes. H_∞ from G (Figure 1.3-8b) has a negative correlation to BY and was best modelled with LME3 (n = 87, R^2 = 0.419) with intercept 115.85 mm (SE = 4.04, p < 0.001) BY as the only fixed factor (-0.66 mm, SE = 0.302, p = 0.0312). All other candidate models had ΔAIC>2.

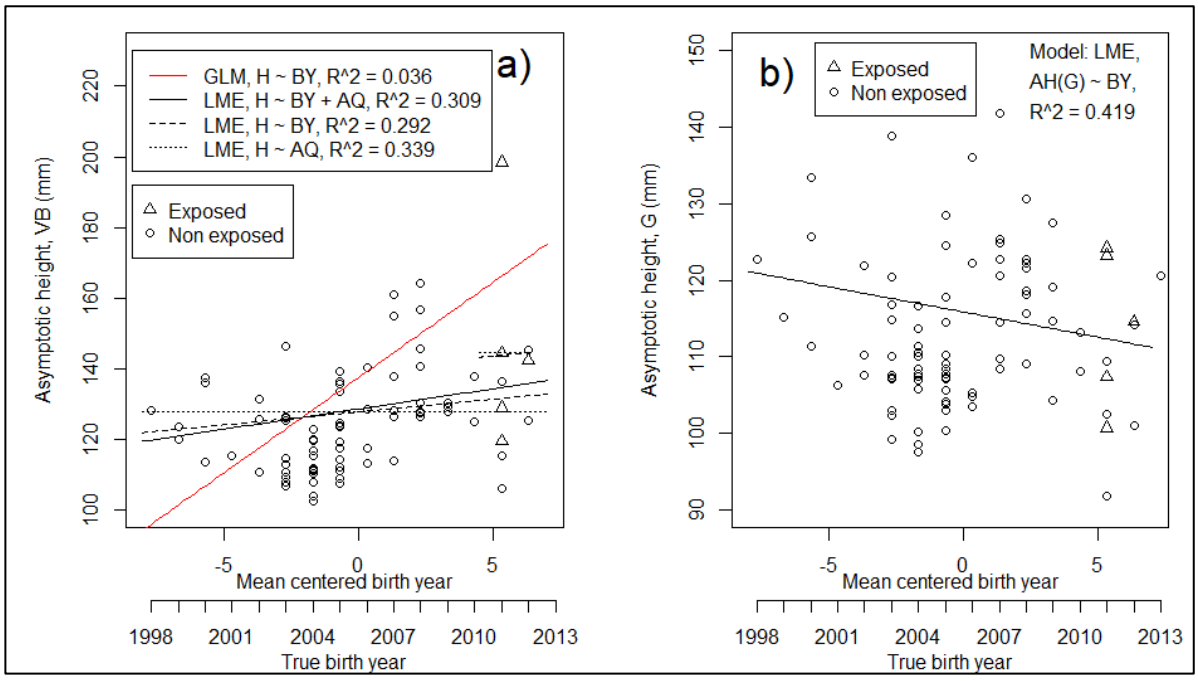

Figure 1.3-8: Asymptotic height (mm) in a) VB and b) G plotted against BY. In a) four models are shown; red line is including all points, whilst two outliers are removed before fitting the black curves (those two points are not shown in plot as they are far out of the range of the plot).

As seen in Figure 1.3-9, there is a correlation between H_∞ for the two growth models, having a correlation coefficient of 0.72 (Pearson correlation coefficient, excluding outliers; n = 85). In all cases the $H_{\infty(VB)}$ is larger than $H_{\infty(G)}$.

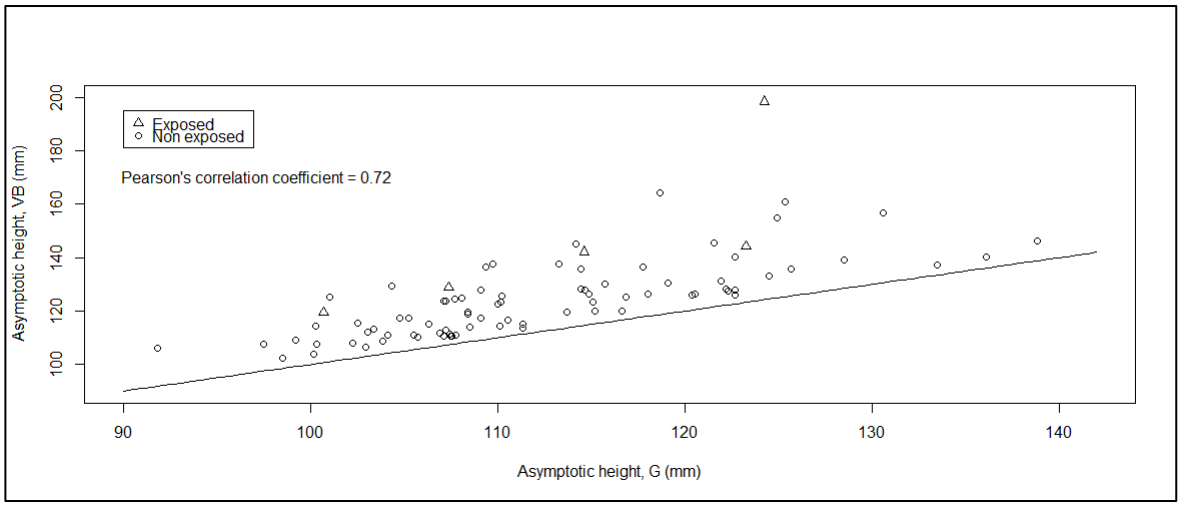

Figure 1.3-9: Correlation plot of H_∞ between the growth models. The black line indicates $H_{\infty(G)} = H_{\infty(VB)}$.

The theoretical age at which height reached 50% of H_∞ in VB (T_{VB50}) was not significantly affected by the BY or AQ in any of the tested models (p-value for those variables ranged between 0.36 and 0.67 in all tested models), thus the global mean is selected as best fit (n = 85, mean = 3.31 years, SD = 0.66, Figure 1.3-10a). For G, the best model was GLM3 (n = 87, R^2 = 0.135) with intercept 3.06 years (SE = 0.050, p < 0.001) and BY (-0.056, SE = 0.015, p < 0.001) as the only explanatory variable (Figure 1.3-10b).

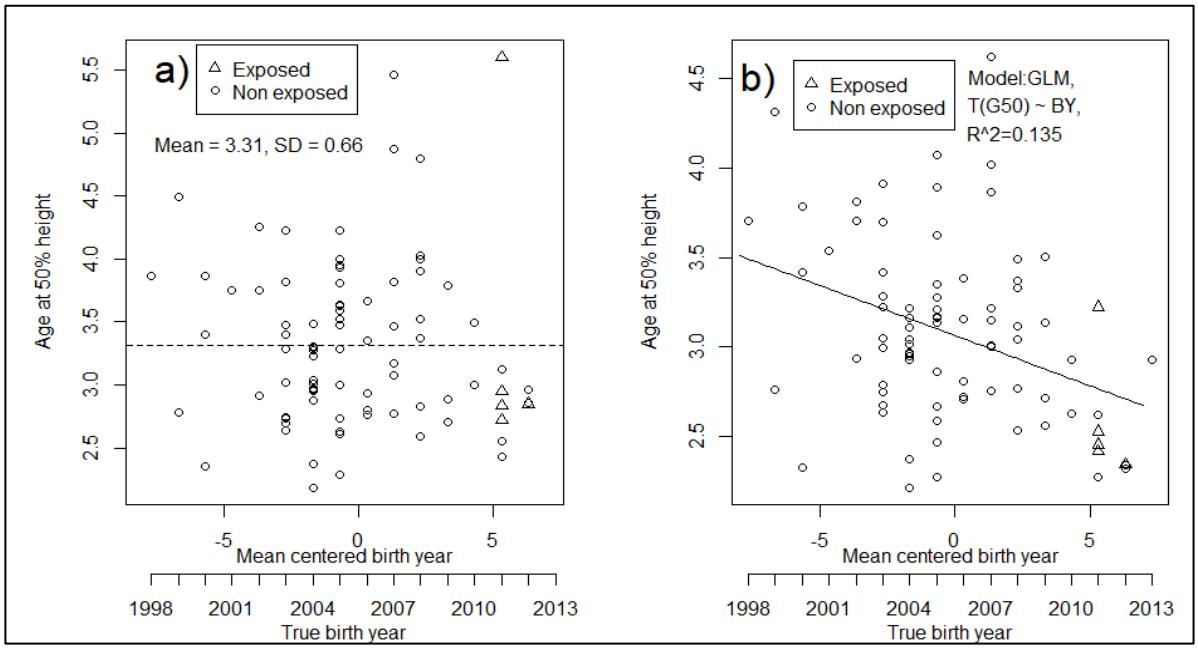

Figure 1.3-10: Age when 50% of H_∞ is reached in a) VB, b) G. in a) dotted line represents the mean T_{VB50}. Solid line in b) represents the best fitted model in T_{G50}.

1.4. Discussion

The Gompertz function proved to be better fitting growth model than Von Bertalanffy, demonstrating this model is better suited for disentangling growth in *P. maximus*. Furthermore, effects of VB and G produced ambiguous and contradicting results of effect of BY and AQ, highlighting the importance of applying the best model when assessing environmental parameters' influence on growth patterns. BY proved to be of high importance for growth of *P. maximus*, while AQ might influence certain characteristics of the growth patterns. However, conclusion on the AQ-effect is weakly supported here due to low sample size and few significant responses. The ultimate factor producing the effect of BY is uncertain, although suggestions are large scale spatial effect of aquaculture, harvest induced selective mortality, temperature increase and the consecutive changes in intraspecific competition and altered plankton communities.

The habitat around Lamøya and Bukkholmen was seemingly well suited for scallops. Their absence, and the presence of large numbers of brittle stars, indicates either some factor(s) making the habitat unfavourable for scallops or conditions favouring brittle stars in outcompeting them. The high abundance of brittle stars also indicates high food availability. This is supported by their behaviour with arms erect into the water masses, probably collecting food particles. These localities are in the sheltered waters inside of Inntian, as well as close to the oldest fish farms in the area. Whether there were scallops present prior to the initiation of the fish farming is not known, but these localities have been exposed for a long time. If aquaculture influences scallop performance, potential individuals in these areas have been affected. Hence, as scallops are absent, this supports that strong exposure might negatively influence scallop populations, but conclusions cannot be drawn on this basis and the absence of scallops can equally well be for other reasons based on these observations.

This study has a major assumption regarding the scallops' migratory behaviour; Adult scallops are assumed to exhibit a minimum of migrating behaviour – limited to few meters. Being able to swim, they can change their location if food availability or environmental conditions are unfavourable. If scallops migrate significantly, this can mask true effects of aquaculture. Experiments have been carried out to investigate how long and how frequent *P. maximus* migrates, indicating very little migration during adulthood and swimming behaviour is mainly linked to escaping from predators. However, the methods for assessing this behaviour have not been very precise and time series are generally not covering the full lifespan of individuals (Brand, 2016, Guderley and Tremblay, 2016). Nevertheless, the behaviour in pectinids has been

known for long, without strong findings of long distance adult migration to the authors knowledge (Baird, 1958, Moore and Trueman, 1971, Buddenbrock, 1911).

1.4.1. Growth models

According to the predefined selection criterion for growth model selection the growth of *P. maximus*, the best model is the Gompertz function; There appears to be a heteroscedastic pattern in the early age classes by Von Bertalanffy, and VB also exhibits higher and more variable MSSQ. The fit of the VB predicts negative height values – down to below -50 mm - from age 0 to age about 1 years which is biologically impossible and demonstrates this model is irrelevant at young ages and small sizes. Shell heights for young individuals predicted by the G function are more biologically realistic, ranging from 0 to 25 mm at age 0, mostly about 0 to 10 mm. This pattern is also found in the residual plots, in which the VB tends to have positive residuals at age 1 indicating underestimation at this age, whilst G does not express this strong pattern in its residual plot, although it seems to have a slight underestimation at older ages. This pattern is as expected from other studies on these models (Lloyd-Jones et al., 2014, Gamito, 1998). The two models also express very different mean- and variance of the MSSQ; the MSSQ from VB is 10.70, twice that of the G at 5.27. The variance around the MSSQ, 116.81 and 41.52 for the VB and G respectively, is much higher for the VB. This demonstrates that the G predicts the growth exhibited by individuals of *P. maximus* more precisely than the VB. Hence, less stochastic variance is introduced by modelling growth with G, conserving the properties of the individual growth data better than the VB. Applying VB, with more residual variance, might mask potential environmental effects. Furthermore, VB made two obvious outliers, as well as two individuals for which the model failed to predict parameter estimates. The G, on the other hand, made no outliers and fitting failed in only one individual. It was not performed a statistical test of outliers per se. However, as the mentioned height values were so biologically unrealistic they were considered as such, produced by the combination of a non-optimal growth function and a special growth pattern of the specific individuals. Even though fit to the same data, G did not produce any such outliers. This underpins the contention that G predicts more biologically relevant parameter estimates and demonstrates that it is more robust towards few data points, as well as conserving the data properties better, requiring less data points and hence conserves the sample size better. This makes it more suitable for analysing growth of this species and possibly other pectinids.

