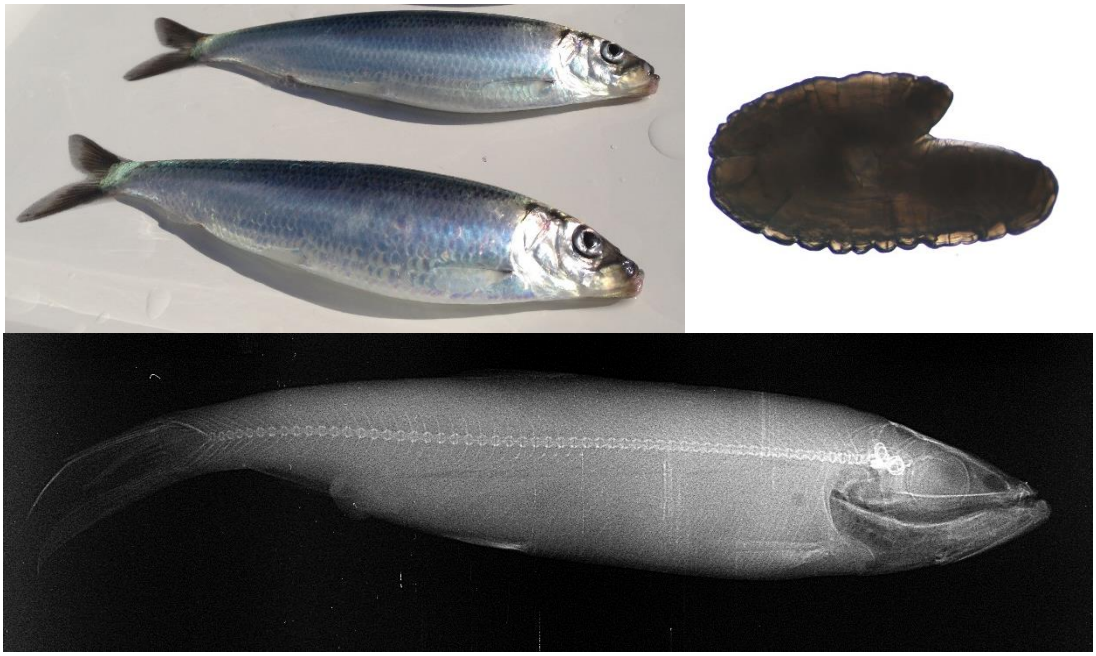
Master of science in Marine Biology

Phenotypic plasticity in Atlantic herring (*Clupea harengus*) juveniles reared at two different salinities



Oda W. Almeland

Department of Biology
University of Bergen

June 2015

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ACKNOWLEDGEMENTS

Foremost, I would like to express my gratitude to my supervisor, Prof. Arild Folkvord at the University of Bergen, for support and guidance. He came up with the original idea for the project, and he offered good advices, great enthusiasm and feedback during the work with this thesis.

I would like to thank Aril Slotte at the Institute of Marine Research Bergen for giving me advice and feedback on my thesis. Prof. Audrey Geffen at the University of Bergen for introducing me to otolith photography and otolith chemical analysis.

I would also like to thank Prof. Leif Andersson at Uppsala University, Sweden, for financial support.

I want to thank Jan de Lange from the Institute of Marine Research Bergen, who taught me how to count vertebrae in herring, and assisted me during the dissection of the parental fish groups.

Thanks to the research technicians, Christel Krossøy, Julie Skadal, Heikki Salvolainen and Frank Midtøy from the University of Bergen (Department of Biology), and Siv Hjorth Dundas from the University of Bergen (Department of Geology) for guidance and assistance.

At last, I want to thank; My good friends Marianne and Magnus who proofread my thesis, encouraged me and gave me advice. My fellow students Sylvelin, Lisa, Rikke, Kristine, Susanna and Kristoffer with whom I have shared many lunch breaks and good conversations. My flatmates Malin and Maria who have been a great moral support during the writing of this thesis. And my family for support and encouragement.

ABSTRACT

A common garden experiment with juvenile herring (*Clupea harengus* L.) was carried out to investigate the influence of salinity on different phenotypic traits commonly used to investigate population structure in this species. Local Atlantic spring spawning herring from western Norway was crossed with Baltic spring spawning herring to create hybrid offspring. Purebred Atlantic spring spawning offspring was also produced. The offspring of both crosses were randomly assigned and co-reared in tanks with two different salinities (16 and 35 psu). The fish were kept in the respective salinities from fertilization to final sampling 20 months later. Samples from both salinity groups were collected at day 187, 279 and 614 days post hatching (DPH). The mean (± SD) vertebrae count was higher in the purebred (56.4 ± 0.6) group than in the hybrid (55.9 ± 0.4) group, while it was not different between the two different salinities. Otolith shape developed over time from being more circular to being more oblong as the fish grew from 9 to 21 cm in total length. The otolith shape was more circular in the hybrid group, which indicate genetic effects on overall otolith shape. The ambient environment and genetic origin influenced the otolith chemistry. As an example, the otolith strontium levels (mg element weight / kg otolith weight) were higher in the 35 psu salinity, with a mean of 978 mg/kg vs 803 mg/kg in the 16 psu salinity. The purebreds within each salinity, also had a higher mean otolith strontium level of 997 mg/kg vs 876 mg/kg in the hybrids. The purebreds showed a lower survival than the hybrids, in the 16 psu salinity, before 187 DPH. The results of this study can be used to understand how the ambient salinity in the nursing areas affect the phenotype of herring and by this contributing to a better understanding of the complex population structure in herring stocks.

Keywords: Atlantic herring, *Clupea harengus*, herring, experimental study, common garden, salinity, phenotypic plasticity, phenotypic traits, vertebrae count, otolith, otolith chemistry, otolith shape, growth, length-at-age, otolith length, SGR, otolith weight, otolith size, population structure, population, strontium, ICP-MS, radiography.

CONTENTS

1 INTRODUCTION

1.1 ATLANTIC HERRING

Atlantic herring (*Clupea harengus*, Linnaeus 1758) is a small silver coloured pelagic teleost fish with a geographic distribution that ranges from both sides of the Atlantic Ocean, including the freshwater influenced Baltic Sea (Grant, 1984). Even though the herring in the Baltic Sea and the Atlantic Ocean are different in some aspects (e.g. size-at-age and genetics), herring in the two areas are considered to belong to the species, Atlantic herring (Lamichhaney et al., 2012). The salinity tolerance of Atlantic herring is wider than most other marine teleost species and ranges between approx. 4.5 to 42.5 practical salinity units (psu) (Blaxter and Holliday, 1962). Atlantic herring together with other clupeids are the most fished species globally. Norway have the highest yearly catches of Atlantic herring in the world, and accounts for approx. 35 % of the annual total catches of approx. 2 million tons (FAO, 2014). Even though the Atlantic herring is a well-studied fish species, a wide range of life history traits, phenotypic plasticity and a complex population structure makes this fish species an interesting study object for many years to come.

1.2 POPULATIONS AND POPULATION STRUCTURE

Atlantic herring spawns annually after they reach sexual maturity. Different populations at different locations have different spawning seasons. The latitudinal locations for spawning grounds ranges from approx. 35° to 71° N, and the temperature for spawning areas can be as low as below 0° C to approx. 15° C (Blaxter, 1985, Vikebø et al., 2012). Even though the different herring populations are separated in different areas during their spawning period, they can be found in mixed aggregations other times of the year (Husebø et al., 2005, Ruzzante et al., 2006). Some feeding areas are also fishing areas for Atlantic herring where mixed catches are common (Hintzen et al., 2015). Along the Norwegian coast, there are several stationary, semi-stationary and migratory herring populations. One example of a migratory herring populations is the Norwegian spring spawning herring (Dragesund et al., 1997). Other examples of smaller less migratory local populations includes Landvikvannet herring, Lindåspollen herring and Trondheimsfjord herring (Eggers et al., 2014, Johannessen et al., 2014). Exactly how many populations of herring there are, and how much gene flow there is between them, have become a question of interest in the later years (Lamichhaney et al., 2012, Limborg et al., 2012, Pampoulie et al., 2015) .

Genetic differences have been found between Norwegian spring spawning herring and different local fjord populations along the Norwegian coast (Pampoulie et al., 2015) and between different populations in the Baltic Sea, and the North Sea (Lamichhaney et al., 2012, Limborg et al., 2012, Teacher et al., 2013). No genetic difference between Islandic summer spawning herring and Norwegian spring spawning herring have been found, even though spawning grounds and mean spawning time is different (Pampoulie et al., 2015).

Population structure in herring stocks is complex (McQuinn, 1997). There are several examples of metapopulation structure with different components with uncertain mixing, partly separated by spawning area and time of spawning (McQuinn, 1997, Ruzzante et al., 2006, Johannessen et al., 2009, Eggers et al., 2014, Johannessen et al., 2014). In a small semi enclosed ecosystem outside of Bergen, Lindåspollen, Johannessen et al. (2014) found that two different herring populations were schooling together right before and after the spawning period. A similar phenomenon was also found in the brackish lake, Landvikvannet, in southern Norway (Eggers et al., 2014). In Lindåspollen, one resident component spent most of its life inside the semi-enclosure while another migrating component only spent the first 5 years of its life in this area. In the 1960's, the resident component was slower growing and had a lower mean vertebrae number than the migrating component. The migrating component in this example was assumed to be Norwegian spring spawning herring, while the resident component was named Lindåspollen herring. In the last 50 years the resident component has increased in size and has become more similar to the migrating component. This indicates genetic exchange between the two populations. These results suggest that the different populations can change genetically and phenotypically over time (Johannessen et al., 2014).

Identification of distinct spawning populations in mixed aggregations is useful when it comes to managing the different populations, because it is important to know which populations that are actually being fished. It is especially important when smaller populations are found in mixed fishery areas, as the small populations are more vulnerable to overexploitation (Blaxter, 1985, Hintzen et al., 2015).

1.3 PHENOTYPIC TRAITS AS A PROXY OF ENVIRONMENT AND GENETICS

The phenotype of an organism can be defined as:

> ” *The complete observable characteristics of an organism or group, including anatomic, physiologic, biochemical, and behavioural traits, as determined by the interaction of genetic makeup and environmental factors.*”

On the other hand the genotype can be defined as:

> “*The complete genetic constitution of an organism or group, as determined by the specific combination and location of the genes on the chromosomes. Alternatively, the alleles situated at one or more sites on homologous chromosomes.* “(Mosby’s Medical Dictionary. 8th edition. © 2009, Elsevier.)

Phenotypic traits can reflect conditions in the nursery grounds and therefore tell us something about origin of the fish (Bekkevold et al., 2005). This is especially relevant when it comes to separate herring populations, since different populations usually have specific spawning areas. However, the phenotype can also be a result of genetic selection (Lamichhaney et al., 2012). Phenotypic plasticity is a term that explains, that a single genotype can give different phenotypes, when influenced by different environmental conditions (Via et al., 1995). The mechanisms behind phenotypic plasticity may be caused by allelic sensitivity, which means that some alleles will be expressed in specific environments. Another possible mechanism behind phenotypic plasticity is gene regulation, were regulatory loci may turn genes on or off in particular environments, this is also termed epigenetics (Via et al., 1995, DeWitt et al., 1998). Since Atlantic herring have a wide range of phenotypes between populations (Runnström, 1941, Silva et al., 2013), it is interesting to test whether the observed variability in phenotypic traits in herring is a result of genetic selection or the environment. In the study of Lindåspollen herring that was mentioned above (Johannessen et al., 2009), phenotype changed over time and revealed a possible change in population structure. If the phenotypes are plastic, such changes over time could be caused by both genetic change and environmental change. It is therefore important to know, which environmental factors that influence the phenotypic traits. It is also useful to know how much phenotypic traits are influenced by the genotype and genetic selection.

1.4 MATERNAL AND PATERNAL EFFECTS

Maternal effects are usually the term used for non-genetic contributions to the egg and offspring by the mother. Examples of such maternal effects in herring include yolk-sac volume and larval weight (Bang et al., 2006). The term non- genetic might not be true for all maternal effects, because it is most likely a combination of non-genetic and genetic contributions, correctly termed female effect (Bang et al., 2006). The genetic contribution to the egg from the sperm is termed paternal effects. The parental genetic contribution to the offspring will potentially increase the fitness of the offspring if the parents are adapted to the environment the offspring is living in (Via et al., 1995). Sudden changes in the environment can demand adaptability of an organism (Reed et al., 2010). In herring populations, the separate populations can have distinct life history traits like growth rate, preferred spawning substrate and area, and different migratory behaviour (Geffen, 2009). The cause of these differences are not fully explored, but are probably a combination of genetic adaptation to the environment and the influence of the environment on the phenotypes of the fish (Jørgensen et al., 2008).

1.5 METHODS USED TO SEPARATE ATLANTIC HERRING POPULATIONS

Several methods can be used to separate different populations of Atlantic herring. Traditional methods rely on a combination of phenotypic characteristics. Typically characteristics that have a distinct mean value within different populations are used (Runnström, 1941). Genetics methods are also becoming more and more useful as new genetic markers are discovered for herring (Lamichhaney et al., 2012, Libungan et al., 2012, Limborg et al., 2012). The most common phenotypic traits used for assigning herring into separate populations are otolith microstructure, otolith shape, maturity stage, and mean vertebrae counts. When there is overlap in these mean values between populations, it can be difficult to separate them.

Different methods can be divided into categories based on the characters of the methods. Morphometric methods include comparisons of shapes and sizes e.g., body shape, otolith shape and scale shape (Begg and Waldman, 1999). Meristic methods includes morphological structures that can be counted, e.g. vertebrae counts, fin rays and gill rakers (Begg and Waldman, 1999). Meristic traits are usually set early in the development, and can be controlled by both genetic and environmental factors (Barlow, 1961, Begg and Waldman, 1999). Other methods include spawning season, length at first maturity, length at age, spawning area, otolith microstructure and otolith chemistry. Maturity is usually divided into 8 different stages in herring. Stage 1-2 represent the immature herring gonads, while stage 3-8 represent the maturing, spawning and resting gonads (Mjanger et al., 2014). The maturity stage can give good

indications of the spawning season of the herring, but cannot be used to separate spawning populations with overlap in spawning periods (Libungan et al., 2015). The length at age can give information about the growth rate of the fish, which can be different between populations. One example of herring populations that used to have different growth rates and length at first maturity are the Norwegian spring spawning herring and the Lindåspollen herring (Johannessen et al., 2014). Distinct growth rates have also been found between juveniles of Norwegian spring spawning herring in different retention areas along the Norwegian coast, which indicates that environment, especially temperature also influences the growth rate (Husebø et al., 2007).

Otoliths are useful for many purposes in fish research; amongst these the understanding of stock structure and dynamics (Campana and Thorrold, 2001). Otoliths are growing throughout the whole life of the fish and are influenced by both the environment and genetics. Otoliths are small crystalline calcium carbonate ($CaCO_3$) structures in the fish ears and play an important role in the fish's ability to detect sounds (Popper and Lu, 2000) and maintain equilibrium (Payan et al., 2004). There are three pairs of otoliths; sagittae, lapillus and ateriscus (Figure 1). The sagittal otoliths are the largest, and as the most commonly used otoliths in fish research. The otoliths can give good indications of earlier life history and growth (Folkvord et al., 1997, Campana and Thorrold, 2001). Due to the nature of otolith calcification, otoliths are constantly formed, but not resorbed (Burke et al., 2008b), thus providing chronologic information about the fishes life history (Campana and Thorrold, 2001). Individual herring can be assigned to spawning season by looking at the microstructure of the otoliths, this can be done for both fish larvae (Moksness and Fossum, 1991), juveniles and adult herring (Clausen et al., 2007).

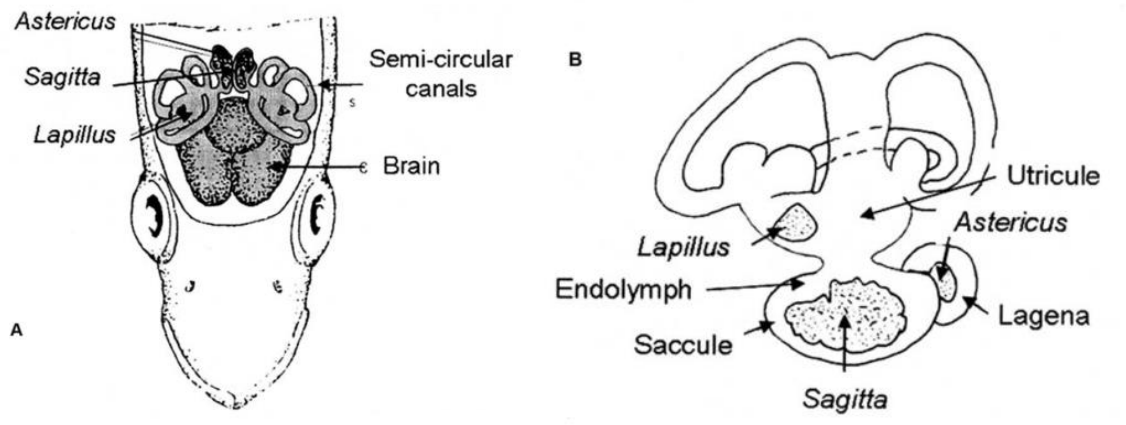


Figure 1: A general illustration of where otoliths are found in fish. A. The vestibular apparatus (semi-circular canals) and their placement relative to the brain in the fish head. The three otoliths, astericus, sagitta and lapillus are indicated with arrows. B. The vestibular apparatus and the relative internal placement of the otoliths. Illustrations borrowed from (Payan et al., 2004).

Otolith shape analysis includes several methods to compare the shape of the otoliths. The sagittal otolith have been pointed out as a better indicator of spawning population than the two other otoliths (Campana and Casselman, 1993). Otolith shape analysis can be done by doing simple measurements like otolith length vs width, or otolith length vs fish length. To better compare complex shapes, more advanced methods like Elliptical Fourier analysis (Burke et al., 2008a) or wavelet transform (Libungan et al., 2015) can be used. Otolith shape analysis has been used to successfully investigate stock structure in several fish species, including blue whiting (Keating et al., 2014), European sardine (Jemaa et al., 2015), horse mackerel (Stransky et al., 2008), Atlantic saury (Aguera and Brophy 2011) and Atlantic herring (Eggers et al., 2014, Libungan et al., 2015). Using the wavelet transform method of otolith shape, Libungan et al. (2015) successfully separated different spawning populations of Atlantic herring, from the Faroe Islands, Ireland, Iceland, Canada, Norway and Scotland UK. Environmental effects or life history traits might influence otolith shape in herring, since the difference between the otoliths could be related to different spawning time (Libungan et al., 2015). The wavelets transform method, together with other methods, could also be used to separate three different herring populations in Landvikvannet (Eggers et al., 2014). Due to the potential environmental influence on the otolith shape, otolith shape analysis can be a useful tool to separate pelagic fish populations. Especially in populations with high gene flow between the them, which can be hard to separate by genetic methods (Hemmer-Hansen et al., 2014, Jemaa et al., 2015)

The amount of trace elements in the otoliths is often described as the otolith microchemistry. Otolith microchemistry analysis is a good method to explore stock structure and dynamics. The chemical and physical characteristics of ambient environment, are reflected in the elemental composition of the otoliths in some extent (Kalish, 1989, Campana et al., 2000). The otoliths consists of approx. 99 % calcium carbonate, only a small amount of other trace elements can be found in the otoliths (Payan et al., 2004). The relative amount of specific trace element (usually compared with the amount of calcium) can tell us something about the previous life history of a fish, and can work as a natural tag in mixed fish aggregations (Campana et al., 2000). Before the elements are being incorporated into the otolith matrix they need to pass through at least two important membranes (Kalish, 1991, Payan et al., 2004). The first membrane separates the blood plasma from the environment and the second separates the endolymphatic fluid from the blood plasma. The elemental concentration in the endolymphatic fluid is more strictly regulated than in blood plasma (Campana, 1999). The physiological adaptations to the environment may influence this regulation. “Contamination” of the otoliths

can occur when divalent cautions like Sr^{2+}, Ba^{2+} and Mg^{2+} replaces the Ca^{2+} during the precipitation process that builds the otolith (Townsend et al., 1992). Some elements are more strictly regulated than others, and are not so much dependent on the environment e.g. S, P, Na, K, and Cl (Thresher et al., 1994). The amount of strontium deposited in the otoliths is dependent on the salinity and the temperature of the ambient environment, while barium reflects the salinity but not the temperature (Bath et al., 2000). Several different methods and techniques can be used to analyse the chemical composition of fish otoliths. Most includes a Mass spectrometer (MS), which creates gas-phase ions from molecules and atoms in a sample and separates them according to their mass-to-charge ratio and measures the abundance of these ions (Robinson et al., 2014). Otoliths can be dissolved whole in acid, or parts of the otoliths can be dissolved and analysed separately (Geffen et al., 2013). Whole-otoliths-chemical-analysis can be used as chemical tags for groups of fish, but are unlikely to remain constant over long periods of time (Elsdon et al., 2008). The life history of the fish can be explored by taking several samples from the same otolith along a transect from the otolith core to the edge (Elsdon et al., 2008). The time the fish spent in freshwater and seawater in anadromous fish can be investigated by otolith chemistry analysis (Quinn et al., 2014).

Mean vertebrae count is influenced by temperature in the ambient environment of the fish in the early larval development, and reflects the conditions in the nursery grounds (Hulme, 1995). Fahy and Ohara (1977) concluded that there is little evidence suggesting that the salinity actually influences the final vertebrae count in fish. In their experiment they did, however not use the respective treatment salinities during the fertilization of the eggs, which may have influenced their results. Hempel and Blaxter (1961) found a positive relationship between salinity and myotome count at hatching in herring, and they also found a negative relationship between temperature and myotome count. There are several herring populations and spawning grounds in the Baltic Sea. In one study a correlation was found between the salinity in the spawning grounds or nursing ground and the final number of vertebrae of herring in the Baltic Sea (Jørgensen et al., 2008). The North Sea herring showed differences in mean vertebrae count between different spawning grounds, probably due to different environmental factors, especially temperature (Hulme, 1995). However, there was a flexibility in the preferred spawning ground location in some of the subpopulations which indicated that mean vertebrae number should not be used to separate different spawning populations alone (Hulme, 1995).

1.6 THE AIMS OF THIS STUDY

A common garden experiment was set up to test the effects of salinity and paternal genetic effect on different phenotypic traits in Atlantic herring. Two populations with different geographic origin (and distinct environmental differences e.g. salinity) and phenotypic traits were crossed in the lab. Later in this thesis these fish will be termed *parental fish*, or *parental fish populations*. Even though the geographic origin of the fish was different, the latitudinal position of the sampling areas was similar (~60°N). To eliminate non-genetic maternal effects, only one female was used as parental fish (Høie et al., 1999). The offspring of the wild caught herring will from this point on be termed *first generation herring*. The first generation herring were exposed to two different salinities; 35 psu and 16 psu from fertilization to final sampling. The purebred Atlantic herring was expected to do better in 35 psu salinity, while the hybrid herring was expected to do better in 16 psu salinity. This was due to potential physiological adaptation to salinity of the parental fish. The temperatures were similar in all of the tanks to eliminate any effect of temperature on the fish. In this study the otolith shape, otolith microchemistry and vertebrae count were investigated. Herring otoliths trace element was analysed using an ICP-MS, measuring the elements Ca, Na, K, Ba, Sr, Mn and Mg.

The aims of this study can be described, by the following hypotheses:

H0: The vertebrae count is not influenced by ambient salinity or by male genetic origin.

H0: The otolith shape does not reflect ambient salinity or male genetic origin.

H0: Otolith microchemistry is not influenced by ambient salinity or by male genetic origin.

In addition to these hypotheses, the survival and growth of the first generation Atlantic herring, and development of otolith shape, will be explored in this thesis.

2 MATERIALS AND METHODS

2.1 SAMPLING AREA – COLLECTION OF PARENTAL FISH

The parental fish were collected from two wild Atlantic herring populations in May 2013 (Figure 2). One group was caught off the Norwegian coast close to Bergen in proximity of Askøy (Appendix A, Figure 1). This group will later in this thesis be termed the Atlantic parental population group. The other group was caught in the Baltic Sea off the Swedish coast north of Stockholm (Appendix A, Figure 2), close to Fagerviken. This group was termed the Baltic parental population group.

Figure 2: Map with the two different sampling locations indicated by red dots. The two sampling areas shared similar latitudinal coordinates (~60°N).

The Atlantic parental population would in their natural habitat experience a more stable environment in regards to salinity and temperature than the Baltic parental population. The Baltic Sea is a large threshold basin with limited connection to the open ocean, and it is the largest area of brackish water in the world ocean system (Pickard and Emery, 1990). The Baltic Sea is more influenced by freshwater runoff from land than the Atlantic Ocean; this makes the marine environment in the Baltic Sea highly heterogeneous when it comes to both salinity and temperature (Pickard and Emery, 1990). There is a salinity gradient from north to south and from east to western parts of the Baltic Sea, which starts at freshwater and gradually increases

to approx. 32 psu in the southwest (Appendix A, Figure 3). It is also a temperature gradient from the north to the south (Al-Hamdani and Reker, 2007, Teacher et al., 2013).

In contrast, the salinity regime of Atlantic and coastal water along the Norwegian coast is much less variable with typical surface salinities of 30 - 33 psu, while at greater depths (> 200m) the salinity is relatively constant around 35 psu (Aure and Østensen, 1993). Both the Atlantic parental population group and the Baltic parental population group was caught with gillnet, with mesh sizes of 36 mm and 18 mm, respectively. Potential parental fish for the experiment were measured for length and weight before the gametes was stripped by hand or dissected out. The fertilisation of the eggs took place in the wet lab facility in the University of Bergen. One female and one male were used as parental fish from both parental population groups. After the parental fish had been chosen and the eggs were successfully fertilized the fish parental fish groups were stored in a freezer for later examination. The goal of these examinations was to get some reference data to describe the population composition and the general phenotypical traits in the populations of the parental fish. Only the Atlantic female gave enough viable juveniles to be used further in the experiment.