Generally, the parameter estimates from the two models show ambiguous patterns in terms of effects of BY and AQ. This demonstrates the importance of selecting the most precise model, as the model selected strongly determines the results and conclusions to draw from the data. This is particularly important for management, as this relies on the knowledge of the management units such as populations or areas. As the VB is widely used in both bivalve- and fish- fisheries ecology, it is more suitable for comparison to other studies. Thus, selecting which model to use is a trade-off between precision and comparability. However, as Ø' is species specific, comparative value is limited to within species (Munro and Pauly, 1983). Additionally, management should aim for incorporating all known knowledge to achieve the best available management practices, which advocates for using the most informative growth model; in this case G (Begon et al., 2006a). As the G is known from literature to underestimate H_∞ there are probably other growth curves that can outperform G (Laslett et al., 2002, Gamito, 1998, Helidoniotis et al., 2011). There are suggestions in literature that inverse logistic functions are better, as well as a combination of G and VB to better model both the young age classes as well as the older age classes (Gamito, 1998, Helidoniotis et al., 2011). Those were not tested her, as they are incomparable to G and VB by using least square criterion due to the number of parameters.

As VB is currently the most widespread growth model for fisheries, there can be practical implications for management of shifting to using G. Management generally aims to determine quotas and potential harvest, and knowledge on size distribution in the population can be a valuable tool; Given an age structure and a generalized growth model, one can estimate harvestable population size or harvestable fraction. G always predicts a lower shell height than VB, thus shifting to using G can give different such estimates. In the case of *P. maximus* management in Norway, there are currently no quotas set for this species in Norway. The scallop fishery is self-controlled through economy of this industry, which is only profitable when population size is big and density high (Strand et al., 2016). Hence, changing to using G for scallop fisheries should not constitute a challenge for Norwegian management of great scallop.

1.4.2. Growth model parameters

Growth performance parameters Ø' and G'_{max} showed positive significant correlation to the birth year. Interestingly, the G'_{max} also showed significant effect of aquaculture exposure which

was not detected by the models for Ø'. The numerical comparability of Ø' and G'_{max} is limited and one should be careful when doing so. Nevertheless, variations in performance indexes should reflect the environmental or demographic variation (Moreau et al., 1986). Results indicate the G'_{max} is more sensitive to environmental influences than the Ø', supporting G as the best model for disentangling the growth of this species. Furthermore, one should keep in mind to be cautious to draw strong conclusions on the effect the aquaculture in this study; there are only 5 individuals defined as exposed, all of which born during or after 2010, and thereby confounded by the apparent effect of BY. On the other hand, the confounding effect should be accounted for by the regression models, and the effect of aquaculture is significant in the GLM for G'_{max}. It is also important to note G and VB detects different effects of both BY and AQ for growth performance parameters.

Interestingly, the asymptotic height H_∞ from the VB is either unaffected or positively affected by the BY, while in the G this is significantly negatively affecting H_∞. In this case growth model selection makes a significant difference when disentangling the true growth patterns of scallops. In VB, it is not possible to disentangle the BY from the AQ in affecting the H_∞, of the three models selected by AIC, one indicates only BY is affecting the height, while another indicates only AQ. The last selected model includes both AQ and BY as explanatory variables, however neither were significant as AQ and BY are likely to confound each other and no conclusions can be drawn here. This shows the data is insufficient to confidently disentangle this pattern in VB. Furthermore, the VB always predicts higher H_∞ than G, probably due to the model structure. The latter is consistent with previous studies on G, demonstrating the presence of challenges also found with using G (Laslett et al., 2002, Gamito, 1998, Helidoniotis et al., 2011). A correlation coefficient of 0.72 between $H_{\infty(G)}$ and $H_{\infty(VB)}$ could generally be described as modest or strong, although interpretation of such coefficients is somewhat subjective and depending on the environmental variables and their effect on the data (Taylor, 1990). However, as this correlation is based on parameters describing the same trait, originating from the same data, these should be strongly correlated here. This difference and in this case weak correlation demonstrates that at the two models introduce different biases when applied on the same data. As G is found to introduce less variance and predicts the growth more precise than VB, this suggests the bias introduced by G is lower and the decrease with BY is more likely than the increase or no effect indicated by the VB– also when considering the underestimation by G at older age classes as this underestimation should be expected to be the same for all values of BY. Furthermore, models for $H_{\infty(VB)}$ excluding outliers had much lower effect of BY and higher

R^2 (about an order of magnitude higher for H_∞ and 5 times higher for Ø') than those including outliers, demonstrating these two points had very strong effect on the model.

There appears to be no change in T_{VB50} with time. Combined with the increasing growth performance (Ø') for increasing BY, this fits well with increasing H_∞ with BY as the height is a function of growth performance and duration of growth – thus increased growth performance within the same duration of time produces larger sizes. For G, there is a decrease in T_{G50} with BY, suggesting a reduction in age of maturation with increasing BY. Together with the increase in maximum growth rate G'_{max} and a decrease in H_∞, this combination in growth development is typical for a live-fast-die-young strategy (Reznick et al., 2002). It appears from this that the *P. maximus* are developing more towards r-selection along the r/K-continuum than in the 1990s/early 2000s. This development is typically seen when the selection regime is shifting towards more stochastic survival and competitiveness is getting less important, commonly associated with increased environmental stochasticity and catastrophic events (Pianka, 1970, Begon et al., 2006b).

Even though BY surprisingly is a strong determining factor for performance of *P. maximus* in most of the selected models, birth year itself is not likely to be the causative factor determining the changes in growth patterns. It is more reasonable to assume is a proxy for some other effect which is not uncovered by this study and this data. Additionally, it is not possible to determine in this study whether this pattern represents an evolutionary response or phenotypic plasticity. Here, I suggest 4 possible explanations for the pattern regardless of having genetic basis or purely being a developmental response; 1) increasing temperatures in the water masses in the study area, 2) all stations are affected by aquaculture activities, 3) increased mortality from harvest lead to development towards more r-selection, and 4) the pattern is an artefact of the data:

1) Considering temperature is known to be a key factor determining growth of this species, the most obvious possible factor is: *is there an increase in ocean temperature within the time and area of this study?* Although the temperature at Bud appears to have no increase with time, there might be patterns and information not easily read from this data; such as the number of days with temperature supporting growth, or degree days increasing with time. Temperature alters the metabolism of scallops, increasing the potential to increase growth in presence of enough food but also increases the cost of maintenance metabolism i.e. the energy required to conserve body functions (Chauvaud et al., 2001, Heilmayer et al., 2004). Also, the temperature is controlling the growth conditions for planktonic algae – thus food

availability is an interaction between energy and species composition which is not assessed in this present study (Chauvaud et al., 1998). A northward distribution shift in planktonic communities are observed in the northern Atlantic and linked to increasing temperatures and climate change, possibly having implications for the food availability for scallops (Gregory et al., 2009, Hays et al., 2005). Thus, temperature should not be excluded as an ultimate factor producing the trend in the growth found in this study. For more confidence, local data on temperature with higher temporal resolution should be analysed. This data might be available from fish farms in the study area, but it has not been provided to this study.

2) The whole study system might be affected by aquaculture and there might be no real control group in the data. Aquaculture activities has been increasing in the area the last 20 years, thus fitting with the trend in the data if the nutrients released are made relatively uniformly available for scallop populations in the area. The release of nutrients might increase planktonic primary production in a larger area than expected, and the subsequent sedimentation of plankton to benthos also has a delay and can be advected by currents – thus the impact of aquaculture might cover a larger area than the spatial resolution of this study, suggesting all stations have been relatively equally affected by aquaculture activities. From literature, however, the effects on benthic communities appear to be within 250 m, supporting classifying KaS1 and HeS1 as non-exposed (Seguel et al., 1998, Brooks et al., 2003, Kutti et al., 2008). The mentioned estimate of 2-2.5 % increase in primary production in Trøndelag during 2012 indicates assumes larger spatial scale, but the data has low resolution and no information on plankton community changes (Taranger et al., 2014).The species composition in the plankton community interacts with the presence of aquaculture, and strongly determines what effect this can have on the scallop populations as this affects its' growth (Chauvaud et al., 2001, Chauvaud et al., 1998). Stensås (2014) found higher growth in scallops close to fish farms, however the experimental design using open-water-cages excludes the previously mentioned potential negative impacts associated with the benthic environment such as changes in oxygen levels, excremental sedimentation and changes in sediment redox potential and thus the spatial succession of sediment chemistry. Therefore, the relevance of this study (Stensås, 2014) is limited for natural habitats but documents that *P. maximus* is capable of consuming energy resulting from fish farming.
If this effect of higher spatial impact of aquaculture than the current conception is true, aquaculture is potentially affecting many ecosystems that are believed to be unaffected. This

should receive scientific attention in order to increase the knowledge base for management of coastal areas. Here, this can explain increased growth performance such as Ø' and G'_{max}, and reducing T_{G50}, although it is not obvious how this explains the reduction in H_{∞} found in G.

3) The commercial harvest developed in the 90s and has been relatively stable for the last 20 years (appendix, Figure A1.12, Strand et al., 2016). Harvest generally tends to lead to increased mortality in a harvested population and depending on strength of the harvest and technique it can also be very selective and thus lead to a strong selection regime (Heino and Dieckmann, 2008, Allendorf et al., 2008). In Norway, smallest allowed catch is 100 mm shell width. Harvest is performed through diving and hand picking, although effort is made to develop mechanical methods (Strand et al., 2016). This harvest is strongly selective, increasing mortality of individuals above 100 mm and reducing their fitness as well as increasing the relative fitness of smaller individuals and individuals with a more r-strategy life history strategy (Jørgensen et al., 2009). This can be expected to lead to more r-selection regimes such as indicated by the results from G and might explain the patterns in the data. However, this is a very quick response to such changed selection regime considering scallops live up to 20 years, and this mortality only acts on the older individuals. Hence, it might be more likely that this increased mortality facilitates increased survival amongst the remaining individuals. Barbeau et al. (1998) demonstrated that higher densities of sea scallops *Placopecten magellanicus* suffered higher predation rates than lower densities, indicating reduced mortality of low density populations. This population-level response to predation is well known in population ecology (Begon et al., 2006c, Mittelbach, 2012). Applied to this study, this response suggests the individuals surviving harvest will have increased survival and performance as predation rate can be expected to decrease after harvest, fitting well with the observed pattern of G'_{max} and T_{G50} within the observed timeframe, suggesting a phenotypic response. However, one might expect increasing shell heights rather than decreasing for such responses. Comparing harvested populations to not-harvested ones might give further insight into this, however populations with high densities are likely to be affected by harvest, thus making it harder to find comparable populations and -densities with differing harvest efforts.

4) Lastly; are the trends really an artefact of the data? All individuals were collected in 2014 and 2017. Therefore, the BY reflects the age and can include a range of life history

strategies. Hence, the pattern found in the growth model parameters might be representing the life history strategies represented in the populations. This can be illustrated by the two extremes of this strategy continuum; at one extreme, long living, slow growing, investing in many reproductive seasons – and in the other end short living, fast growing and maximizing effort in fewer reproduction seasons. Obviously, the oldest individuals are in the long living end of the range, but the short living end is not as easily identified as the young individuals can have either strategy. However, if this hypothesis is true, one should expect higher variance of the parameters in the younger ages as all life history strategies should be present in the younger individuals. This should be visible in the scatter plots and produce heteroscedasticity visible as wedge shapes in the residual plots of the models. Neither of those patterns are found, indicating this is not important for the observed patterns of growth performance. Another way the pattern might be an artefact of the data is by growth model precision. Older individuals have more data points of height-at-age data which the model can be based on than younger individuals have. This can influence the fit of the growth models, especially in individuals that are so young that growth has not clearly started levelling off. To test for this, the growth models were fit to size-at-age data for ages up to 6 years old only, from which the results indicate the same patterns and effects of BY and AQ are present in models based on few data points. Hence, this can also be excluded as the factor producing the effect of birth year.

It is likely that one or more of these suggestions are contributing to the observed patterns. The potential implications differ between them. The temperature increase and shift in plankton communities are difficult to control, hence the implications are the need of adapting management of coastal areas to consider such changes for a range of management units. If *P. maximus* have increased growth because of aquaculture activities, which the some of these results indicate as well as point 2) above might support, there are a few implications. Firstly, it shows aquaculture has effects on the local environment either locally (as found in G'_{max} and ambiguously in $H_{\infty(VB)}$) or regionally (as suggested by 2) above). This pattern should also be expected to be found in other species, affecting the whole ecosystem and altering competition and community compositions in the area. This has potential implications not only limited to the coastal areas, but as many pelagic shelf species has spawning- and nursery grounds in coastal waters and habitats this can affect community composition in a larger area. Secondly, it shows that there is potential for Integrated Multi Trophic Aquaculture (IMTA) systems, which have received increasing attention in later years. This concept is synergistic production of aquatic

species together across niches and trophic levels aiming to increase total energy out:in ratio and reduce waste to the environment. It is, politically and economically speaking, very likely that aquaculture in Norway will persist, thus finding methods to improve the efficiency of aquaculture as well as reducing waste and pollution will be of importance for increased sustainability of coastal management. Lastly, if harvest affects this pattern, management should consider the need of setting quotas in Norway to limit the effects on the populations. As this study adds no new evidence, the status of the harvest is unchanged and is still considered sustainable as according to Strand et al. (2016). However, this study suggests extending the monitoring of this species to increase confidence in management recommendations.