2.2 FIRST GENERATION HERRING

2.2.1 Experimental design

To study genotypes and phenotypic plasticity in fish, the best method would be to conduct a common garden experiment, where offspring of known parents are kept in the same tanks, experiencing the same repetitive treatments (Conover and Schultz, 1995). Common garden experiments with fish have previously been done by e.g. Otterlei et al. (1999), Lankford jr. et al. (2001) and Folkvord et al. (2009). By doing a common garden experiment, the effect of salinity on phenotypic characters, survival and growth in the first generation herring could be investigated.

The experimental set-up in this study is shown in Figure 3. In the start of the experiment, there were three salinity treatments (6, 16 and 35 psu) and two replicates of each treatment (Figure 3). The offspring from two different parental combinations were mixed in each of the six tanks. The ratio of different crosses in the start of the experiment was 333 Atlantic x Atlantic cross (purebreds) and 667 Baltic x Atlantic cross (hybrids) (Arild Folkvord, pers. com.). Later in this thesis, the two *genetic groups* will be referred to as *purebreds* and *hybrids*. The fish in the 6 psu group did not have a satisfactory survival throughout the larval stage, and were not included

in the juvenile experiment. When the fish grew larger, the original salinity replicates eventually had to be merged due to lack of space and large tanks at Industrilaboratoret (ILAB) (Figure 3).

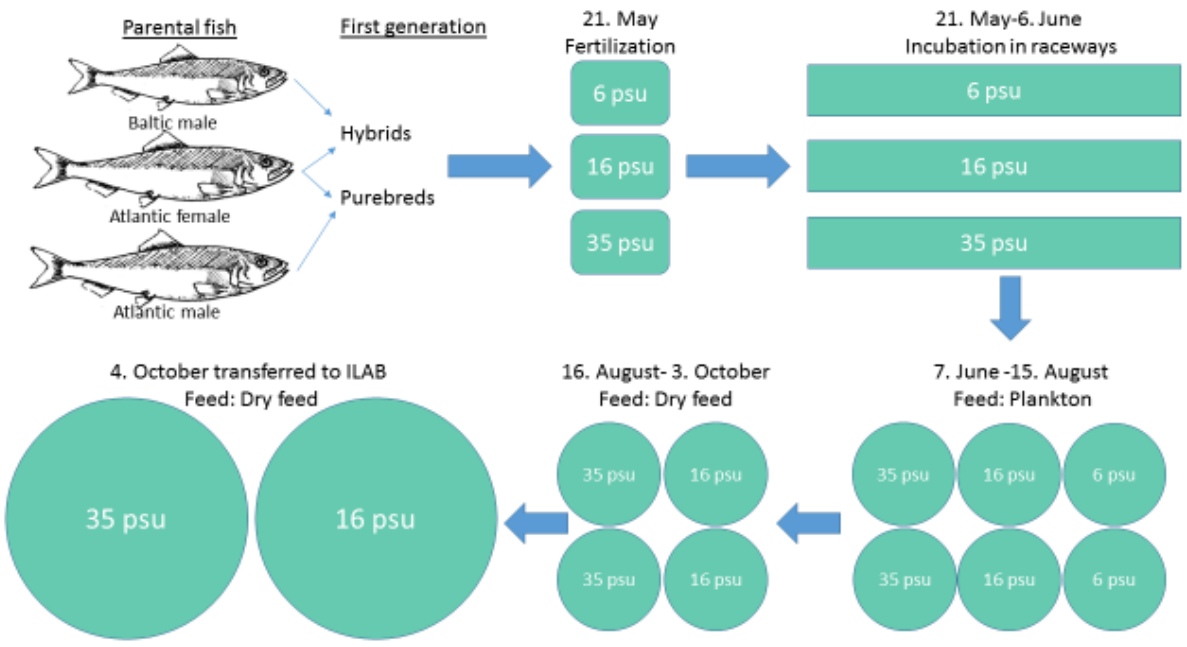


Figure 3: An overview of the tanks at different times of the common garden experiment in 2013. Since October 4th 2013, the fish were held in Industrilaboratoriet (ILAB). Hybrids and purebreds were co-reared in the different salinity treatments to control for tank effects. The first generation fish described in this thesis were sampled from the tanks at ILAB.

2.2.2 Production of herring juveniles in the lab

Three fish were used as parental fish; one Atlantic female, one Atlantic male and one Baltic male (Table 1). Gonads from both males and females were stored dry in glass containers before fertilization. The age of the Atlantic male was uncertain due to unclear annual increments.

Table 1: Measurements of the parental fish.

	Origin	Total length (cm)	Weight (g)	Vertebra count	Age
Female	Atlantic	30.5	295	57	5
Male 1	Atlantic	32.5	320	57	5-6
Male 2	Baltic	20.5	66.8	55	8

The fertilisation of the herring eggs took place on May 21st 2013. Glass plates were immersed in water in plastic trays. First the eggs were stripped from the gonads and distributed evenly over the glass plates. The seawater would make the eggs sticky and after a short while the eggs were "glued" to the glass plates. The male gonads were then emptied over the eggs at the

respective salinities, and the water was gently whirled over the eggs by hand to make sure that the eggs would be fertilized. After the mixing, the plastic trays were left still for 15-20 minutes, before the glass plates were transferred to an incubation raceway system. The raceway system consisted of three separate raceways, each with its own salinity of either 6, 16 and 35 psu respectively. The water temperature was set to 8.0-8.5 °C. On the 4th of June 2013, the glass plates were placed into separate buckets to ensure known timing of hatching and the knowledge of genetic origin of the herring larvae. At the 5th of June, the hatched larvae (50 % hatching) were transferred to larger circular fish tanks with water of the corresponding salinity. The water volume of the fish tanks were gradually increased from 200 to 400 L during the first two weeks, by adding water with the live feed and by adding water. On 24th of July, continuous water flow of appropriate salinity was introduced to the tanks (Arild Folkvord, pers. com.).

First feeding started on June 9th, 4 days post hatching (DPH). The feed consisted of live algae (*Rhodomonas* and *Isochrysis*), rotifers (*Brachionus* spp.), and natural zooplankton. Natural zooplankton was collected at Espegrend field station in Bergen and were fed to the fish larvae on a daily basis. On June 28th (23 DPH), *Artemia* spp. was added together with zooplankton until fish larvae started to feed on formulated feed, Gemma micro (Skretting), on August 15th (71 DPH) (Arild Folkvord pers. com.). On October 3rd 2013, the juvenile herring were transferred to ILAB. The herring were placed into two large round tanks (3 meters in diameter), with salinities corresponding to their previous fish tanks. The temperature in these tanks was approx. 9 °C, and the light regime used was natural light corresponding to a latitude of 60°N (Sindre Grimen, ILAB, pers. com.). Both hybrids and purebreds, where mixed in the two tanks to remove tank effects between genetic strains. The fish origin was later determined using genetic methods (TaqMan Genotyping Assay from Applied Biosystems).

2.2.3 Handling and sampling

In total, 675 juvenile herring were sampled from the first generation herring at ILAB. Before handling and sampling, the juvenile herring were sedated with aqui-s (clove oil) and euthanized with an overdose (approx.1 g/ 2 L of seawater) of finquel (metacain, MS-222). DNA samples, weight and length were collected or recorded before the fish were stored in a freezer (-18 °C) for later counts of vertebrae and extraction of otoliths. There were three main sampling occasions where substantial samples from both salinities were taken, these included 187, 297 and 614 DPH. The 187 DPH sample was collected on the 09.12.13, while the 297 DPH sample was collected between 27.03.14 and 06.04.14, the 614 DPH sample was collected on the 05.02.15 and 13.02.15 (Table 2).

Table 2: Number of samples from the first generation herring, from the three main sample dates.

Sample date/DPH	Salinity	Genetic background	Total number of fish	Weight and length	Genetic analysis	Vertebrae counts	Otolith shape	Otolith chemistry
09.12.2013/ 187	16 psu	Hybrid	85	85	85	79	73	19
		Purebred	14	14	14	13	12	14
		Undetermined	3	3	3	3	3	0
	35 psu	Hybrid	69	69	69	61	61	22
		Purebred	30	30	30	25	24	17
		Undetermined	1	1	1	1	1	0
29.03.2014/ 297*	16 psu	Hybrid	37	37	37	36	31	0
		Purebred	3	3	3	3	2	0
	35 psu	Hybrid	31	31	31	27	24	0
		Purebred	19	19	19	18	18	0
13.02.2015/ 614**	16 psu	Hybrid	27	27	27	27	23	0
		Purebred	3	3	3	3	2	0
	35 psu	Hybrid	16	16	16	14	14	0
		Purebred	14	14	14	14	12	0
Total			352	352	352	324	300	72

* This sample date represents the three sample dates 27.03.14, 29.03.14 and 06.04.14.
** This sample date represents the two sample dates 05.02.15 and 13.02.15.

Extra samples from the 35 psu salinity were taken during the sampling period to reduce total biomass in the tank or other commitments (Table 3). Because of an outbreak of tenacibaculosis in the 35 psu salinity tank the last main sample from this tank was delayed to 13.02.15 (614 DPH). Tenacibaulosis is an infection which has been associated with ulcers, lesions on the fish body surface, necrosis and eroded mouth (Avendaño-Herrera et al., 2006). It can be treated with antibiotics or by reducing the salinity of the water for a short period. In this experiment, salinity was one of the main effects, and it was important that the salinity be kept the same during the experiment. To treat the infection, antibiotics were administered through the feed. The infection was discovered 15.01.15 (590 DPH), and most weakened or damaged individuals were taken out from the 35 psu salinity tank (Table 3). These fish were thinner due to disease, and were not representative for the general growth of the fish. Some of the herring from this sample were used in diagnosing the fish and find the right treatment.

Table 3: Number of samples of each variable from extra samples taken from the first generation herring. No vertebrae counts or otolith chemical analysis was made of these samples.

Sample date/ DPH	Salinity	Genetic background	Total number of fish	Weight and length	Genetic analysis	Otolith shape
30.09.2014/ 483	35psu	Hybrid	76	76	76	24
		Purebred	37	37	37	19
10.11.2014/ 523	35psu	Hybrid	20	20	20	0
		Purebred	10	10	10	
11.11.2014/ 524	35psu	Hybrid	36	36	36	0
		Purebred	24	24	24	
18.11.2014/ 531	35psu	Hybrid	17	17	17	0
		Purebred	8	8	8	0
		Undetermined	1	1	1	0
15.01.2015/ 598*	35psu	Hybrid	77	77	77	28
		Purebred	16	16	16	11
		Undetermined	1	1	1	1
Total			323	323	323	83

* The 15.01.2015 (589 DPH) samples represent sick and dying fish, that was sampled out during an outbreak of tenacibaculosis.

2.3 VERTEBRA COUNTS

Vertebrae counts were performed to investigate the anatomical differences between the two parental populations and between the genetic groups in the first generation herring. Dissection was used as the main method, while radiographs were used as a control method for the smallest fish. Counting vertebrae by dissection includes a risk of causing vertebral damage and rupture. This problem became a motivation for using radiography as a method that could be more efficient and produce more accurate vertebrae counts in small herring.

2.3.1 Dissection

Fish from the parental fish population groups were defrosted in room temperature for approx. 24 hours. First, the peritoneal cavity was opened, and the gonads were taken out and examined for sex and maturity stage by using a 1-8 stage system (Mjanger et al., 2014). The filet on one side of the herring was cut away with a knife so that the vertebra column became visible, then half of the remaining filet on the other side was cut away, following the vertebral column. A small cartilage structure covering the first vertebra was removed. Any flesh left on the visible side of the vertebrae column was scraped away with a scalpel. The vertebrae were counted manually as described in Mjanger et al. (2014). The urostyle was included in the vertebra count (Figure 4).

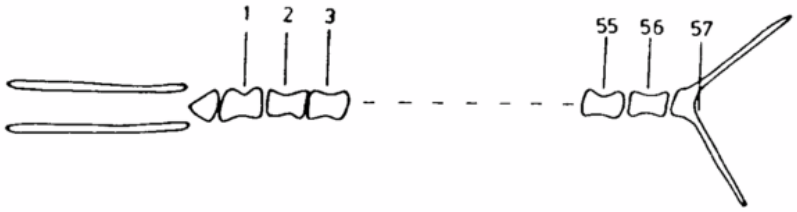


Figure 4: Schematic drawing of the herring vertebra column with indicators of where the counting started and ended. The last vertebra counted was the urostyle, in this illustration nr 57. Drawing from the Institute of Marine Research manual for sampling fish and crustaceans (Borge et al., 2000).

For the first generation herring, the dissection method was very similar to the one used on the parental fish population groups depending on the size of the fish dissected. Due to the more fragile state of the smallest fish, these where counted from the side so that the whole filet on the other side was intact. This was necessary to maintain the vertebral column unbroken during the counting. To make sure that the first vertebra counted actually was the first vertebra, a scalpel was used to remove tissues from the vertebrae in the head region. The first vertebra could be identified by being jointed to the cranium. The first generation herring were counted twice. If the counts did not match, a third counting was performed to assure that the counts were trustable.

Deformations in the vertebral column could make the counting difficult and were categorized as mild or severe fusion of vertebrae. Mild fusion was defined as three or two fused vertebrae, which all could be counted as separate vertebrae. Severe fusion was defined as more than three fused vertebrae. In very difficult cases, the vertebrae count was defined as very unreliable and were excluded from the data. For mechanical damage due to handling and accidental cutting of the vertebrae with a scalpel, the vertebrae count was included or excluded depending on the severity of the damage and the ease of counting.

2.3.2 Radiography

In the juvenile herring, the vertebra were counted manually on a computer screen by using x-ray images taken with a digital x-ray apparatus (HI-Ray 100, Eickenmeyer Medizintechnik für Tierärzte e.K., Tuttlin-gen, Germany) at the IMR research station in Matre. To get a clear image, the filet on one side of the fish had to be removed with a scalpel. This was necessary to reduce reflection produced by the fish skin in the x-ray machine. The final exposure strength used was

40 kV and 16 mAs. The fish were placed on digital x-ray plates. After the x-ray was taken, the digital x-ray plate was scanned into a computer. The contrast in the image was adjusted to a level where the vertebral column was most clearly visible (Figure 5). The vertebrea counts form the x-rays corresponded well with the vertebrae counts from the dissection.

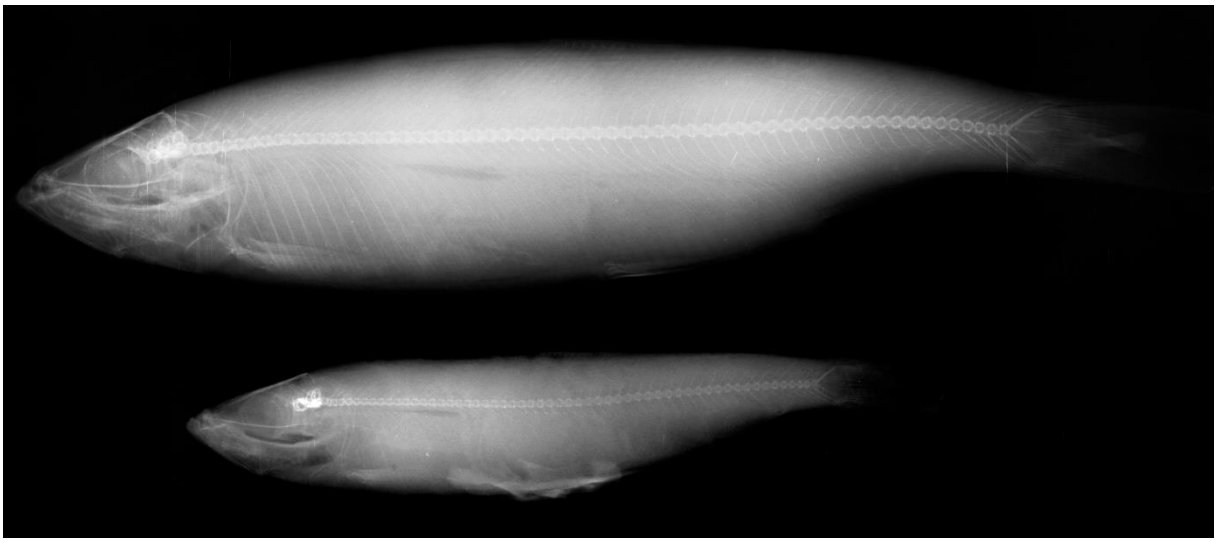


Figure 5: Radiography of fish from the parental fish populations, showing the large size difference of ripe Atlantic herring originating from the Norwegian coast (top) and Atlantic herring originating from the Baltic Sea, off the coast of Sweden (bottom).

2.4 OTOLITH ANALYSES

Both otolith shape analysis and chemical analysis were used to investigate the otoliths from the first generation herring. Shape analysis is a morphometric method, which was used to compare the mean shape of the herring otoliths. Otolith chemical analysis was used to determine the levels of trace elements in the otoliths at 187 DPH. This was done to explore how the environment and fish physiology, of the two genetic groups, influenced the otolith chemistry in herring.

2.4.1 Extraction and cleaning procedure

Both of the largest otoliths (sagittae) were removed from the fish, cleaned in fresh water, and dried in clean plastic containers. In the parental fish population groups, the otoliths were removed from the ventral side by separating two bones in the cranium with the thumb nail. This was not possible on the smallest herring of the first generation fish, due to their small size and fragile state. The otoliths on these fish were removed by cutting of the dorsal part of the cranium

with a scalpel and removing the brain, which covered the otoliths. In both groups, metal tweezers were used to extract the otoliths, except for the herring that were going to be used for otolith chemistry analysis.

The otoliths that was going to be analysed for otolith chemistry required special handling. These otoliths where carefully extracted using plastic tweezers and plastic gloves, then rinsed in milliQ water (Quality: 18.2 MQ cm at 25 °C). The otoliths were first placed in one small petri dish with milliQ water, then into an Eppendorf ® tube (1,5 ml) with new milliQ water. the eppendorf ® tube was then vortexed on an analogue vortex mixer (VWR TM analogue vortex mixer) for approx. 10 seconds at speed 3, before the otoliths were put into another petri dish with new milliQ water. The vortex mixer was used to remove remaining tissue from the otoliths. After the last milliQ bath, the otoliths were placed in a clean 24 chamber NUNC ® plastic container to dry.

2.4.2 Otolith shape analysis

The otoliths were photographed using a Leica MZ95 stereo microscope mounted with a Nikon digital sight DS-U1, microscope camera. NIS- elements F 2.3 software was used to photograph and to adjust the microscope camera. Auto white was used to define the white balance of the background to white, contrast was set to enhanced and the auto exposure with AE compensation was set to -0.6 to -1.0. When the AE compensation was set to negative, the more transparent parts of the otoliths did not look like holes, but were part of the otolith shape. It was very important to get the outline of the otolith as clear as possible, so the focus was always at the edge. Some otoliths were curved at the edge, and it was therefore not possible to get the whole otolith edge in focus. In these cases the most complex part of the edge was focused on. To make the images standardised, a rubber band around the computer screen was used to align the placement of the otoliths in the pictures. The otoliths were placed in a clean plastic petri dish, or a NUNC ® container, with the sulcus side facing downwards before photographing. Sulcus side down was the best positioning of the otolith, as it was the flattest side. The plastic petri dish was only used for the samples from 187 DPH, to make the image background completely free from noise (e.g. dust and scratches in the plastic container). Transmitted light was used when photographing the otoliths, this was the best way to avoid shadows around the otoliths. It was also the most efficient way to produce standardised images (Figure 6). The two different plastic containers used were tested to see if they affected the measured length of the otoliths, but the difference was minimal (± 0.48 %).

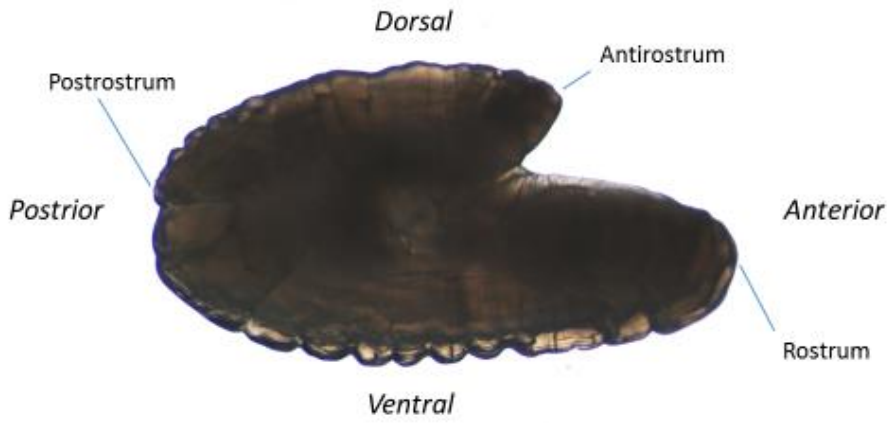


Figure 6: Picture setup of the right sagittal otolith from juvenile herring (482 DPH). The otolith was placed with sulcus medial side facing downwards and rostrum to the right.

The software ImageJ 1.43 was used to analyse the otolith images, this was done using a ImageJ macro called Shape analysis (Audrey Geffen, University of Bergen. pers. com.). The macro inverted the colours of the pictures, so that the pictures showed white otoliths on black backgrounds. After this, a silhouette image based on the inverted image was created. From this image, an otolith outline was created (Figure 7), and several measurements were made (Table 4). To be able to compare the otolith sizes from different sampling dates, the images were calibrated to pixels per mm.

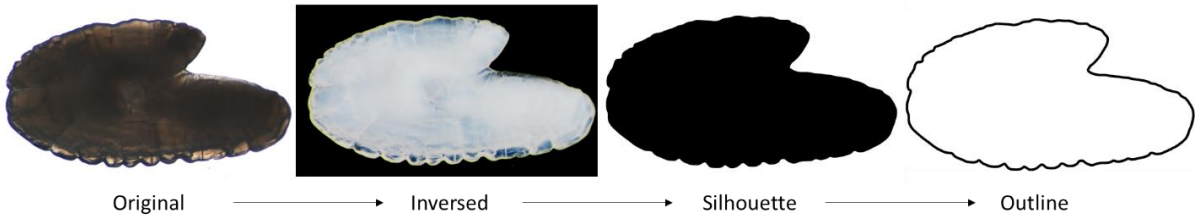


Figure 7: The original shape picture with the three different outputs from the shape analysis macro.

Table 4: Parameters measured by the otolith shape analysis macro in ImageJ, descriptions are modified from the ImageJ 1.46r user guide (Ferreira and Rasband, 2012):

Shape Parameter	Formula	Description
Area		Area of selection in mm^2, in this case mm^2 because the pictures where calibrated to pixels/mm.
Perimeter		The length of the outside boundary of the selection, in this case the circumference of the otoliths.
Circularity	4π × ([Area]/ [Perimeter]2)	Ranges from 0 (infinitely elongated polygon) to 1 (perfect circle).
Feret (Feret's diameter)		The longest distance between any two points along the selection boundary, also known as maximum caliper. This is the longest diameter possible to measure in-between two parallel lines.
MinFeret		Minimum feret (or caliper) diameter
AR (Aspect Ratio)	[Major Axis]/ [Minor Axis]	The aspect ratio of the particle's fitted ellipse

To further investigate the shape of the otoliths, a subset of the total number of otolith pictures were randomly chosen from each of the three main sample dates. Twenty fish from each salinity and each genetic combination (80 fish from each sample date, when this was possible) were used in the analysis (Table 2). There were 300 fish from the three main sample dates included in the otolith shape analysis in total.

Some otoliths had deformations, these where recognized by translucent parts of the otolith with irregular shape (Tomás and Geffen, 2003). This was most likely due to inclusion of vaterite. To describe the amount of vaterite in the otoliths, codes were assigned to describe how much of the otolith consisted of vaterite, and whether one or both otoliths were affected. Otoliths were classified into three groups; less than 10 %, between 10-50 % and more than 50 % vaterite. Only the otoliths with less than 10 % vaterite were included in the shape analysis in ImageJ.

2.4.3 Otolith chemical analysis

In total, 72 otoliths were analysed for chemical elemental composition using an inductively coupled plasma mass spectrometer (ICP-MS). Up to 40 otoliths from each salinity treatment with 20 fish from each genetic group were randomly chosen to be analysed for otolith chemistry (Table 5). The fish used for this analysis were sampled from both salinity groups on 09.12.13 (187 DPH). Two samples were excluded from the statistical analysis due to errors in measurements. This was done because the measured values where extreme when compared to the other values, and must have been the results of instrumental or preparation error. Two individual observations (Na and Mg) was also excluded from the statistical analysis due to extreme values.

Table 5: Number of fish from each combination used in the otolith chemical analysis. The purebreds in the 16 psu group represent all the purebred fish in the sample.

Salinity	Genetic background	Number of fish
16 psu	Hybrid	19
	Purebred	14
35 psu	Hybrid	22
	Purebred	17
	Total	72

*Excluded from the analysis, also subtracted from the number of fish column.

One week before the analysis, the otoliths were washed and decontaminated. The cleaning process was similar to the one used by Arslan and Secor (2008). First, the otoliths were soaked in milliQ water and left for one hour, and then soaked in 3 % H_2O_2 for 5-20 minutes to remove any organic residues. After this, they were rinsed again in milliQ water. To clean the otoliths from any possible other contaminations, the otoliths were dipped in 1 % NHO_3 for two seconds. Then they were triple rinsed in milliQ water and put in an ultrasonic bath for five minutes. When the otoliths were properly cleaned, they were dried overnight in a drying cabinet at 50 °C. This was done to be sure that they were completely dry, before weighing the otoliths. The otoliths where weighed on a Sartorius micro weight and put back into the Eppendorf tubes they were washed in.