1.4.3. Methodical- and statistical challenges

One challenge of in the data is the classification of individuals as either exposed- or not exposed to aquaculture activities. As the aquaculture activities at Hofsøya Fish Farm (close to InS1) was initiated in 2014, the exposure time is shorter than the pre-exposure time giving an unbalanced study and a low sample size of exposed individuals. Furthermore, individuals of different age are not equally affected by aquaculture, and as growth of *P. maximus* is not linear it is challenging to include the degree of exposure. Thus, treating aquaculture as a factor across ages is not methodically preferable, but it is the best available.

The sample size to conclude and generalize on whether aquaculture influences growth of *P. maximus* is 1, as only one fish farm has been investigated. For being able to generalize this potential effect, more fish farms need to be investigated, as well as more balanced time span pre- and post-initiation of aquaculture at the investigated sites, including more data on exposed individuals than in this present study. Alternatively, a series of transects from a farm could be used to investigate this effect further. A setup of before/after exposure within one locality is a strong test of the effect at that site, if one has control of the confounding effects. As the BY unexpectedly proved to be significant and important, this strongly confounded the potential effect of the aquaculture. Although this confounding effect should be handled by including both BY and AQ in the statistical models, the data is insufficient with the very limited sample size of only 5 exposed individuals.

The AIC is a good method for model selection when there is a fixed number of candidate models. The punishment of unnecessary parameters helps avoiding overfitting, however this punishment is prone to being influenced by low sample size. If the sample size is low, the AIC

will be more likely to favour a model with more parameters, hence overfitting the model. In such cases one should use a special case of AIC with correction for small sample size called AICc. The sample size of this study is very close to the rule of thumb for when to use AICc. One should consider using AICc if sample size (n) divided by number of parameters (K) is below the threshold value 40, i.e. $n/K < 40$ (Burnham and Anderson, 2004). In the set of candidate models, K is either 1,2 or 3 (BY, AQ and their interaction) and the sample size is 85 or 87 for the different models. Even when being prone to overfitting, the AIC did not favour the complicated models in this case, and the interaction was not selected in any model selection procedure. Thus, this was probably not a problem for this study. However, the difference between AIC and AICc becomes negligible as the increased punishment for unnecessary parameters approaches 0 as the sample size increases(Burnham and Anderson, 2004). Hence, selecting AICc is not erroneous with high sample size, in contrast to selecting AIC at small sample size. As this study was at the threshold between AIC and AICc, perhaps selecting AICc would have been preferable.

The candidate models including the interaction between AQ and BY are rightly included, as the presence of aquaculture can interact with the drivers behind BY. However, as there are only 5 exposed individuals, one can hardly imagine it is possible to obtain any significant estimations of such interactions. This also goes for the mixed effect models having (AQ|Station) as random factor. As 4 of the 5 exposed individuals are born the same year, it is likely that producing a significant slope for aquaculture alone, unrelated to the other individuals, is statistically not feasible. Hence, given this structure of data, these candidate models were unlikely to be favoured by AIC.

1.5. Conclusions

In the basis of these results, I conclude that the Gompertz growth model is preferable over the Von Bertalanffy for modelling the growth of *Pecten maximus*. G's residual variance is much lower and more constant, and this model appears to be less prone to producing outliers. Hence, the properties of the data are preserved, making G more suitable for detecting factors affecting the growth patterns than the VB. This study has found ambiguous effects of birth year and aquaculture in growth parameters from the two growth models. Hence, this highlights the importance of using the best fitting growth model when aiming to disentangle environmental and biological factors affecting the growth performance. For both scientific purposes and management, one should aim to use a model that is precise, with parameters that are biologically relevant and interpretable, and requires data which is easily recorded. Here, I demonstrate that the G is more suitable for such purposes than the VB. As this demonstrates that model selection can give very different results, this also suggests evaluating the usage of different growth models in other species to determine which model is the best in specific cases. This can be particularly important for assessing growth in harvested species and management of vulnerable areas and populations.

Due to insufficient data I will not draw strong conclusions on to what degree aquaculture are affecting the growth of *P. maximus* in the waters around Frøya, although there appears to be significant effects in some of the growth model parameters. The most important factor influencing the growth in this study is by far the birth year of the individuals. Birth year per se has no biological influence other than being a proxy for some environmental parameter. I suggest that one or more of the following factors are the ultimate factors behind the birth year effect: 1) temperature increase, 2) regional effects of increasing aquaculture activities, or 3) altered selection regime because of increasing harvest or otherwise changed mortality. Temperature and aquaculture can also affect the plankton community, which can affect growth of *P. maximus.* Further studies are required to disentangle the relative importance of these, and other factors may also be of strong importance.

These growth data can be further analysed in several ways; there is probably temperature data available in some database either at fish farms or from some other research activities, or it might be possible to access precise estimates. This can be combined with the growth data and model estimates. Here, growth models are aligned along the age, which allows for evaluating the model in terms of its general trends and tendencies to over- or under-estimate the growth. For further analyses, the models can be aligned along the BY-axis, allowing for analysing whether

the residuals reveal potential patterns of temperature effects, by correlating characteristics of the residuals to characteristics of the temperature data.

For future studies, it would be preferable to have control stations that are more certainly true controls or transects with increasing distance to fish farms to further disentangle the growth of *P. maximus* – including plankton sampling for community analysis and combining this with diet preferences. The sampling areas should have been exposed for longer than in this present study to make room for higher sample size of exposed individuals to strengthen conclusions. Furthermore, nutrient analysis of water masses along the transects will greatly increase the understanding of spatial effect of fish farms and should be included. Preferably this would also include diet analysis to investigate whether scallops consume excess fish feed, either lipid content analysis or stable isotope analyses of tissue or stomach content. Temperature time series, as well as sampling in an area with a minimum of historical and current harvest effort is crucial to be able to disentangle the confounders.

2. Chapter two: Microplastics in *Pecten maximus*

The original aim of analysing plastic content in this study was to include plastic content as a variable in the statistical models for growth model parameters to determine whether plastic influences growth performance. As the lab work progressed, it became clear that this aim would not be achieved due to methodical challenges and low success. It was decided to change the aim into further adopting this method of plastic content analysis in marine invertebrates. The adaptations were generally made to be for rapid screening, and to be of use for educational and citizen-science at a Norwegian high school. At Guri Kunna High School, there was already an initiative for piloting these methods, and this thesis is contributing to their project.

2.1. Introduction

Plastic materials have been in use for a range of every day products around the world for more than 60 years. Since the apparent boom in usage during the late forties, the usage has expanded to almost every kind of products all over the world. As plastic has no counterparts in nature, few organism groups have evolved to cope with presence of plastic in the environment, although there are reports of bacterial and fungal degradation (Mueller, 2006, Shah et al., 2008). Breakdown in nature is slow, and plastic waste follow water pathways through rivers and lakes before entering the oceans. Sediments seems to be environments in which plastic debris accumulates (Nerland et al., 2014). Photo- and mechanic erosion breaks the waste into smaller pieces, producing particles in all sizes from micro meters to meters (Shah et al., 2008). Having a diverse range of size, colour and shape facilitates misidentification of plastic particles as food items for many consumers in natural ecosystems. Ingestion of plastic particles has been detected in representatives from most groups in the food web; amongst other Copepods, krill, shrimp, bivalves, fish and sea birds (Nerland et al., 2014, Cole et al., 2013, Li et al., 2015, Romeo et al., 2015, Devriese et al., 2015).

Consumption of plastic constitutes several possible threats to the consumers and the associated food chain. Those have several different possible pathways; 1) Time and energy spent on foraging on plastic particles might reduce the time and resources spent on foraging on food items, reducing foraging efficiency. 2) Having plastic particles in the digestive system might reduce uptake of nutrients due to clogging and reduced concentration of nutrients, further reducing efficiency of energy acquisition. 3) The physical and chemical structure of plastics facilitates adherence of a range of other pollutants and heavy metals such as PCBs

(polychlorinated biphenyls), POPs (here: persistent organic pollutants), copper and zinc, many of which impact survival or reproduction of organisms (Brennecke et al., 2016, Ziccardi et al., 2016). This can more important in higher trophic levels, as concentration increases with each trophic level through biomagnification (Setälä et al., 2014). Content of plastics and associated pollutants is also expected to increase with age of the organism through bioaccumulation. The magnitude of this problem of plastic particles is not yet fully understood and quantified but is currently a hot topic in science and media. Scallops are suspension feeders and thus typically prone to feeding on plastic particles in the epibenthic water masses, as well as being a commercially harvested species for human consumption. It is not known whether their selective feeding behaviour favours plastic particles, however Beninger et al. (2004) found that the speed of this particle selection can be exceeded by the natural flow of food items, leading to reduced precision of the selection process. This suggests that in areas of extreme food availability, selection of undesirable food items is more likely. Hence, it is possible there is an interaction between plastic consumption and increased food availability from aquaculture activities.

As the extent of plastic waste in the oceans are increasing with human activities, it is important to increase the baseline knowledge. One of the ways of increasing such knowledge is by facilitating citizen science programmes and platforms. Such programmes can be incorporated in already existing activities such as educational programmes at various levels. Here, I contribute to facilitation of citizen science at a high school by adopting a method of rapid screening of plastic content in marine invertebrates. This is achieved by prioritizing practical functionality, easily accessible materials and tools as well as environmental and health hazards of chemicals used in the analysis.

2.2. Materials and methods

The method is an adaptation for assessing micro plastics in marine invertebrates at a high school in Frøya. It is based on recommendations from other studies and the availability of chemicals, lab facilities and equipment, as well as their practical applicability and feasibility at a Norwegian high school. Due to practical limitations and time consumption, tap water used for rinsing was not filtered prior to usage and the squirt bottle used for rinsing was also plastic. Sediment samples were also taken at InS1, HeS1 and KaS1 to document whether plastic is present in the natural habitat of *Pecten maximus*.

Plastics are polymers of organic compounds, commonly with synthetic or semi-synthetic origin. It is highly capable of absorbing different chemical substances, especially non-polar ones. This capacity makes it easy to achieve different physical properties for different uses by adding different additives. For detecting plastics, this ability can be utilized by adding staining compounds to increase visibility. Maes et al. (2017) found Nile Red (NR) to be the best dye for detecting plastic in marine sediment samples. When illuminated with blue light, NR is excited, emitting red-orange light. By observing through an orange light barrier filter, this can be detected visually. Prior to this detection, the plastic particles must be isolated from the sample by removing the biogenic material by chemical, oxidative or enzymatic digestion (Lusher et al., 2017b).

2.2.1. Dissection and digestion

A subsample of the collected scallops was dissected for analysis of plastic content in tissue. Dissection was performed by knife, scalpel and forceps. Care was taken to avoid contaminating the samples, limiting usage of plastic tools to a minimum and covering all glasses with aluminium foil whenever possible. Organs were separated and measured to nearest 0.01 g. Organs were then stored in paper bags at -18^{o}C before further analysis.

Samples of muscle tissue and hepatopancreas (stomach) (Figure 2.1-1) was degraded separately through oxidation using concentrated hydrogen peroxide (35% $H_2O_{2(l)}$), as recommended by Lusher et al. (2017b), Kolandhasamy et al. (2018) and Masura et al. (2015). Hydrogen peroxide is also cheap, easily available, and the waste is manageable within reasonable quantities. 1000 mL glass beakers were filled with 10 mL hydrogen peroxide per g tissue, approximately 100-300 mL per sample. Approximately 10 mL of Iron(II)sulphate (0.05M, $FeSO_{4(aq)}$) was added to

each beaker to catalyse the reaction (Masura et al., 2015). Beakers were then incubated in an oscillating water bath (20-80ºC, the water bath was made by irregularly adding hot water to the water bath in which the beakers were incubated) until samples were completely degraded (Figure 2.1-2, left). Muscle samples were, due to practicalities in availability to lab, digested for about 5 months. The digestion process was not performed for the sediment samples as the biogenic material is negligible (Masura et al., 2015, Maes et al., 2017).

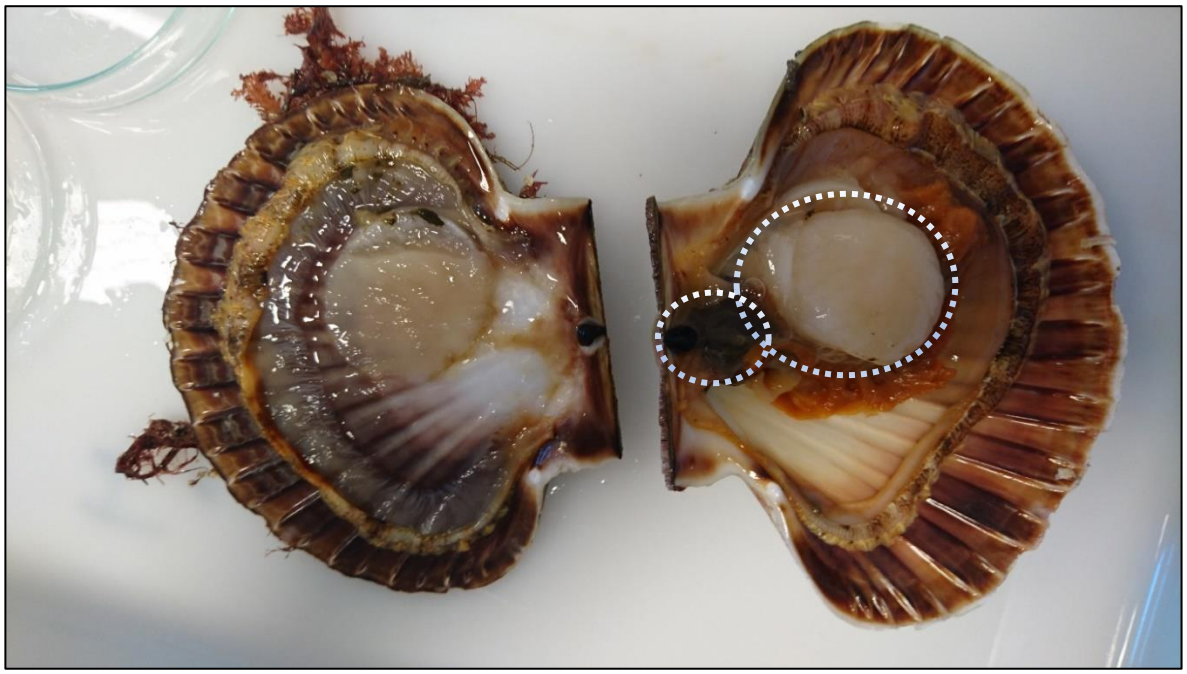

Figure 2.2-1: Newly opened scallop. Circles indicating location of abductor muscle (right circle), and stomach/hepatopancreas (left circle). Photo: Ådne Messel Nafstad

Figure 2.2-2: Left: Digestion of stomach tissue, early phase. Right: Density separation of digested muscle tissue using separation flasks. Picture taken after 3:30 hours of sedimentation. Photo: Ådne Messel Nafstad

2.2.2. Density separation and filtration

After tissue degradation, the density of the solution was increased by adding solid salt. Two salts were tested on the muscle samples; NaCl (1000 g, 58.44 g/mole, mineral origin, Jozo) and $ZnCl_2$ (250 g, 136.3 g/mole, AnalaR NORMAPUR ACS). NaCl is cheap and easily available, and the waste constitutes no health or environmental hazard. Saturated solution of NaCl makes approximately 1.2 g/mL, which is a higher density than many plastic types found in the marine environment and makes this salt suitable for the density separation (Masura et al., 2015). However, some plastic types are heavier than 1.2 g/mL. Maes et al. (2017) suggest using $ZnCl_2$ to reach a density of 1.35 g/mL, by adding $ZnCl_2$ corresponding to approximately 35% of the sample solution mass. This allows to increase the range of plastic types that are retrievable. On the other hand, it is more expensive, and handling- and waste constitutes health- and environmental hazards. For stomach- and sediment samples only NaCl was used, both salts were tested on muscle samples to test if the increased density of ZnCl2 give a different result. The solution was then transferred to separation flasks for density separation and left for

sedimentation for minimum three hours (Figure 2.1-2, right). After sedimentation, the precipitate was discarded.

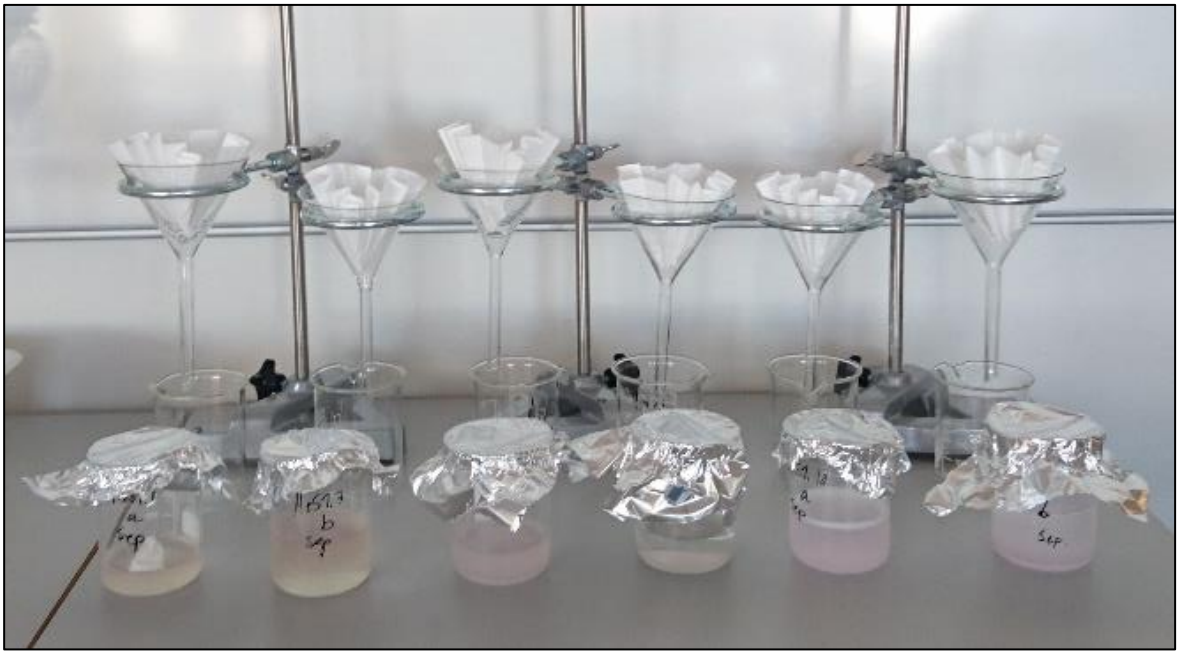

Figure 2.2-3: Filtration setup. Photo: Ådne Messel Nafstad

Three subsamples were taken from the sediment samples from each station. Separation flasks are not usable for sediments, thus other techniques had to be done for density separation based on recommendations from Maes et al. (2017). Density separation was performed by adding saturated NaCl (sat. aq) and additional solid salt to saturate the sample solution, followed by stirring for at least 3 minutes to allow plastic particles to float through the sediments. Then samples were left for sedimentation in the glass beakers overnight. After this first sedimentation, the supernatant was poured into a new beaker leaving the sediments. After this initial separation the procedure was repeated on the remaining fraction, this time left for sedimentation for 3 hours. After the second sedimentation the remaining sediments were discarded. The supernatant fraction was then separated using separation flasks to remove any unwanted sediments in the supernatant.

After density separation the supernatant was stained by Nile Red (Acros Organics). In addition to being recommended by Maes et al. (2017), the needed light source and filters were available for this study. Muscles, sediment sample and a subsample of the stomach samples were stained by 0.10 mg/L as recommended by Maes et al. (2017). Preliminary results from tissue samples indicated this concentration was too high for these samples, and remaining stomach samples

were stained using 0.03 mg/L. The stain was added about 60 minutes before filtration. Filtration was performed in Whatman 595 ½ paper folding filters (pore size 4-7 µm, Figure 2.2-3). These filters were chosen based on price and practical usability, although nitrate cellulose filter and glass membrane filters are recommended for this purpose (Maes et al., 2017, Kolandhasamy et al., 2018).

2.2.3. Excitation and visual analysis

The filters were illuminated using a Nightsea SFA light head (blue light, wavelength 440-460 nm) and analysed using a Motic smz-143 stereo microscope with w10x/20 ocular fitted with light barrier filter (orange, 500 nm). The light excites NR, which is seen as red-orange glowing colour when observed through the light filter. Excited particles distinguishable from background light noise were counted through the microscope. Particles were classified according to their visual appearance as either "plastic fibres" or "other shapes/particulate plastic".

2.2.4. Control samples

Multiple control samples were made; to identify possible sources of contamination, samples of pure tap water, saturated solutions of $ZnCl_2$ and NaCl separately was tested parallel to digestion samples. Additionally, sediment samples (precleaned of plastic by density separation process) and stomach samples were spiked with stained plastic particles (vinyl and High-Density Polyethylene (HDPE)) to determine the recovery rate using this method:

5 stomach samples were spiked with a known number of particles of HDPE, a common plastic material with a broad range of usage. The particles were irregularly shaped, made by slicing small pieces of a piece of HDPE. The size of spiked particles ranged around 0.5-1 mm. Smaller particles were unmanageable with the available tools. Particles were added to the digestion solution and normal tissue sample protocol was followed.

Sediment spiking samples were made by cleaning discarded sediments taken from all three stations HeS1, KaS1 and InS1. Cleaning was performed by the same density separation protocol as used in the sediment samples. After cleaning, a known number of pre-stained HDPE particles and flakes of vinyl was added into the sediments using a glass pipette, ensuring the spiked plastic was buried in the sediments. After spiking, samples followed normal sediment protocol,

except not further stained to avoid staining potential un-stained particles present in the cleaned sediment samples.

2.2.5. Verification of plastic particles

To verify that the method detects plastic, and not detecting non-plastic particles, a sample of particles found through this method needs to be analysed to determine plastic type or other content. Such analyses would have been performed at an external laboratory, preferably by FT-IR spectroscopy (Fourier-Transform infrared spectroscopy, Martin Wagner, pers. comm.). Due to the low success of this study and few particles successfully isolated and detected, this step was not performed.

2.2.6. Statistical analyses

It is possible, perhaps likely to find plastic in blank controls as well as in the tissue samples. To test whether the detected plastic particles from samples is due to the process, one can use statistical methods. In a paired sample study with one control sample and one tissue or sediment sample, this can be done by a one-sided t-test. Due to the low success this was not performed in this study.

Alternatively, one can perform GLM model with Poisson error structure (as plastic particles are count data, which is likely to follow a Poisson distribution. A negative binomial error structure could be selected if the Poisson distribution produces heteroscedastic residuals) with number of plastic particles as a function of age, to test whether older individuals have higher content of plastic particles due to bioaccumulation. If the dataset has a nested structure, such as sampling from different depths at different locations or similar, one should consider using mixed effect models.

2.3. Results

2.3.1. Tissue samples

Muscle samples were not digested completely and there were several practical challenges by digesting tissue by using 35% hydrogen peroxide. Heavy foaming initially led to loss of parts of some samples (Figure 2.3-1, left). Foam mostly decreased with time, but often there were remnants of the foam on the glass walls which was hard to get off and bring to the next step. Muscle samples were not digested fully after four days. After about two weeks they appeared visually to be fully digested and there was no visual change the rest of the five-month digestion of these samples. Filters from muscle samples showed a layer of fine undigested material fluorescing when enlightened (Figure 2.3-1, right). Furthermore, filters themselves were fluorescent, being stained by NR. No particles were distinguishable from this background fluorescence. Stomach samples appeared fully digested after about 70 hours.

Figure 2.3-1: Left: Formation of foam during digestion of muscle tissue. Right: Typical view of filters from muscle samples through light barrier filter, no microscope magnification. Photo: Ådne Messel Nafstad

Little undigested material was visible on the filters, although some particles appeared dark under light barrier filter (Figure 2.3-2, left). As filters from muscle samples - the filters from stomach samples stained by 10 mg/L NR showed strong background fluorescence whilst samples stained by 0.03 mg/L NR showed less. No distinct particles were distinguishable in either subsample. Beakers and equipment used for tissue analyses all showed an oily substance

adhering to the glass wall which did not wash off with water and was therefore lost before filtering. This substance dissolved in acetone, suggesting lipid content. Mean recovery rate of HDPE for spiked stomach samples was 0.42 (SD = 0.14), ranging between 0.3 and 0.6.