Before the chemical analysis, the otoliths were transferred to 15 ml VWR metal-free polypropylene centrifuge tubes. These tubes had been decontaminated in 10 % NHO_3 overnight in a clean hood, and then dried in a Laminar flow box FBS (Spetec) for approx. 72 hours. The otolith was analysed using the *lab B method*, as described by (Geffen et al., 2013). The otoliths were dissolved in the tubes by adding 1 ml of 70 % NHO_3, and left overnight in room temperature to be digested. The next day the acid digested otoliths were diluted with milliQ water until the solution had a total volume of 10 ml. The trace elements measured in the otolith solutions were Na, K, Ba, Sr, Mn, Ca and Mg. In addition to the 72 otoliths analysed, 4 extra test otoliths, 24 tubes with reference material and 3 blank samples were analysed. Two commercially available reference materials (NIES-22 Yoshinaga et al. (2000), and FEBS-1 Sturgeon et al. (2005)) for otolith chemistry were used to calibrate the analysis.

2.5 OTOLITH AGEING AND GROWTH ESTIMATION

Otolith age readings of the parental fish, were performed by counting annual increments in otolith photographs. The annual increments were counted following the procedure described in Mjanger et al. (2014). Only the wild fish used as parental fish in the experiment were aged. No treatments like polishing or grinding were done.

To estimate average weight growth rate per day, specific growth rate (SGR) which describe the % body weight gain per day, was calculated according to formula (1).

$$\text{SGR} = \frac{(\ln(w_2) - \ln(w_1)) * 100}{T_2 - T_1} \qquad (1)$$

The formula contained several parameters where, w = weight (g), T = time (days), 2 = final and 1 = initial.

Growth rates for otolith length and fish length was calculated in µm/day or mm/day according to formula (2):

$$G = \frac{L_2 - L_1}{T_2 - T_1} \qquad (2)$$

The formula contained several parameters where, L = length (µm or mm), T = time (days), 2 = final and 1 = initial.

2.6 STATISTICAL ANALYSES

ANOVA was used to compare the measurements in the different genetic and salinity groups. This was done using the R statistics software, version 3.0.3 (R Core Team, 2014). The various R packages used to create tables and figures will be summarized in the end of the chapter. Several methods were used to explore the data, before the actual analysis was performed. This was done to reduce the possibility of type I and type II error when testing the different null hypotheses in this experiment. The methods applied were inspired by a protocol for data exploration by Zuur et al. (2010).

Due to lack of space for large tanks, true replication of main effects could not be achieved throughout the whole experiment (Figure 3). The effects of genetic origin were therefore analysed on a per tank basis, contrasting hybrid and purebred strains at each salinity regime.

The effects of salinity on phenotypic expression will be analysed simultaneously, for both crosses assuming no cross interaction within tanks.

2.6.1 Assumptions of ANOVA

Analysis of variance (ANOVA) can be employed to test if multiple group and factor means are equal simultaneously (Whitlock and Schulter, 2009). ANOVA was used to compare the means of different biological characteristics in both the parental fish groups and in the first generation herring. This is a parametric method and makes assumptions about the distribution of the variable analysed. Assumptions about the variables that should be analysed include:

1) Measurements represent a random sample from the population
2) There is homogenous variance in each population
3) The error shows a normal distribution in each population compared

To check for general errors and outliers in the dataset, linear regression models were fitted to the continuous variables like fish length and weight, fish length and otolith length or amount of Mg and K. All these variables would be expected to show a relationship. Diagnostic Q-Q plots were made to see if the data displayed a normal error distribution and to investigate if there were any outliers in the data in general (Appendix C, Figure 1). The outliers were easy to detect in the diagnostic plots, but some further investigation was needed to see if the suspected outliers actually where outliers. A few outliers were detected in the otolith shape analysis data and in the otolith chemical analysis data. These where outliers due to errors in the measurements due to technical difficulties, and were excluded from the dataset. To test the homogeneity of variance in the different variables tested, a residuals vs. fitted values plot was made (Appendix C, Figure 1).

2.6.2 Comparing mean values with factorial ANOVA

A two-way factorial ANOVA was used to compare the means of the different parental fish, with catch date as a within-groups factor with three levels and the catch site as a between-groups factor with two levels. The sample sizes per catch date and catch site were quite unequal, leading to an unbalanced design. There was however most important to describe the last catch from these catches, because the last catch contained the parental fish and other ripe herring from their respective spawning population. When the sampling design is unbalanced, the order of where the effects appear in the ANOVA formula becomes important for the results (Kabacoff, 2011). More fundamental variables should be listed first in the formula. In the case of the parental fish, the catch sites were more fundamental than the catch dates and were placed first

in the ANOVA formula. In the model (3), Y represent the tested variable, while the following words represent two main effects and a possible interaction effect. If the higher order interaction was not significant, it was removed from the model. Interactions are mentioned in this thesis only if they were significant.

$$Y\sim \text{Catch site} + \text{Catch date} + \text{Catch site} : \text{Catch date} \quad (3)$$

The first generation herring data were also analysed using a two-way factorial ANOVA, one analysis per sampling date. Only the three main samples from both salinity groups were used in the analysis, this was done to avoid missing treatment data. The main effects in the analysis were the salinity and genetic background. The model can be described with this formula (4).

$$Y\sim \text{Salinity} + \text{Genetic background} + \text{Salinity} : \text{Genetic Background} \quad (4)$$

Several extra packages were used to create figures and tables in R statistics. The most frequently used packages with reference to their developers are summarized in Table 6.

Table 6: Name of the R-packages most used to create figures and tables in this thesis:

Type of plot or table	r-syntax	Package name with reference to developers
Mean plots	plotmeans(…)	gplots (Warnes et al., 2015)
Histogram	hist(…)	lattice (Sarkar, 2008)
Scatterplot	xyplot(…)	lattice (Sarkar, 2008)
Otolith outlines combined	*	Momocs (Bonhomme et al., 2014)
Summary tables	ddply(…)	plyr (Wickham, 2011)

* Cannot be summarized by one command

3 RESULTS

3.1 CHARACTERISTICS OF THE PARENTAL FISH GROUPS

There was a large size difference between the herring caught outside the coast of Bergen and the herring caught outside the coast of Stockholm (Table 7). The parental fish group that was caught off the Norwegian coast (Atlantic) had a higher mean vertebrae count than the parental fish group that was caught off the coast of Sweden (Baltic). Otolith shape was different in several aspects, between the parental populations, and between the parental fish.

Table 7: Summary of the biological characteristics of the parental fish groups caught at the respective sample areas off the coast of Norway (Atlantic) and Sweden (Baltic), at the three different sampling dates. The parental fish used were caught on the 21.05.13. sd = standard deviation.

Parental group	Catch date	Weight (g)		Total length (cm)		Vertebrae count		Number
		mean	sd	mean	sd	mean	sd	of fish
Atlantic	26.03.13	307.4	12.2	32.1	1.5	57.4	0.5	51
	13.05.13	304.8	44.7	33.1	1.9	57.6	0.9	9
	21.05.13	271.2	37.7	32.1	1.3	57.2	0.7	109
	Mean	284.1		32.2		57.3		169
Baltic	13.05.13	59.2	9.2	20.6	0.9	55.3	0.6	42
	21.05.13	49.1	5.7	19.8	0.8	55.1	0.6	48
	Mean	54.4		20.2		55.2		90
Total								259

Both parental population groups were mostly ripe and running (Table 8), but there were also some herring in the Atlantic group that had just spawned and were classified as spent or resting. The fish were caught at approx. the same place in each sample. No fish were caught in the Baltic Sea at 26.03.2013 in due to ice cover. At 13.05.2013, the deliverance of the Baltic herring gonads to Bergen was delayed, and the crossing were unsuccessful. A second attempt done on 21.05.2013, this was successful and resulted in the first generation herring used in this study.

Table 8: The maturity stages of the parental herring divided into Atlantic and Baltic origin. Stage 5 = Maturing, Stage 6 = Spawning, Stage 7 = Spent and Stage 8 = Resting.

Catch date	26.03.2013		13.05.2013		21.05.2013	
Maturity stage	Atlantic	Baltic	Atlantic	Baltic	Atlantic	Baltic
5	5	0	0	0	1	9
6	43	0	9	42	83	39
7	2	0	0	0	13	0
8	1	0	0	0	11	0

The sex ratio in the fish examined from the parental fish population groups was close to 1:1 in both the Atlantic and Baltic catches (Table 9). These fish do not represent the whole catch, however.

Table 9: The amount of males and females in the parental fish groups.

Catch date	26.03.2013		13.05.2013		21.05.2013	
	Atlantic	Baltic	Atlantic	Baltic	Atlantic	Baltic
Females	21	0	4	25	52	23
Males	30	0	5	17	56	25

The number of vertebrae were higher among the Atlantic parental population group than the Baltic parental population group (ANOVA $p << 0.001$). There was no difference in vertebrae number between the sexes (ANOVA, $p < 0.6$). Approx. 57 % of the Baltic parental population group had 55 vertebrae while more than 77 % of the Atlantic group had 57 or 58 vertebrae (Figure 8). There was however some overlap between the two populations. Individual herring could therefore not be assigned to belong to a population solely on the number of vertebrae. Both the Atlantic parental fish had 57 vertebrae, while the Baltic parental male had 55 vertebrae. These vertebrae numbers seems to be the most common phenotype within their respective populations.

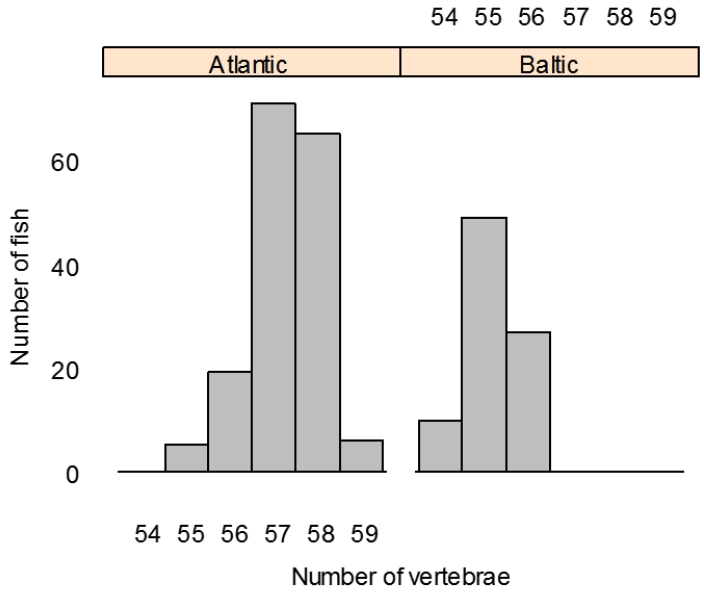


Figure 8: The distribution of number of vertebrae in the parental population groups.

The otolith shape analysis showed that the otoliths from the two populations were different in several aspects (the mean values are summarized in Appendix A, Table 1). The circularity of the Atlantic parental population otoliths was in general lower, than the circularity in the Baltic parental population otoliths (ANOVA, $p << 0.01$, Figure 9a). This means that the Baltic otoliths were more similar to a perfect circle than the Atlantic otoliths. The aspect ratio (AR) is the major axis divided on the minor axis. The Atlantic parental population had a higher AR than the Baltic parental population (ANOVA, $p << 0.01$, Figure 9b). This means that the Baltic parental population had wider otoliths than the Atlantic parental population. The perimeter/area was significantly different between the parental populations, this indicates that the Baltic parental population had more lobed otolith edges, than the Atlantic parental population (ANOVA, $p << 0.01$, Figure 9c).

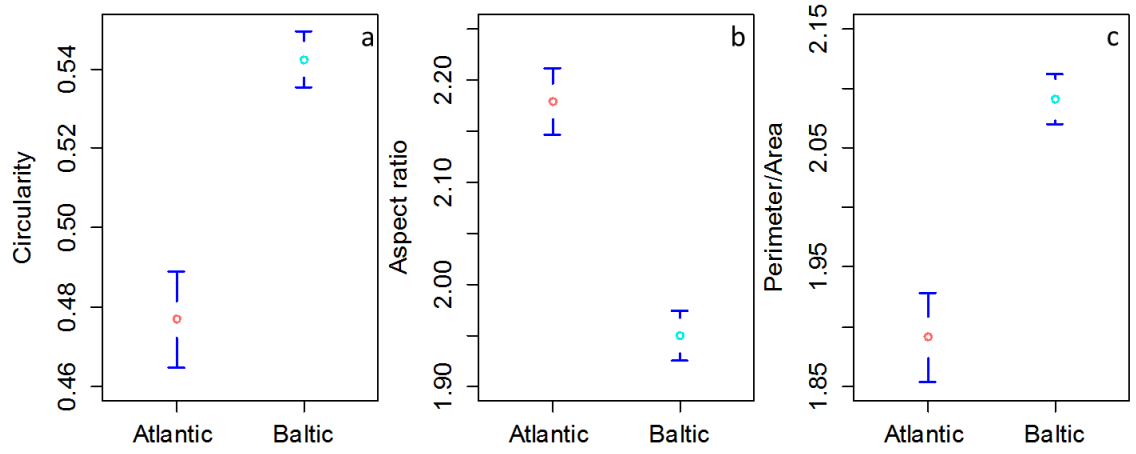


Figure 9: Mean otolith measurement values for the Atlantic and Baltic parental fish groups. The whiskers represent 95 % confidence intervals. Circularity ranges from 0-1, where 1 is a perfect circle. The aspect ratio is the major axis divide by the minor axis. Perimeter/Area is describing the smoothness of the otolith outline, the higher value the more lobes are potentially found in the otolith outline.