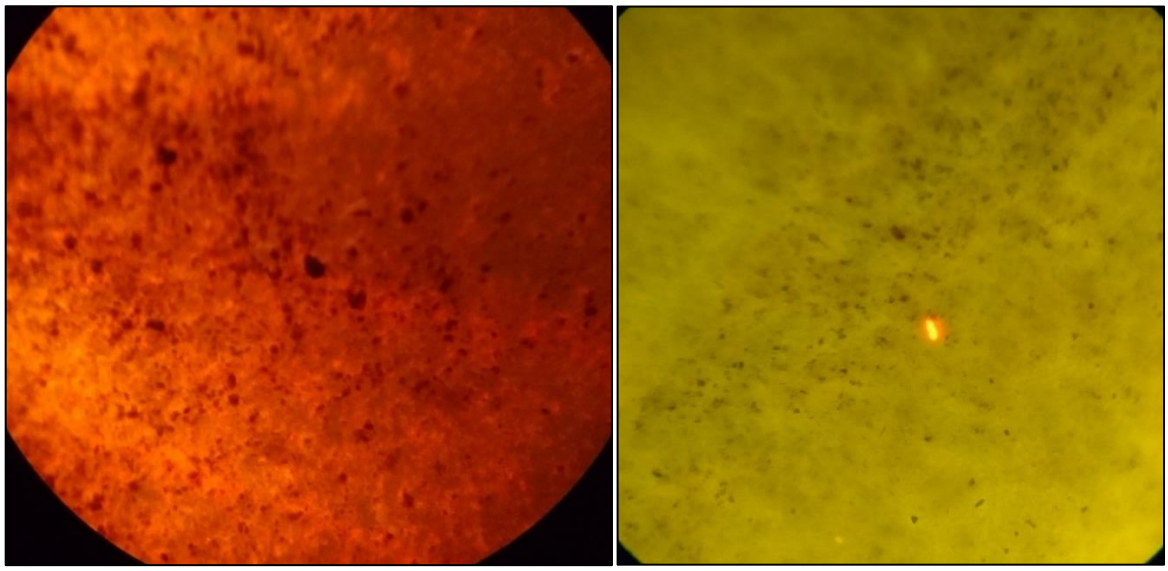

Figure 2.3-2: Left: Typical view through microscope, stomach samples; filters showing background fluorescence, and some particulate materiel not stained by NR appear dark. Right: Small plastic particle on filter from KaS1.S1B as seen through microscope during excitation. Photo: Ådne Messel Nafstad

2.3.2. Sediment samples

Sediment samples from the three stations appeared to be relatively similar in structure and fragment size, being composed of shell fragments, sand and gravel up to about 5 mm diameter - although InS1 appeared to have a higher content of finer particles. Filters with sediment samples showed little background fluorescence, allowing for accessible counting of stained particles (Figure 2.3-2, right). Numbers of particles per gram wet weight were similar at HeS1 and KaS1, but InS1 differed in composition of particles (Figure 2.3-3). There were several plastic fibres present in the InS1 samples (Figure 2.3-4), whilst almost absent in the others. Particles of other shapes were small (<0.5mm) and showed irregular-round shape (e.g. Figure 2.3-2, right). Mean recovery rate of HDPE and vinyl for sediment samples were 0.42 (SD = 0.025) and 0.67 (SD = 0.064) respectively. During density separation of sediment spiking samples, a few of the spiked particles of HDPE were observed on the surface of the sediments.

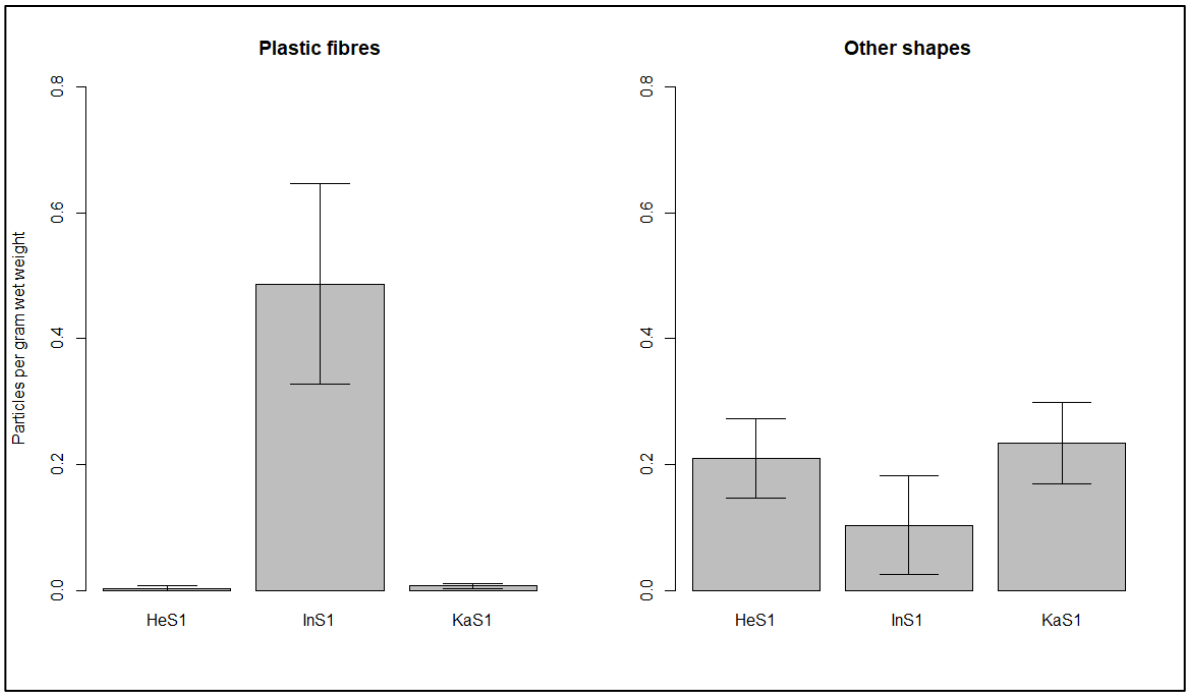

Figure 2.3-3: Mean number (± 1 SE) of plastic particles of the two shape classifications per gram (wet weight) of sediments in sediment samples.

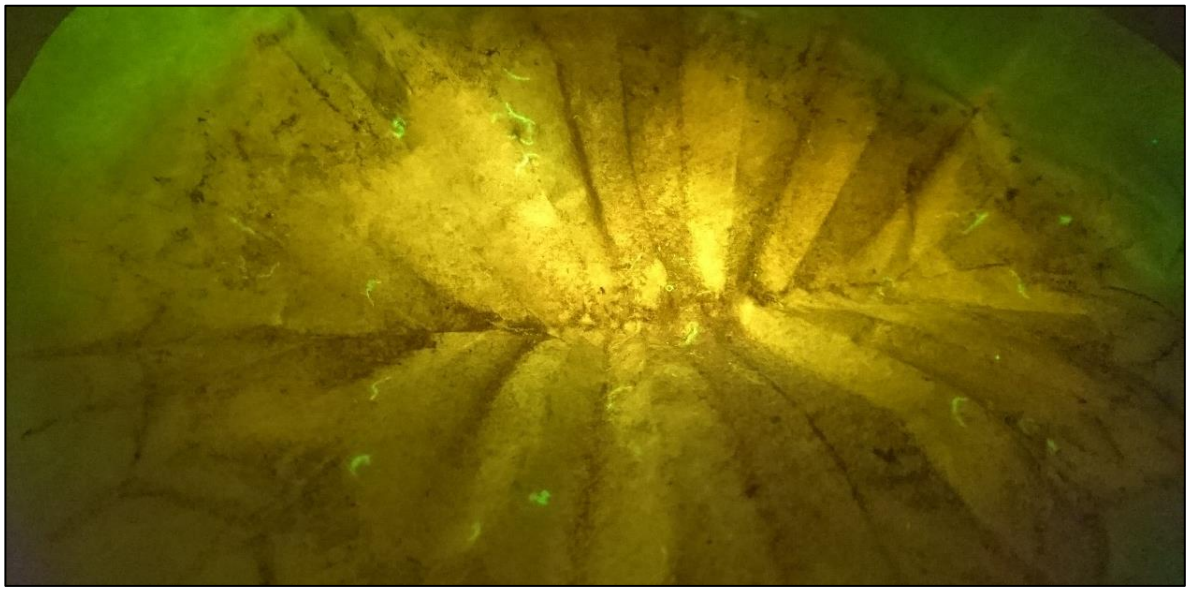

Figure 2.3-4: Filter from sediment sample InS1.S1A during excitation. Plastic fibres seen here as green illuminating lines. View through microscope revealed red fluorescence from fibres, which were partly covered in material emitting green fluorescence when enlightened with the blue light. Photo: Ådne Messel Nafstad

2.3.3. Control samples

Control samples showed content of some small fragment of micro plastic (table 3.3.1). It does not appear to be a clear pattern to which factor that has the most influence on the contamination of samples.

Table 2.3-1: Detected plastic content of control samples. Mass refers to content of solid salt in sample except in control.aq1 and -2, in which it refers to water content.

ID	Mass (g)	Plastic fibres	Other fragments	Number of fragments per gram
control.nacl	28.31	0	2	0.071
control.nacl	24.18	1	0	0.041
control.nacl	27.26	0	1	0.037
control.zncl2	44.73	0	2	0.045
control.zncl2	34.24	0	6	0.175
control.zncl2	85.95	0	1	0.012
control.aq1	218.3	1	0	0.005
control.aq2	203.49	0	2	0.010

2.4. Discussion

The adopted method proved to have low suitability for analysing biological tissue as these samples were largely unreadable due to the background fluorescence from the filters and remaining biogenic material. Based on the observations made during processing, the problems are likely to be due to an insufficient digestion process. Muscle tissue did not degrade fully even when digested for several months, and it appeared that much of the lipid content did not degrade fully, making a high content of lipids in the final solution for filtering. The NR stain is commonly used for lipid staining, hence contributing heavily to the background light noise on the filters (Fowler and Greenspan, 1985, Greenspan et al., 1985). The digestion process also led to potential loss of material through the foaming during initiation of digestion. Furthermore, the spiked tissue samples had a low recovery rate even though the spiked particles most likely were bigger than one should expect that scallops possibly consume. The loss of spiked particles can be associated with the digestion process, adhering to lipids on the equipment, or during density separation which is supported by observation during sedimentation of sediment samples as plastic particles were observed on the sediment surface thus being discarded along with the rest of the sediments. It can also be due to low visibility and distinguishability against the background fluorescence during visual analysis. Additionally, the spiked particles were relatively big compared to the food particle size for scallops, and recovery rate for the smaller particles might differ from the rate found here. Hence, a digestion agent that also digests lipids as well as produce less foam would be preferable, especially when using NR to reduce background fluorescence on the filters. Other agents have support in literature, such as HNO_3 and KOH (Lusher et al., 2017b, Vandermeersch et al., 2015, Lusher et al., 2017a). KOH was recommended for monitoring programmes of plastics in *Mytilus* spp. along the Norwegian coast, as well as having a high potential for digesting lipids as it is a strong base (Lusher et al., 2017a). Different concentrations of NR were tested, and it appeared that background fluorescence in tissue samples was lower with 0.03 mg/mL of NR, although plastic particles might also have less fluorescence at this concentration. 0.10 mg/mL proved suitable for sediment samples, as was also found by Maes et al. (2017).

The method proved more usable for analysing sediment samples, although not surprising as these analyses were very similar to those tested and recommended by Maes et al. (2017). The results from this study suggest plastic is present in the sediments at the sampling stations. However, statistical tests were not performed and the number of plastic particles per gram salt is not comparable as this to the number of plastic particles per wet weight of sediments. It

appears that there is a difference in number of plastic particles per gram between the controls and the samples, but it cannot be concluded on this basis. The difference in plastic content between stations suggest there is plastic in the samples as the plastic introduced through the analysis process is expected to be similar in all samples. These differences can either be differences in sources of plastic; InS1 is located close to a fish farm, and fish farms are likely to release high amounts of plastic particles from wear of equipment (Christensen, 2017). The similar structure of plastic particle composition in KaS1 and HeS1 contrasts with the structure of plastic particles in the InS1 sample. However, an important contribution of such plastic pollution is the wear of the tubes transporting feed to cages. Such wear of the tubes is rather expected to produce particulate plastic fragments than fibres, which is the dominating in the InS1 sample – and content of particulate plastic is rather lower in the InS1. Thus, it is more reasonable to believe the structural differences in plastic content has other origins, such as water current regime. This latter hypothesis is supported by the observation of higher content of finer particles and silt in the InS1 than the other stations, indicating sedimentation of smaller and lighter particles here. Additionally, the most common plastic type in such tubes is polyethylene, which has a density of 0.88-0.96 g/mL and will therefor most likely not reach the benthos but stay in the surface waters. The spiked samples showed low recovery rates compared to Maes et al. (2017), probably partly due to lower density of the solution in this study, using NaCl instead of the $ZnCl_2$ in their study. The observed content in this study is likely to be an underestimate of the true content. The observations of the spiked HDPE-particles during density separation of sediment samples indicate that the density separation using NaCl is not sufficient for this type of plastic and is likely to be an important reason for low recovery rate. Unexpectedly, even within samples plastic particles cut from the same piece of plastic showed different density as some precipitated to the sediments and some particles floated up to the surface. This indicates that the plastic either has a varying content of additives between surface or similar or the original plastic piece was not as homogenous as it appeared. In any case one should consider alternatives to NaCl for density separation to increase the recovery rate.

Plastic content in the blank control samples was lower than that of the sediment samples. Furthermore, plastic content in NaCl, $ZnCl_2$ and pure tap water did not differ either qualitatively nor quantitatively (table 2.3-1). Tap water was not filtered prior to the addition of the solid salts for the salt controls, thus the salt controls are effectively controls of the additive contribution of tap water and salt during the process. Even though not statistically tested and assuming the particles detected are truly plastic, this indicates plastic particles are present in the habitat of *P.*

maximus. As no data was collected on plastic content in samples from natural populations of scallops, it cannot be included as a factor in the models for growth parameters and the effect of plastic on growth of scallops is still unknown.

As verification analysis of the particles was not performed, it is not possible to conclude strongly on whether this method is prone to false detections. Considering NR stains lipids and plastics, the detected particles contain either of those. Hence, detected particles might be organic material, such as shell- or exoskeleton fragments in the sediment samples. Although a benefit of the use of NaCl is that such particles are not likely to float and is not detected as plastic (Maes et al., 2017). To be sure, one must perform verification analysis. An alternative method to the relatively expensive and logistically more challenging external lab analysis is to freeze dry or oven dry the samples. Non-plastic, typically biogenic material, will in most cases easily wither through this process, while plastic will not (Lusher et al., 2017b). Hence, this technique can help verify plastic or non-plastic but not plastic type. It is also cheap and usable at most labs, and therefor practical for plastic analysis at high school education programmes. However, it is not a very good method on small particles, as determining if they wither is probably challenging. Also, it only verifies plastic from non-plastic.

The methods and materials used here to adopt a method to assess plastic content in marine invertebrates are highly influenced by the changing aims of the study as the work progressed. If the aim was all along to adopt such method, the priorities would have been different. For one, only stomachs should have been assessed. This is where it is most likely to find naturally occurring plastic particles, and they are easier to digest chemically. Analysing muscle samples was chosen as muscle tissue is the main commercially utilized and consumed part of this species and detecting plastics here would probably have received attention and contributed to awareness of the problem of plastic pollution of the oceans as well as demonstrating that humans are exposed to this pollution. In retrospect this is clearly a choice which was not realistically feasible, as the method needed development. Next, there should have been more spiking samples to get more secure estimates of recovery rates. A wider range of plastic types should have been present in the spiking samples, as well as spiking tests for both salts in both tissue samples and sediment samples. However, due to time limitations and limited availability of $ZnCl_2$, the final priorities were as described in the method section. Also, other digestive agents should have been tested, instead of assuming it would work just because H_2O_2 has support in literature.

2.5. Conclusions and recommendations

No plastic was detected in *P. maximus*. However, this does not demonstrate absence as it is impossible to conclude due to the strong limitations of the method, making the tissue samples unreadable. It appears to be plastic in the sediment samples as the composition of plastic particles differed, however the fragments found has not been verified as plastic by chemical content analysis. Using hydrogen peroxide as digestive agent proved insufficient, leaving lipids and biogenic material in the solution. For assessing plastic content in invertebrates, it is suggested to use other digestive agents such as KOH or HNO_3, which has not been tested here but both have support in other studies. It is not possible based on this study to conclude on which salt is the best for the density separation, $ZnCl_2$ has the advantage of producing the recommended density of the solution (1.35 g/mL), increasing the recovery rate of plastic particles and the range of retrievable plastic types. However more safety precaution is required when using this salt. NaCl require less safety precautions and is much cheaper. For plastic content assessment for educational purposes at high school, NaCl might be preferred even though the density reaches a maximum of only 1.2 g/mL. Nile Red proved promising as a tool to increase visibility of plastic particles, but it requires full digestion of biogenic content and lipids. Testing different concentrations in the range of 0.03 to 0.10 mg/mL to determine the best compromise between fluorescence of plastic particles and background fluorescence is recommended. The Whatman 595 ½ paper folding filters appeared well suited for filtration of these samples, although no comparisons were made towards other potential filters. Other filters such as glass membrane filters and nitrate cellulose filters are recommended in literature, however many such filters are expensive. Metal sieve can probably also be used for bigger particles. Verification of plastic composition is necessary to be able to conclude whether the method is qualitatively accurate, and when performing this analysis to determine plastic content in sediments and organisms, this needs to be performed to be able to conclude on potential presence of micro plastics.

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Appendix 1: External data

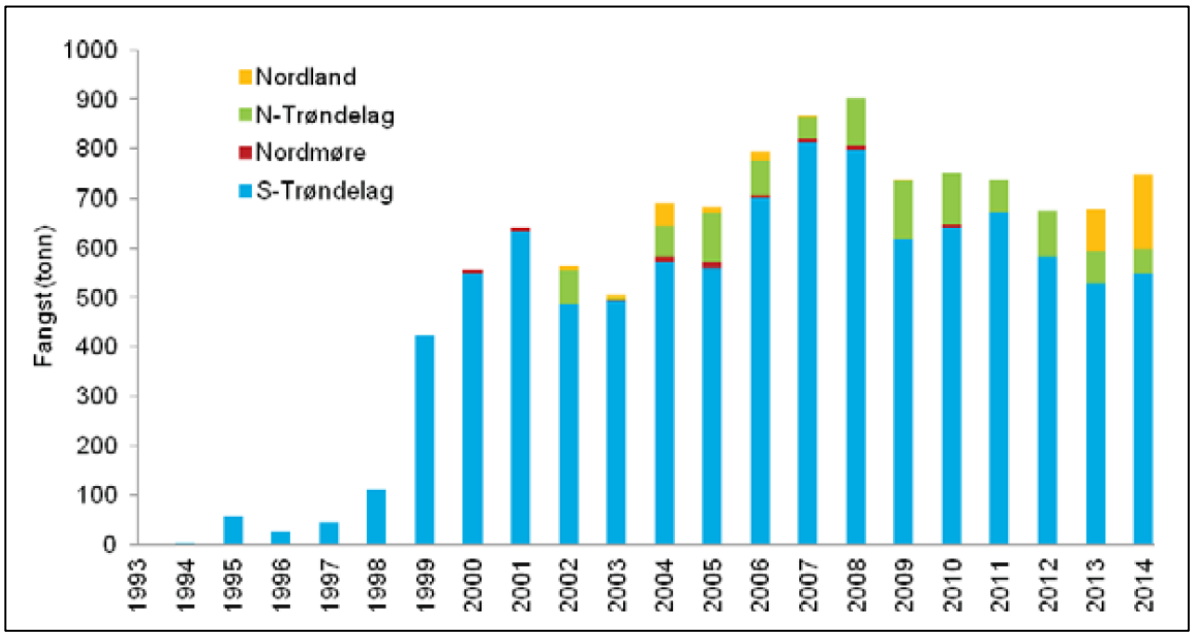

Figure A1.1: Development of commercial harvest of P. maximus in Norway. Blue bars indicate Sør-Trøndelag County (prior to merging Sør and Nord-Trøndelag), in which Frøya is an important part of the harvest grounds. From Strand et al. (2016)

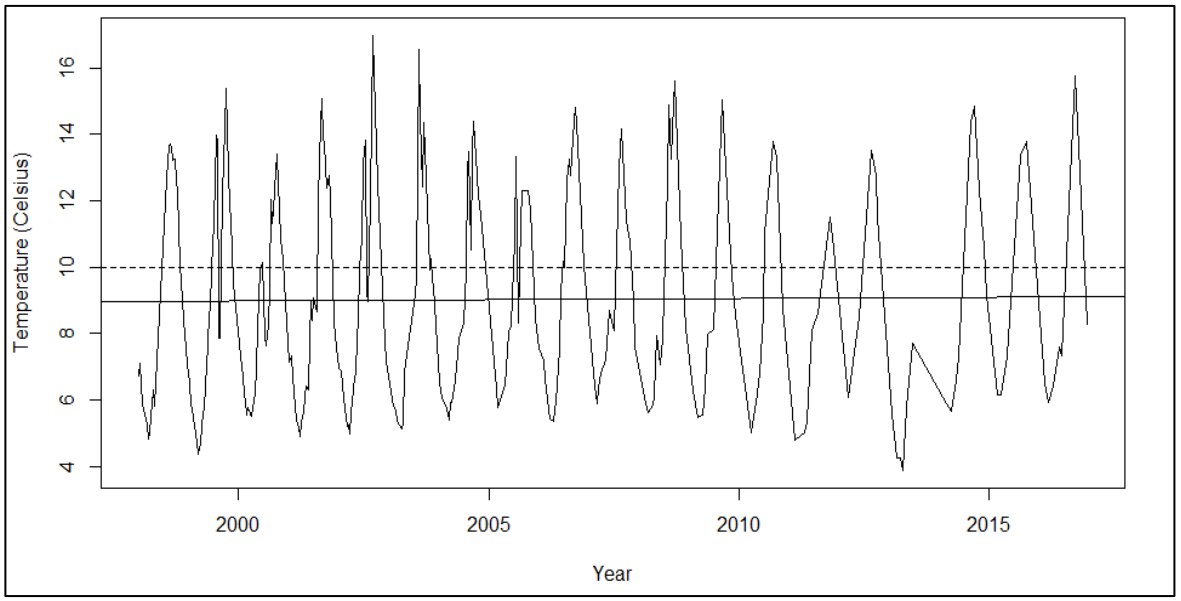

Figure A1.2: Temperature at Bud, Nordmøre between 1998 and 2017. Dotted line indicates 10°C, above which scallops grow. Solid line is the fitted linear increase with time. Data: Norwegian Institute of Marine Research (2018)

Appendix 2: Data analysis and model verification

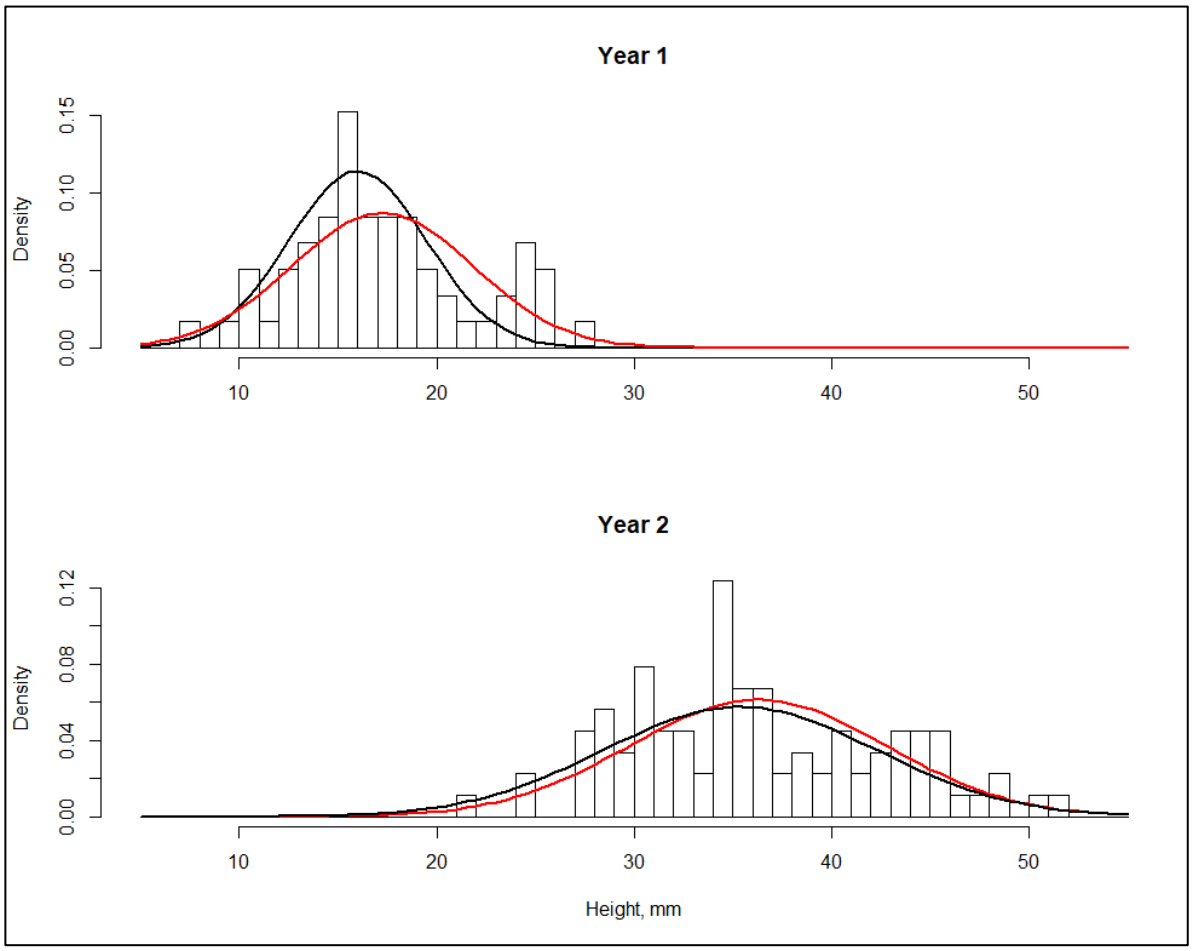

Figure A2.1: Distribution of shell heights at age 1 and 2. Red lines are the distribution of data including the likely misidentifications, black curve illustrates the distribution using 24 mm as separating height between year class 1 and 2.