The otolith length (Feret's diameter) of the Atlantic parental population was higher than the Baltic parental population (ANOVA, $p << 0.001$), this was expected since the mean body size also was different. When otolith length was standardized by dividing on the total fish length of the individual fish, the Baltic parental population had longer otoliths, compared to body length, than the Atlantic parental population (ANOVA, $p << 0.001$, Figure 10).

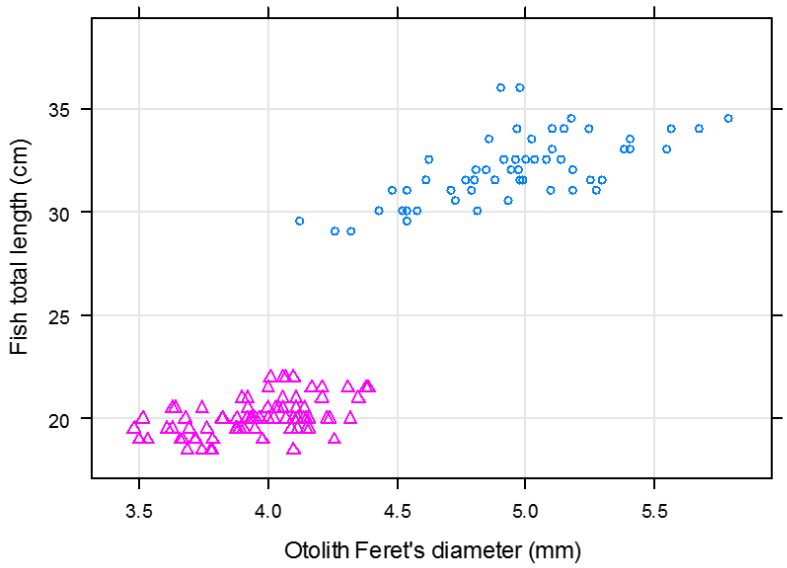


Figure 10: The relationship between herring total length and Otolith Feret's diameter. Triangles represent the Baltic parental fish group. The circles represent the Atlantic parental fish group.

The otolith shapes of the three parental fish used in this experiment is shown in Figure 11. If shape was strongly influenced by genetics, the offspring of the two different crosses would show similarities in otolith shape to their parents.

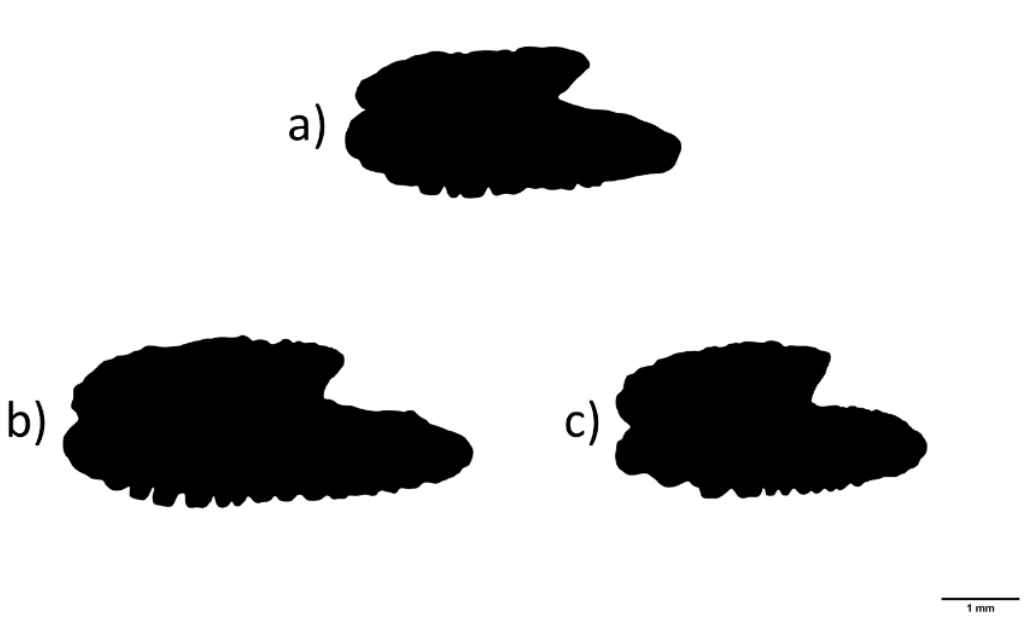


Figure 11: Otolith shapes of the parental fish used in this experiment. Atlantic female (a), Atlantic male (b) and Baltic male(c). The otolith outlines used in the shape analysis were based on silhouettes like these. The length of the scale bar equals 1 mm.

3.2 SIZE-AT-AGE AND FISH GROWTH OF THE FIRST GENERATION HERRING

In the first generation herring, several parameters were measured and compared, the mean values are summarized in Appendix B, Table 1. In the 187 DPH sample, an interaction effect was observed between salinity treatment and genetic group for fish weight (ANOVA, $p < 0.04$, Figure 12a). This interaction effect was also significant for fish total length (ANOVA, $p < 0.03$, Figure 12b). The hybrid genetic group was larger in the 16 psu salinity, while the purebred genetic group was larger in the 35 psu salinity. There was no significant difference in otolith size between the groups (ANOVA, $p < 0.3$, Figure 12c).

In the second sample, 297 DPH, the purebreds were heavier than the hybrids (ANOVA, $p < 0.01$, Figure 12d). In addition, the fish reared in the 35 psu salinity were heavier than the fish reared in 16 psu salinity (ANOVA, $p < 0.01$, Figure 12d). However, no significant difference in fish length could be found (ANOVA, $p < 0.3$, Figure 12e). No difference in otolith length between the groups was observed (ANOVA, $p < 0.3$, Figure 12f).

At 614 DPH, both hybrids and purebreds reared at 35 psu salinity were significantly longer and heavier than the fish reared in 16 psu salinity (ANOVA, $p < 0.01$ and $p << 0.01$, Figure 12h and 12g). Within each salinity treatment, the purebreds were also significantly longer and heavier than the hybrids (ANOVA, $p << 0.01$ and $p << 0.01$, Figure 12h and 12g). The purebreds reared in 35 psu salinity were approx. 5 % longer than the purebreds in the 16 psu salinity, while the hybrids reared in 35 psu salinity were approx. 3 % longer than the hybrids reared in 16 psu salinity. There was no difference in otolith size between the groups (ANOVA, $p < 0.2$, Figure 12i).

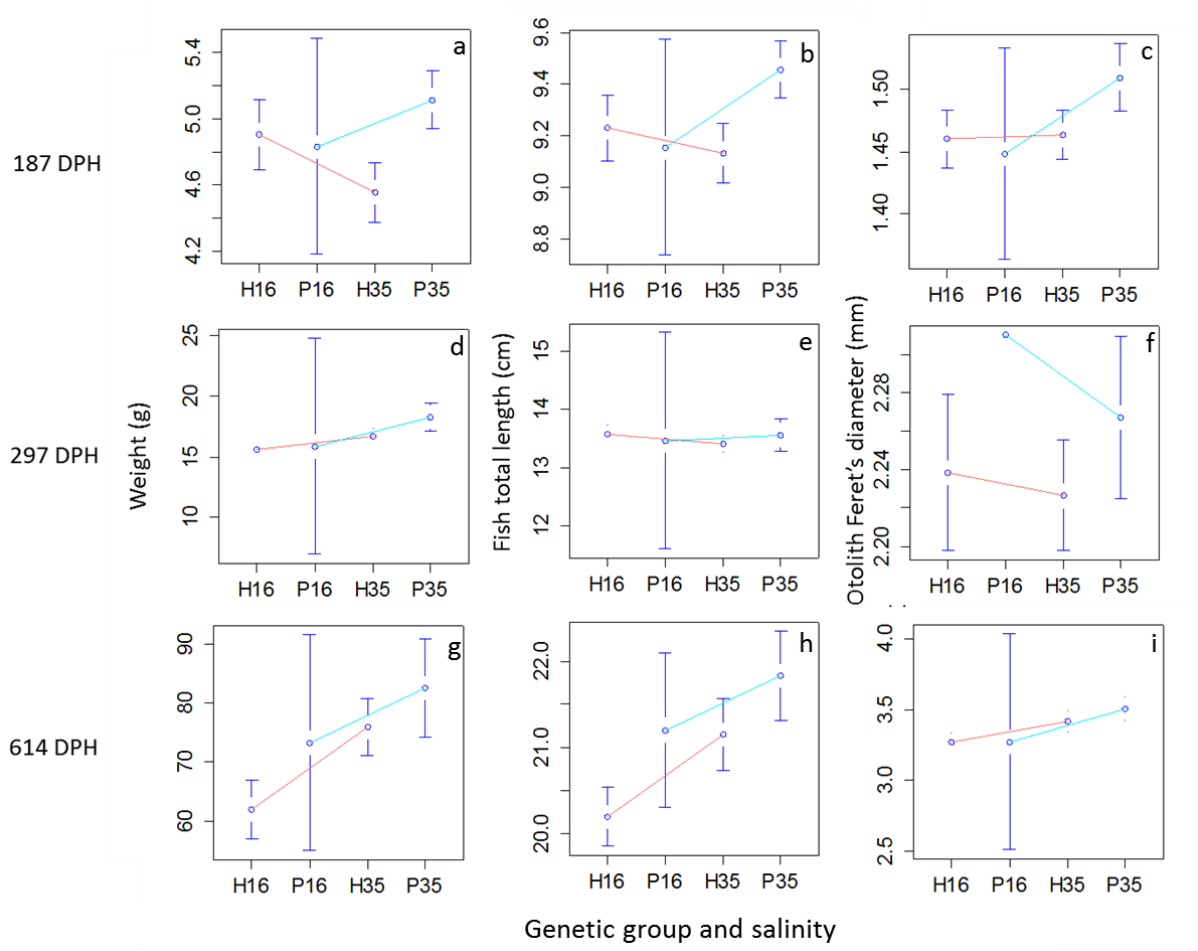


Figure 12: Mean values of fish weight, total length and otolith length in the first generation herring in the three main samples. Fish age is presented as days post hatching (DPH). Whiskers represent 95 % confidence intervals. H16 = Hybrids reared in 16 psu salinity, P16 = Purebreds reared in 16 psu salinity, H35 = Hybrid reared in 35 psu salinity, and P35 = Purebreds reared in 35 psu salinity. The pink line connects the mean values of the hybrids, while the blue line connects the mean values of the purebreds. This makes it easier to see differences within the genetic groups reared in different salinities.

The specific growth rate (SGR) was similar in the different salinities and genetic groups (Table 10). It appeared to be slightly lower in the 16 psu salinity treatment, at least from 187 - 297 DPH. From 297 - 614 DPH, there was very little difference in the specific growth rate between groups.

Table 10: The Specific growth rate (SGR, % body weight/day) of the different genetic and salinity groups. The SPG calculated for samples from 614 DPH was calculated using their real DPH of 610 DPH (16 psu) and 618 DPH (35 psu).

Genetic group and salinity	187-297 DPH	297-614 DPH
H16	0.46	0.19
P16	0.47*	0.21*
H35	0.51	0.20
P35	0.50	0.20

*May be unreliable due to small sample sizes (n = 3)

The mean length growth was not very different between the groups (Table 11). Nevertheless, it was a change in growth pattern between the two periods. At 187-297 DPH, the hybrid group and the 16 psu salinity group was growing faster, but at 297-614 DPH the purebred genetic group reared in the 35 psu salinity grew faster.

Table 11: Mean length growth (mm/day) in the different genetic and salinity groups.

Genetic group and salinity	187-297 DPH	297- 614 DPH
H16	0.40	0.21
P16	0.39*	0.24*
H35	0.39	0.24
P35	0.37	0.26

*May be unreliable due to small sample sizes (n = 3)

Otolith growth (µm/day) from 187 DPH to 297 DPH, were higher in the 16 psu salinity than the 35 psu salinity. This changed in the sampling interval, between 297 DPH to 614 DPH, where the herring reared in 35 psu salinity had a higher otolith growth than the others.

Table 12: Mean otolith growth (µm/d) of the different genetic and salinity groups.

Genetic group and salinity	187-297 DPH	297- 614 DPH
H16	7.1	3.2
P16	7.8*	3.0*
H35	6.9	3.8
P35	6.9	3.9

* May be unreliable due to small sample sizes (n = 2)

The fish grew steadily during the experiment as seen in the fish lengths in the different samples (Figure 13). Nevertheless, two exceptions could be observed. The sample collected at the 30th of September 2014 (482 DPH) contained several herring with jaw deformities (marked with green squares in Figure 13). These fish had not grown as well as the other fish

in this sample. On the 15th of January 2015 (590 DPH) sick or weakened fish (marked with orange squares in Figure 13) were specifically collected from the 35 psu salinity treatment, this group appears to be a little shorter than earlier and later samples.