Table A2.1: summary of vital data from the selected models from the AIC model selection. ±SE in parentheses. P-values; «.»<0.1, «»<0.05, «**»<0.01, «***»<0.001.*

Response	Model	Intercept (if no AQ)	effect of BY	Intercept (AQ exposed)	Intercept (AQ non-exposed)	P-value, AQ	R^2	Removed outliers
Ø'	GLM	4.52 (±0.039)***	0.04314 (±0.012)***	-	-	-	0.126	No (n=87)
Ø'	LME	-	0.016 (±0.0054)**	4.614 (±0.0961)	4.519 (±0.067)	0.202	0.514	Yes (n=85)
Ø'	LME	4.53 (±0.069)***	0.019 (±0.0047)***	-	-	-	0.520	Yes (n=85)
G'_{max}	GLM	-	0.87 (±0.12)***	24.59 (±1.59)	21.22 (±0.36)	0.044*	0.501	No (n=87)
H∞(VB)	GLM	137.39 (±10.10)***	5.41 (±3.162, p= 0.0907) .	-	-	-	0.036	No (n=87)
H∞(VB)	LME	-	0.73 (±00.55, p=0.18)	139.92 (±8.45)	127.66 (±4.65)	0.111	0.309	Yes (n=85)
H∞(VB)	LME	128.51 (±4.69, p=0.0303)*	1.144 (±0.490, p=0.022)*	-	-	-	0.292	Yes (n=85)
H∞(VB)	LME	-	-	144.6 (±8.03)	127.7 (±5.18)	0.0141*	0.339	Yes (n=85)
H∞(G)	LME	115.85 (±4.04)***	-0.66 (±0.302, p=0.031)*	-	-	-	0.419	No (n=87)
T_{VB90}	Grand mean	3.31 (±0.66)	-	-	-	-	-	Yes (n=85)
T_{G90}	GLM	3.06 (±0.0.05)***	-0.056 (±0.015)***	-	-	-	0.135	No (n=87)

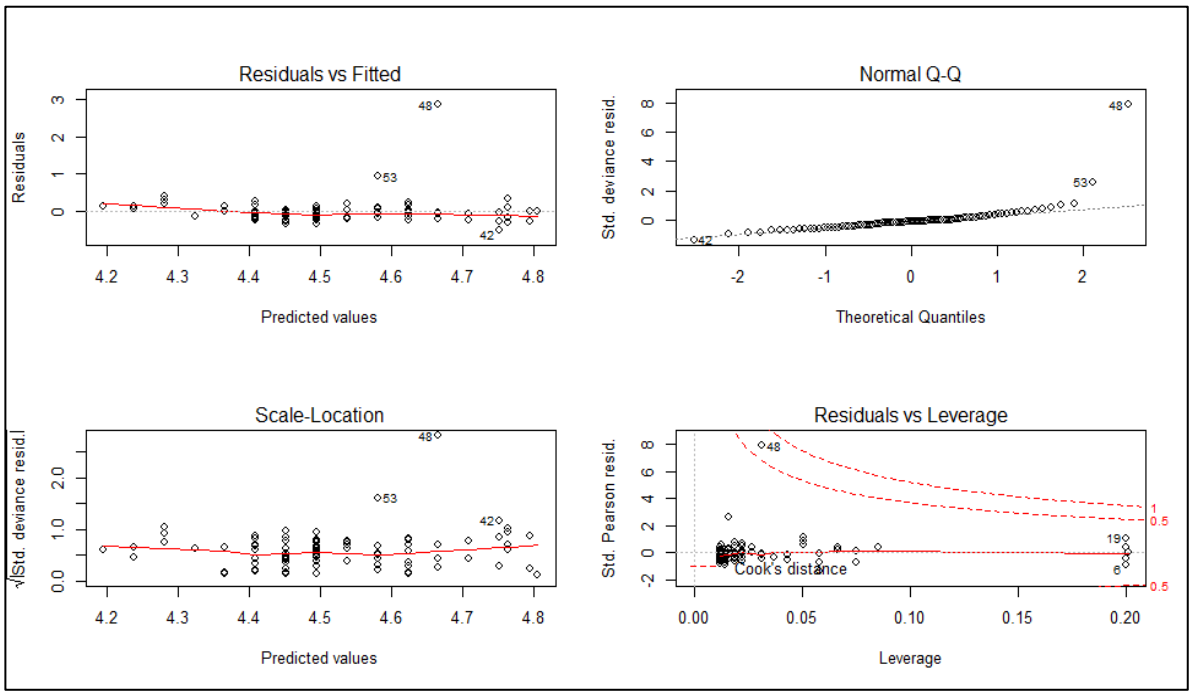

Figure A1.2: model check for Ø': `plot(glm(Ø~Yearborn+factor(Aq.exposure), family=gaussian)`, two datapoints appear to be outliers (n=87).

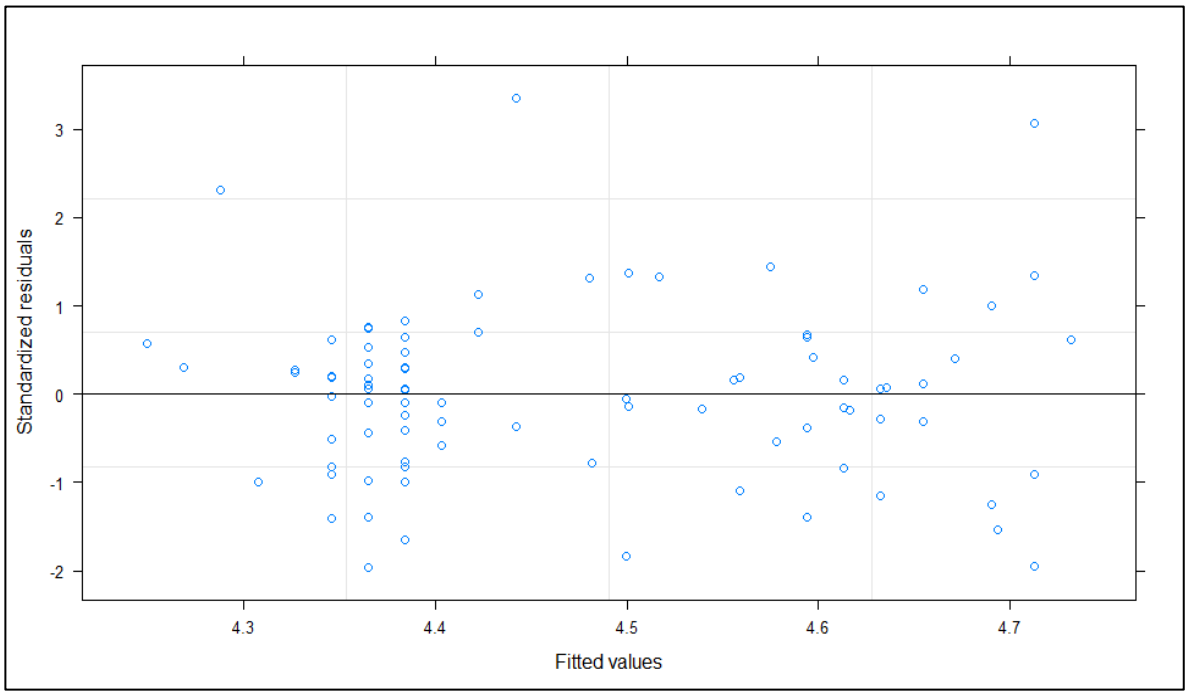

Figure A2.3: Model check of the selected LME for Ø': `plot(lme(Ø ~ Yearborn,random=~1|Station, method="REML",na.action=na.omit))`, *without the outliers (n=85).*

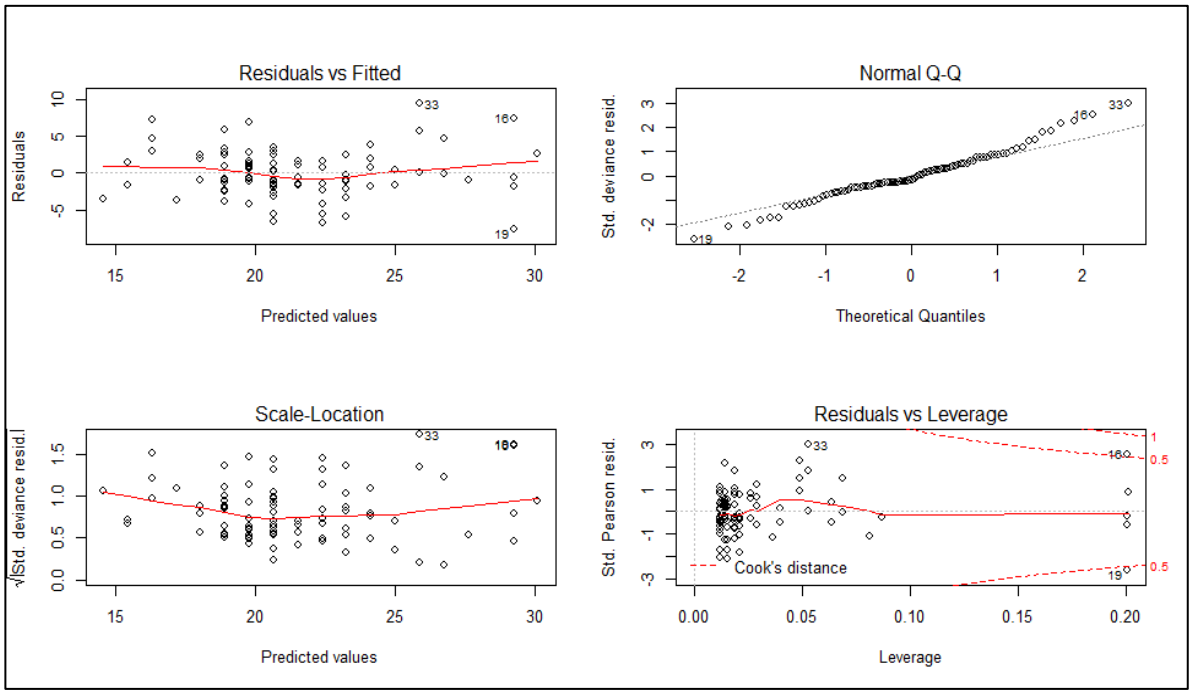

Figure A2.4: Model check of G'max: `plot(glm(Gmax ~ Yearborn +factor(Aq.exposure),family=gaussian))`, *n=87.*

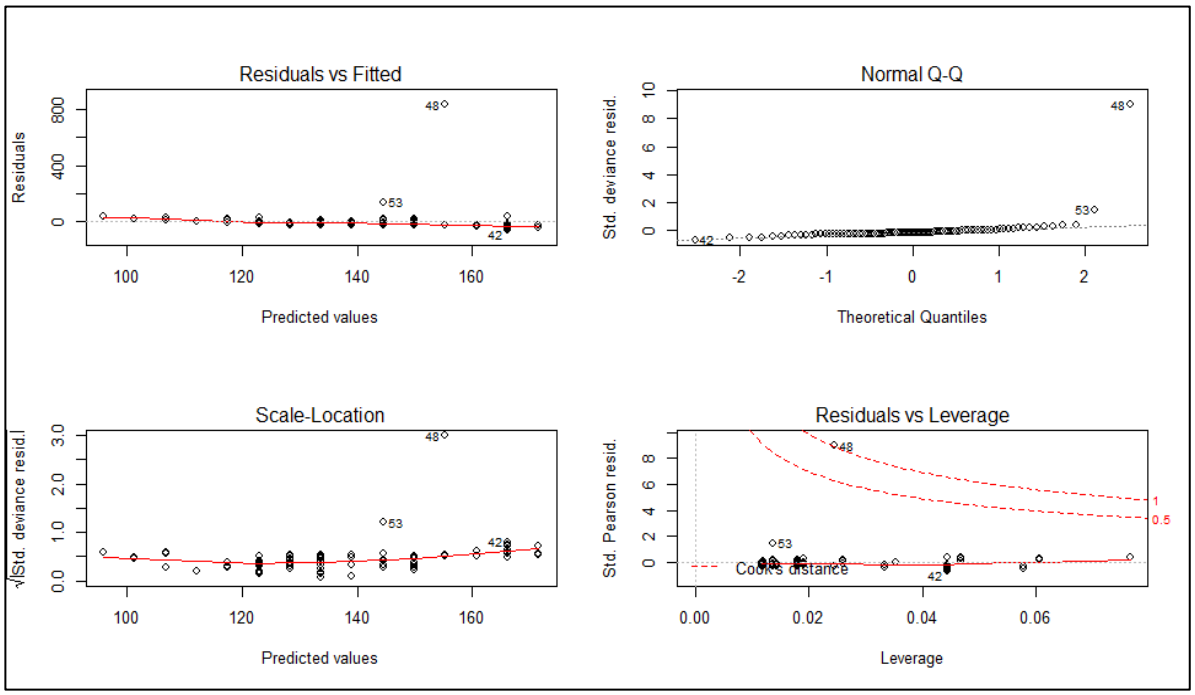

Figure A2.5: Model check for H∞(VB): `plot(glm(a.VB ~ Yearborn+factor(Aq.exposure),family=gaussian,data=table))`, *n=87 (including outliers).*