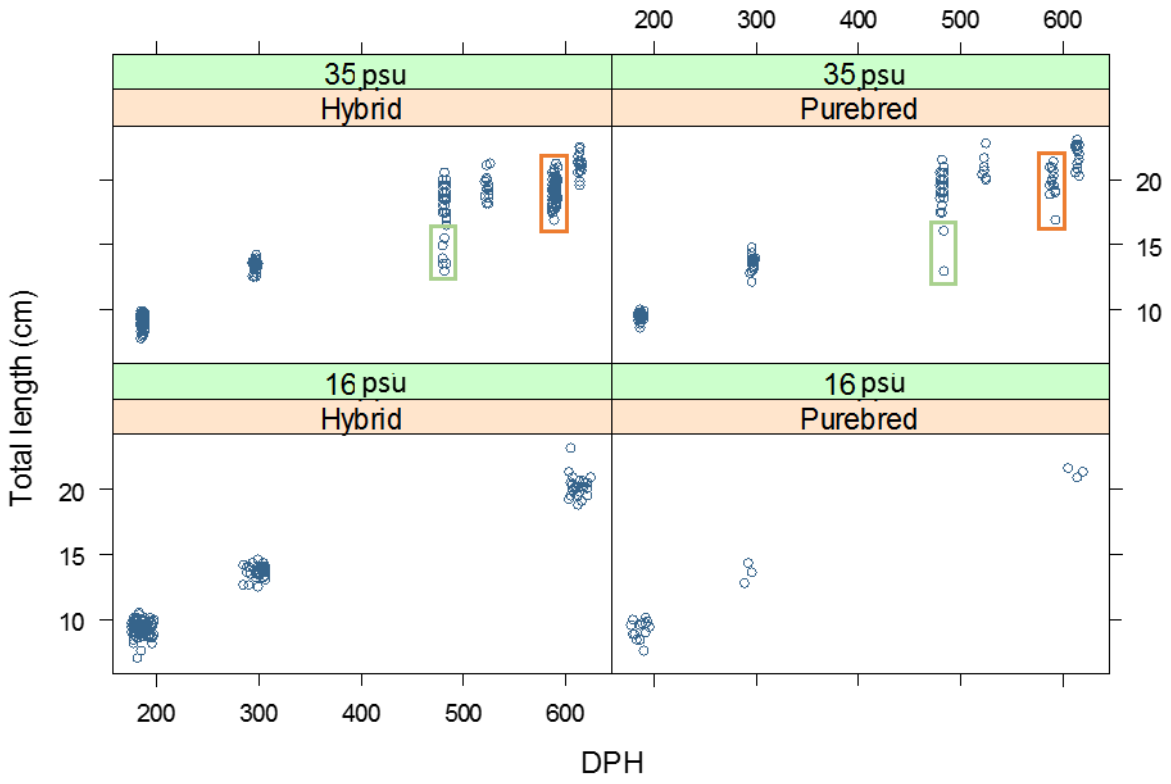


Figure 13: Total length of the first generation herring at all the different sampling dates, where each plot represent one genetic group in one salinity treatment. The green squares shows jaw deformed herring collected 482 DPH, the orange squares shows the sick and weakened fish collected 590 DPH. A Jitter setting of 1.6 has been applied in both directions, to better display the data points.

3.3 SURVIVAL AND SELECTIVE MORTALITY

In total, 489 hybrids and 178 purebreds were sampled from the two salinities. The original ratio between hybrids and purebred was 2:1 in both salinities. In the 35 psu salinity group, the ratio did not change much for the first two samples at 187 and 297 DPH. At 614 DPH the ratio had evened out closer to 1:1 (Table 13). The ratio changed most likely due to the Tenacibaculum infection (589 DPH sample, Table 14).

In the 16 psu salinity group, selective mortality had already taken place before the 187 DPH sample was sampled. At 187 DPH the ratio had changed to 7:1 hybrid and purebreds, at 297 DPH it was 12:1 and at 614 DPH it was 9:1. However, this was not a significant change between the three main samples (Table 13).

Table 13: The numbers and the percentages of purebred and hybrid first generation experimental fish in the samples collected at 187, 297 and 614 DPH.

Salinity	Genetic group	187 DPH*		297 DPH		614 DPH	
35 psu	Purebred	30	30 %	19	38 %	14	46 %
	Hybrid	69	70 %	31	62 %	16	53 %
	Total	99		50		30	
16 psu	Purebred	14	14 %	3	8 %	3	10 %
	Hybrid	85	86 %	37	93 %	27	90 %
	Total	99		40		30	

* Four fish was not possible to determine genetically in the 187 DPH sample.

Besides, the three parallel samples shown in the table above, three extra samples were collected from the 35 psu salinity. The ratio of hybrids and purebreds in these samples was similar to the original ratio of 2:1 (Table 14). This was, however, not the case for the 589 DPH sample, where the ratio was approx. 6:1 (Table 14). Nevertheless, this sample was not a random sample of all the fish in that tank, as sick and weakened fish were specifically selected.

Table 14: The number and percentage (of total number fish) of hybrid and purebreds from extra samples collected from the 35 psu salinity tank.

Salinity	Genetic group	483 DPH		524 DPH		589 DPH*	
35 psu	Hybrid	76	67 %	73	63 %	77	83 %
	Purebred	37	33 %	42	37 %	16	17 %

* Sick (moribund) and weakened fish sampled out for diagnosing infection.

In the first two samples, it was not possible to assign the juvenile herring into male and female due to their very small and immature gonads. In contrast, this could be done for the 614 DPH sample, where the ratio of males and females was relatively close to 1:1 in all the groups (Table 15). There were however some more females than males.

Table 15: The number of male and female fish in the 614 DPH sample.

Salinity	Genetic group	Males	Females
16 psu	Hybrid	10	16
	Purebred	0	3
35 psu	Hybrid	7	9
	Purebred	7	7

3.4 VERTEBRA COUNTS

The average vertebrae counts in the first generation herring from the two salinities were quite similar at 187, 297 and 614 DPH. There was no significant difference in mean vertebrae number between the salinity groups (ANOVA, $p < 0.3$, Appendix B, Table 2). The purebreds had a higher vertebrae count than the hybrids, with a mean vertebrae count of 56.4 vs 55.9 (ANOVA, $p << 0.001$, Figure 14). This difference was significant when the samples was analysed per sampling date and combined. When the samples were analysed combined a significant difference between the sample dates was observed (ANOVA, $p < 0.01$, Appendix B, Table 3). This could be due to relatively small sample sizes in the last two samples. The majority of the hybrids had 56 vertebrae (83 %) while the purebreds were almost equally divided on 56 (45 %) and 57 (51 %) vertebrae. Both groups had a proportion of fish with 55 vertebrae (Figure 14).

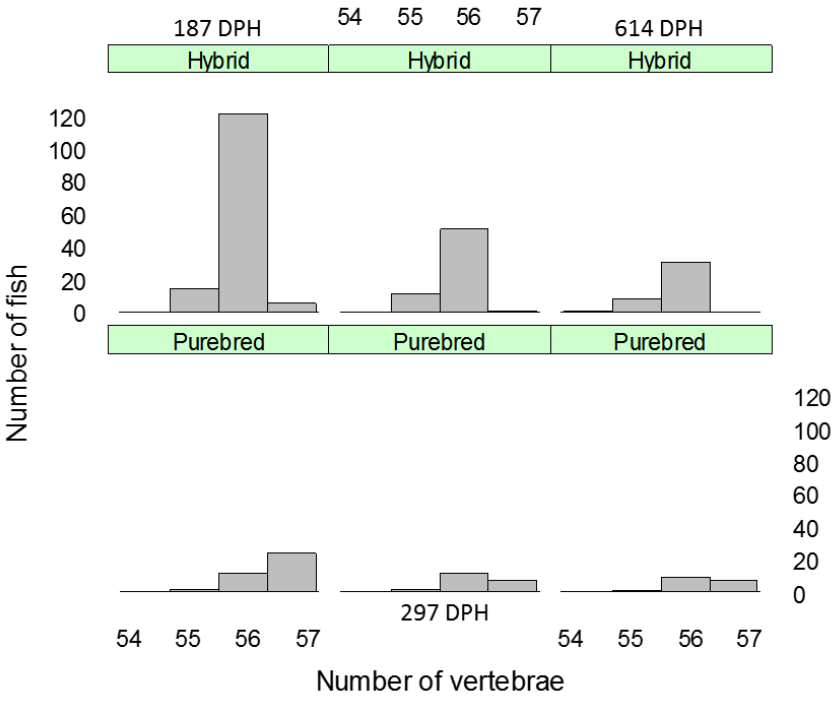
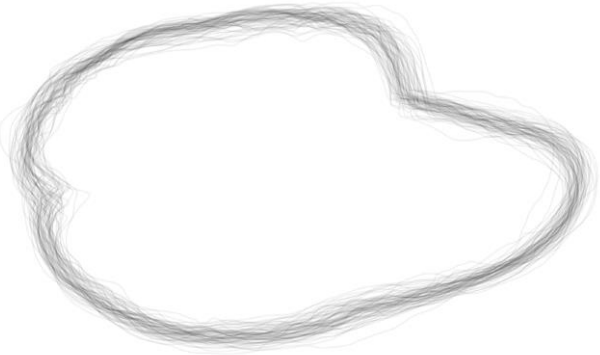


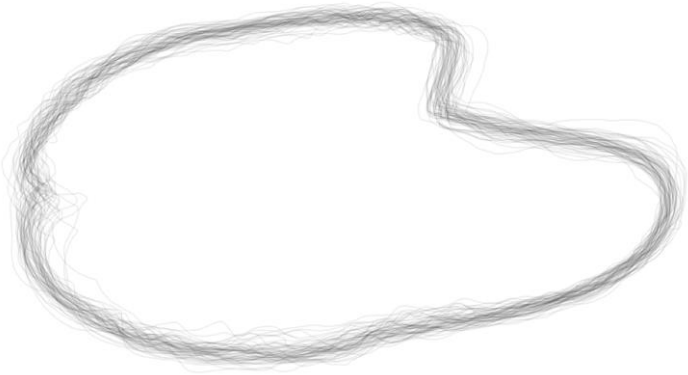
Figure 14: Vertebrae counts in the first generation herring, divided into genetic group and the three main sampling dates.

3.5 OTOLITH SHAPE AND WEIGHT

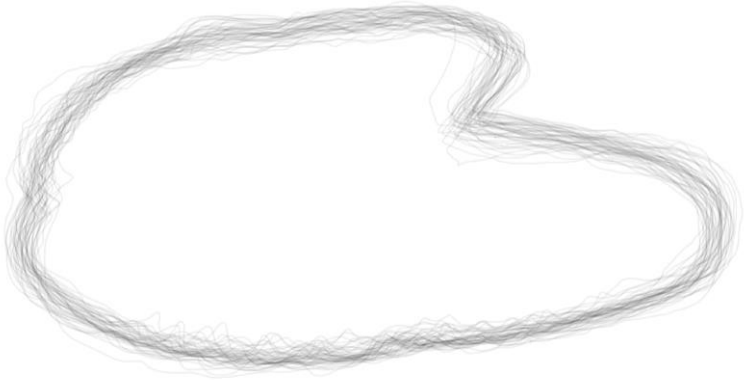
The herring were sampled from both salinity groups at 187, 297 and 614 DPH. All the samples were taken over a 14 months' time period. The development of the otolith shape in herring can therefore be described by looking at the data collected during this experiment. By combining all the outlines from the otolith shape analysis, Figure 15 a, b and c, represent the general development of shape in herring at 187, 297 and 614 DPH, respectively. The different genetic and salinity groups have not been separated into different figures. The figures gives an overview of where there might be more or less variation in otolith shape in herring otoliths. All the otoliths were scaled and aligned to each other in R statistics, size is therefore not a factor in these comparisons (Figure 15).



a)



b)



c)

Figure 15: A general development of otolith shape in juvenile herring, created using a subset of otolith outlines from the three main samples of the first generation herring. All otolith outlines were scaled and aligned to match each other. The areas of most variation can be seen as areas where the lines are more spread out. Each thin line represents the outline of an individual otolith. The different genetic groups and salinity treatments have not been separated into separate figures. The age of the fish at the different samples were a) 188 DPH, b) 483 DPH and c) 614 DPH. The 614 DPH sample contain only otolith outlines from the 35 psu salinity due to technical problems.

A general change in shape with age is clearly seen, while other factors are less evident. The following analyses were therefor undertaken on an age by age basis. The mean values are summarized in Appendix B, Table 4.

The otolith circularity was higher in the hybrids than the purebreds at 187 DPH (ANOVA, $p << 0.001$, Figure 16a). This means that the outlines of these otoliths were more similar to a perfect circle. This trend was also evident at 297 DPH (ANOVA, $p << 0.001$, Figure 16d) and at 614 DPH (ANOVA, $p << 0.001$, Figure 16g). No significant difference in otolith circularity between the salinity treatments was observed (ANOVA, $p < 0.2$).

The otolith aspect ratio was significantly higher for the purebreds, than the hybrids at 187 DPH (ANOVA, $p << 0.001$, Figure 16b). This means that these otoliths where more elongated than the others. The trend was also seen in the next samples at 297 DPH (ANOVA, $p << 0.001$, Figure 16e) and at 614 DPH (ANOVA, $p << 0.001$, Figure 16d). No effect of salinity on aspect ratio was observed (ANOVA, $p < 0.5$).