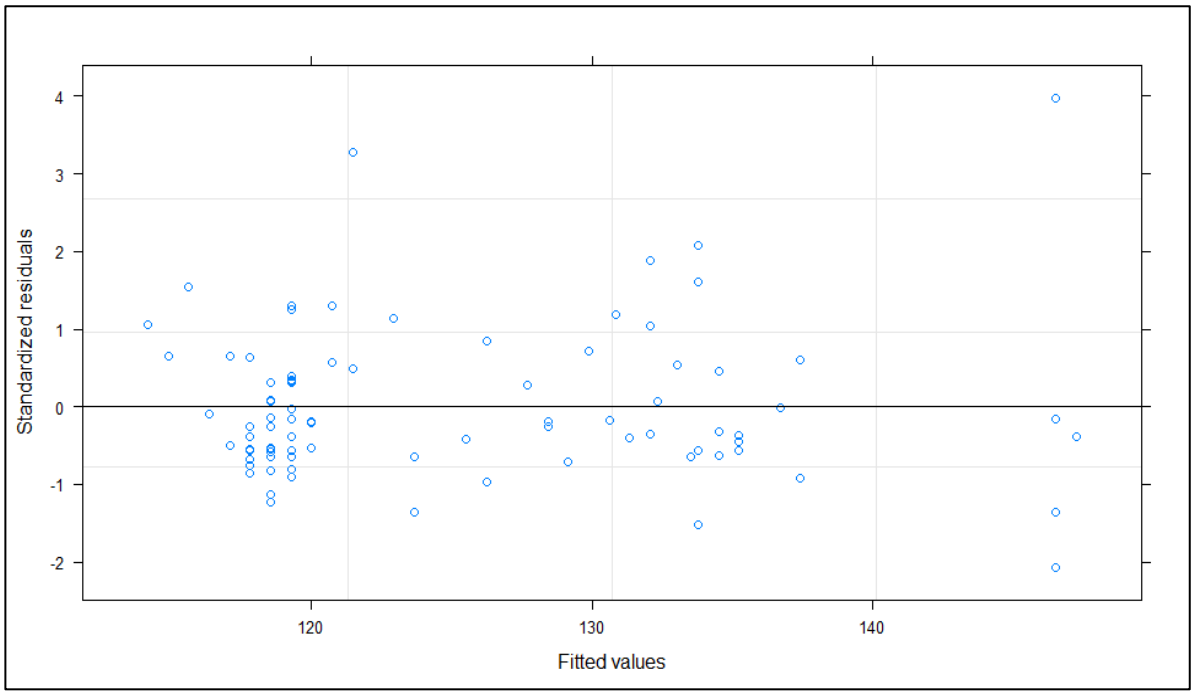

Figure A2.6: Model check for H∞(VB): `plot(lme(a.VB ~ Yearborn+factor(Aq.exposure),random=~1|Station,method="REML", na.action=na.omit))`, *without outliers (n=85).*

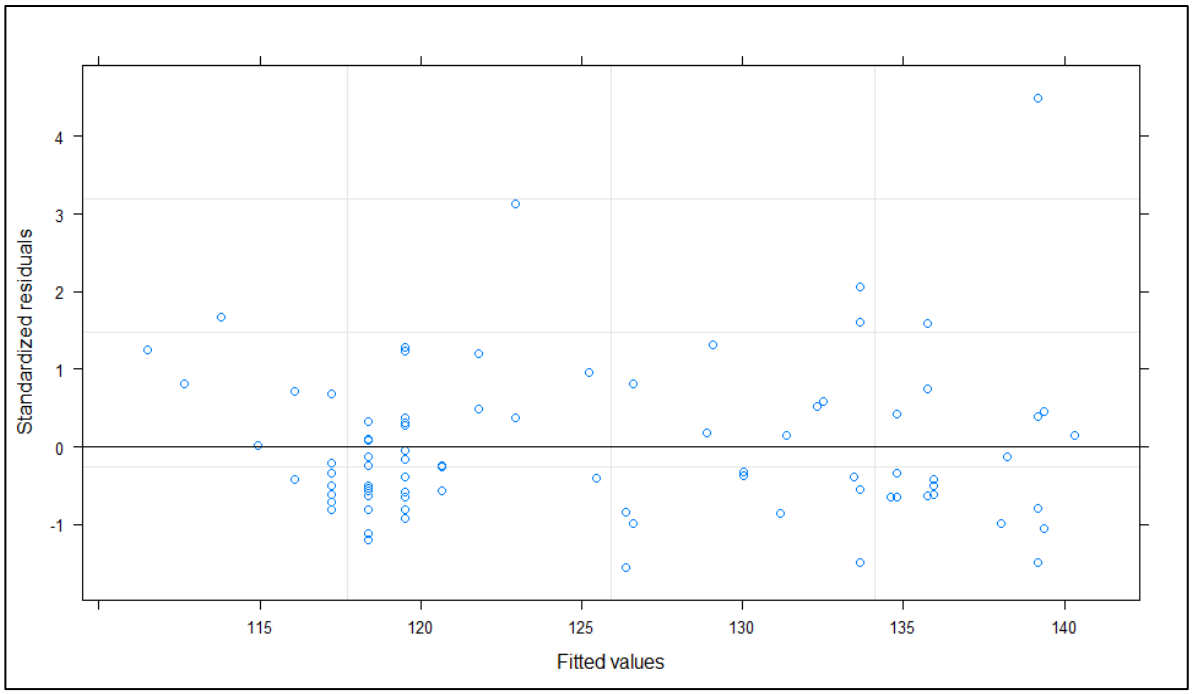

Figure A2.7: Model check for H∞(VB): `plot(lme(a.VB ~ Yearborn,random=~1|Station, method="REML",na.action=na.omit))`, *without outliers (n=85).*

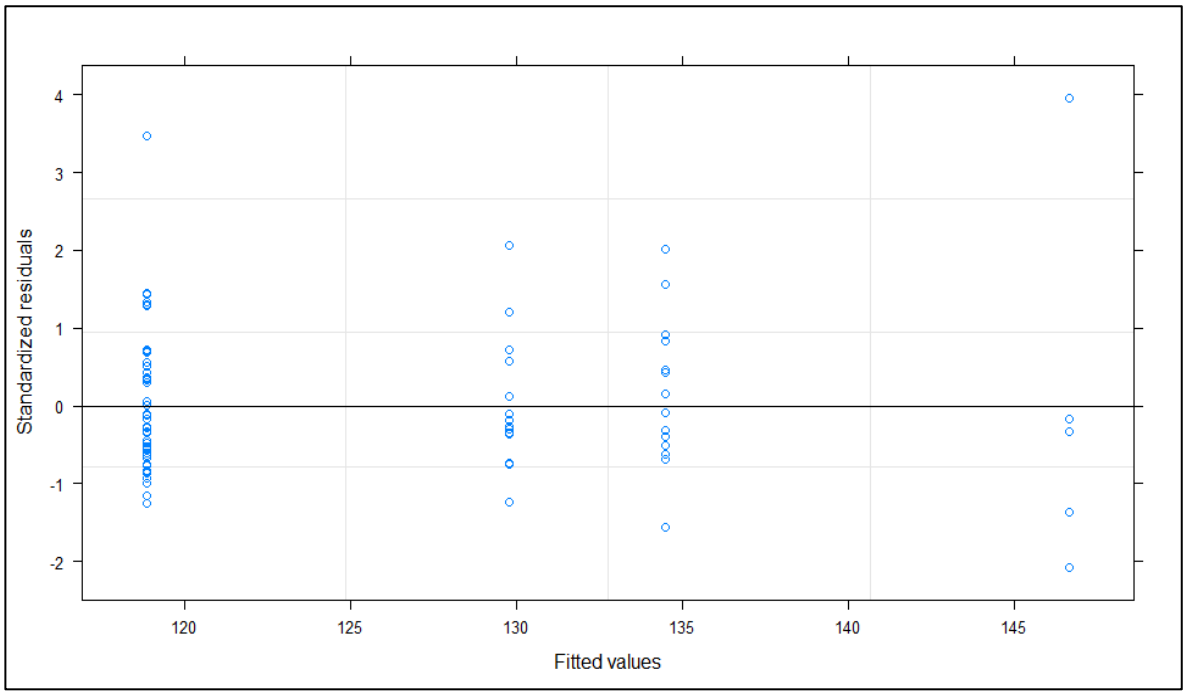

Figure A2.8: Model check for H∞(VB): `plot(lme(a.VB ~ 1+factor(Aq.exposure),random=~1|Station,method="REML",na.action=na.omit))`, *without outliers (n=85).*

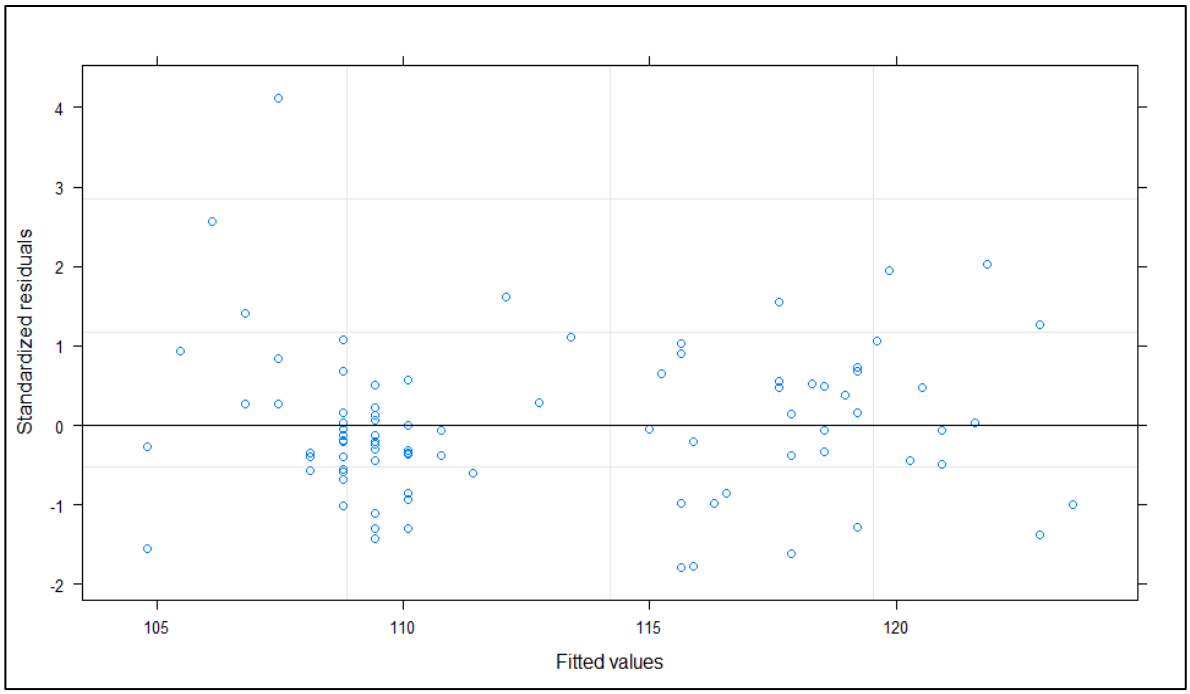

Figure A2.9: Model check for H∞(G): `plot(lme(a.G ~ Yearborn,random=~1|Station, method="REML",na.action=na.omit))`, *(n=87).*

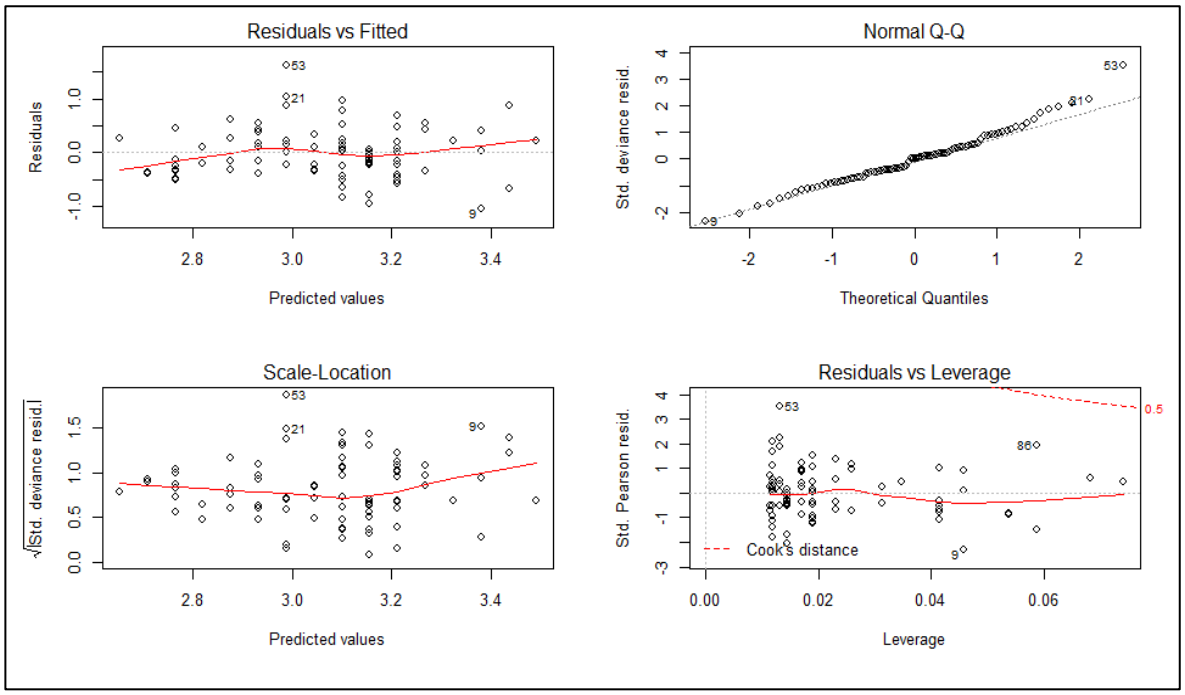

Figure A2.10: Model check for T_{G50}: `plot(glm(T50.G ~ Yearborn,family=gaussian))`, *n=87.*