The perimeter divided by area, indirectly describes how lobed the edges of the otoliths are. A significant interaction between genetic background and salinity on the amounts of lobes in the otolith edge, was observed at 187 DPH (ANOVA, $p < 0.01$, Figure 16c). The purebreds had more lobed otolith edges in the 16 psu salinity than in the 35 psu salinity, while the hybrids had a similar amount of otolith edge lobes in both salinities. At 297 DPH the amount of lobes in the otolith edge was significantly higher in the purebreds than in the hybrids (ANOVA, $p < 0.05$, Figure 16f). There was no difference between the salinity groups (ANOVA, $p < 0.07$). In the 614 DPH sample, the otolith edge was more lobed in the 16 psu salinity than in the 35 psu salinity (ANOVA, $p < 0.01$, Figure 16i). No significant difference was observed between the genetic groups (ANOVA, $p < 0.6$, Figure 16i).

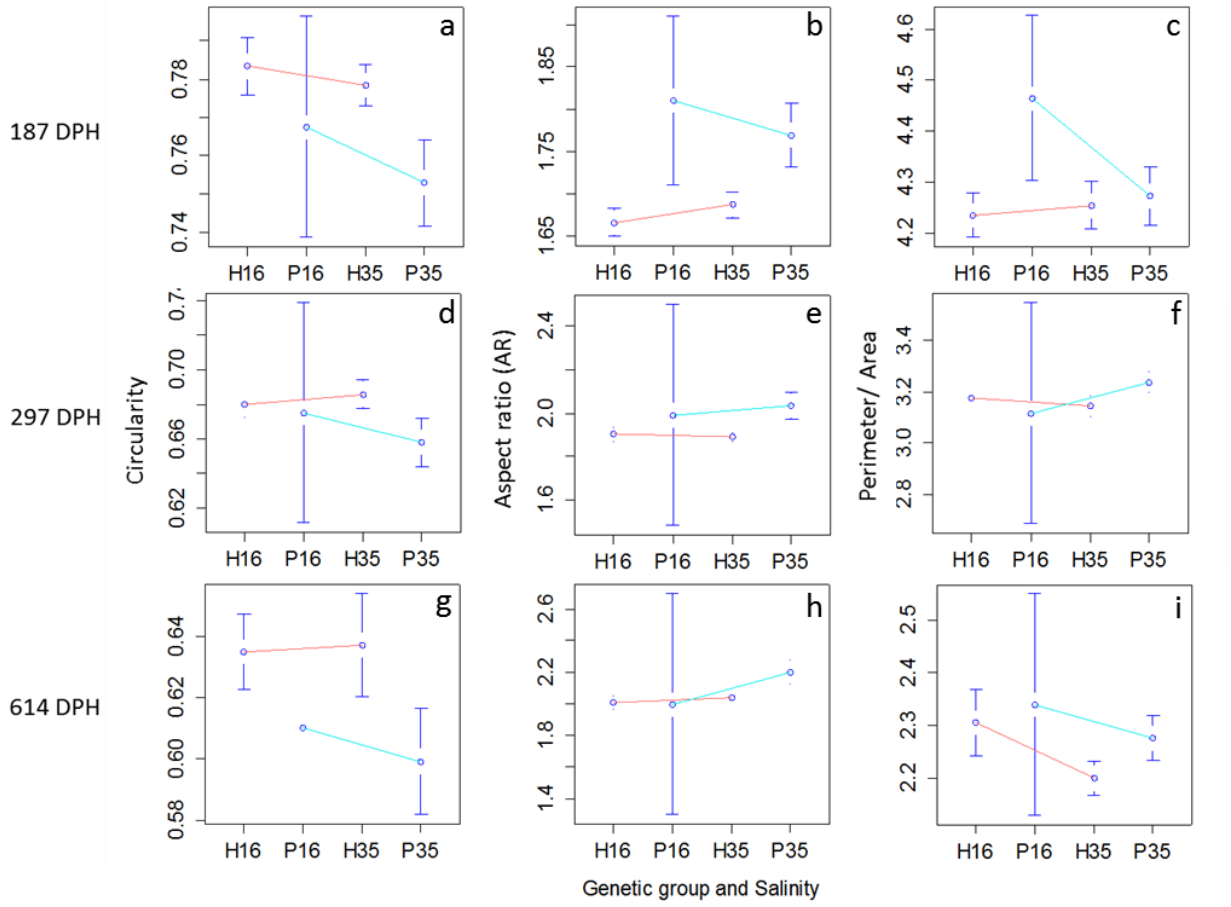


Figure 16: Mean values of otolith measurements of first generation herring divided into genetic groups, salinity, and age (DPH). Whiskers represent 95 % confidence intervals. H16 = Hybrids reared in 16 psu salinity, P16 = Purebreds reared in 16 psu salinity, H35 = Hybrid reared in 35 psu salinity, and P35 = Purebreds reared in 35 psu salinity. The pink line connects the mean values of the hybrids, while the blue line connects the mean values of the purebreds. This makes it easier to see differences within the genetic groups reared in different salinities.

Otolith weight was only measured for the otoliths used in the otolith chemistry analysis. These were sampled at 187 DPH. There was a significant interaction between salinity treatment and genetic background on otolith weight (ANOVA, $p < 0.01$, Figure 17). The purebreds had significantly heavier otoliths in the 35 psu salinity, than in the 16 psu salinity treatment, while the hybrids did not differ much in otolith weight between the two salinities.

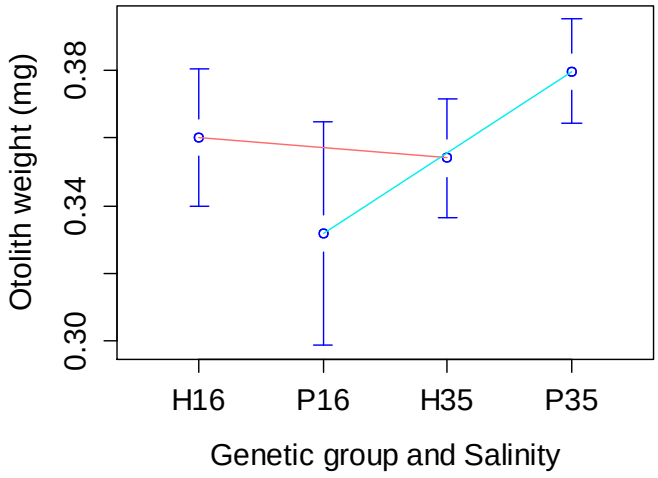


Figure 17: Otolith mean weight at 187 DPH in the first generation herring. Whiskers represent 95 % confidence intervals. H16 = Hybrids reared in 16 psu salinity, P16 = Purebreds reared in 16 psu salinity, H35 = Hybrid reared in 35 psu salinity, and P35 = Purebreds reared in 35 psu salinity. The pink line connects the mean values of the hybrids, while the blue line connects the mean values of the purebreds.

3.6 OTOLITH CHEMISTRY

The otoliths were analysed for their content of the following elements: Na, Sr, K, Mg, Mn, Ba and Ca, all the mean values are summarized in Appendix B, Table 5. All the otoliths analysed originated from the 187 DPH sample. The levels of sodium (Na) in the otoliths were in general high compared to the other elements investigated. Na levels were significantly higher in the 35 psu group than in the 16 psu group (ANOVA, $p < 0.05$, Figure 18a). There was no significant difference in otolith Na content between hybrids and purebreds (ANOVA, $p < 0.1$). The amount of strontium (Sr) was significant higher in the 35 psu salinity group (ANOVA, $p << 0.001$), and there was also a genetic effect where the purebreds had a higher Sr otolith content than the hybrids (ANOVA, $p < 0.05$, Figure 18b). An interaction effect between salinity and genetic group on the otolith potassium (K) levels was found (ANOVA, $p < 0.01$, Figure 18c). This means that both salinity and genetics together influence the K levels in the otoliths, yielding higher K for the purebred genetic group reared in 35 psu salinity and for the hybrid genetic group reared in the 16 psu salinity.

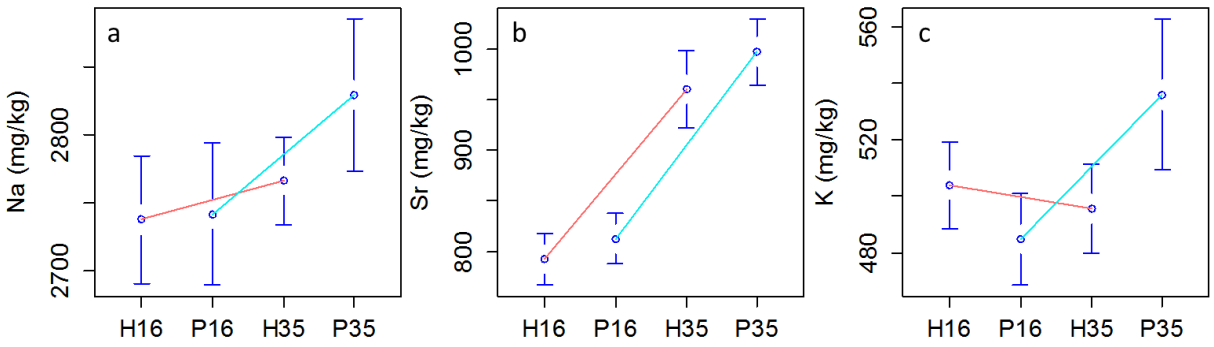


Figure 18: Mean levels of Na, Sr and K, mg element weight / kg otolith weight, in otoliths of the first generation herring (187 DPH). Whiskers represent 95 % confidence intervals. H16 = Hybrid genetic group in 16 psu salinity, P16 = Hybrid genetic group in 16 psu, H35 = Hybrid genetic group and P35 = Purebred genetic group in 35 psu salinity. The pink line connects the mean values of the hybrids, while the blue line connects the mean values of the purebreds.

The 35 psu salinity group had a significant higher magnesium (Mg) otolith content than the 16 psu salinity group (ANOVA, $p < 0.01$, Figure 19a). There was no significant difference between the purebred and hybrid genetic groups (ANOVA, $p < 0.3$). The amount of manganese (Mn) was not significant different in any of the salinity and genetic groups (ANOVA, $p < 0.6$, Figure 19b). When it comes to otolith calcium (Ca) levels, an interaction effect was observed between salinity and genetic groups (ANOVA, $p < 0.05$, Figure 19c). Barium (Ba) was found in low levels in all the otoliths, usually < 1 mg/kg with a few exceptions. Barium levels were therefore not compared with ANOVA.

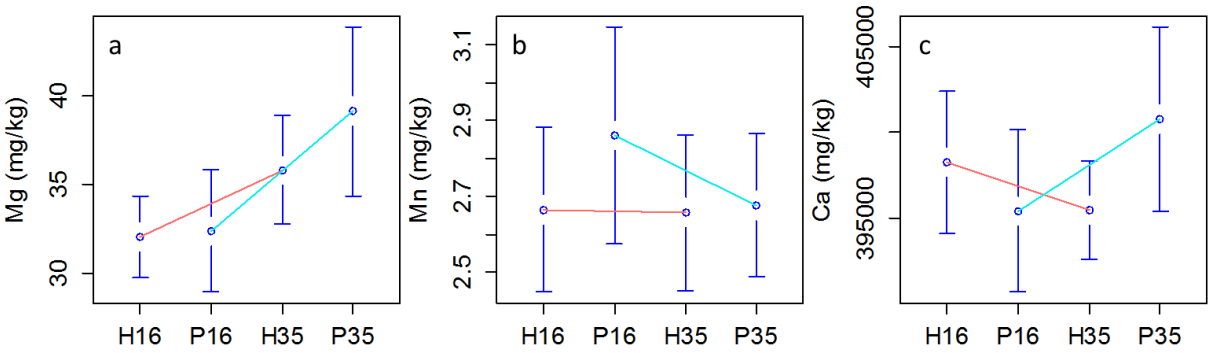


Figure 19: Mean levels of Mg, Mn and Ca in the first generation herring otoliths 187 DPH, in mg element weight / kg otolith weight. Whiskers represent 95 % confidence intervals. H16 = Hybrid genetic group reared in 16 psu salinity, P16 = Hybrid genetic group in 16 psu, H35 = Hybrid genetic group in 35 psu salinity, and P35 = Purebred genetic group in 35 psu salinity. The pink line connects the mean values of the hybrids, while the blue line connects the mean values of the purebreds.

4 DISCUSSION OF METHODS

4.1 EXPERIMENTAL DESIGN

In this experiment only one female and two males were used to produce the first generation herring. This was done to restrict the genetic variation in the offspring as much as possible, so that potential environmental influence on the phenotypic traits could be explored. Non-genetic maternal effects have previously been shown to affect otolith size at hatching in herring larvae (Høie et al., 1999). If there was more than one female parental fish it would have been harder to distinguish maternal effects from salinity effects and male genetic effects. There is however, a risk of choosing a parental fish that could be an outlier compared to the population it is supposed to represent, which could also influence the results. In the current study, the female seemed to be close to the average of the population, at least when it comes to length, vertebrae count and other measurements. Significant male parental effect were found in several measurements in the first generation herring in the current study. Paternal effects can cause difference in yolk-sac volume, larval length, lapillar area and RNA:DNA ratio (Bang et al., 2006). Høie et al. (1999) found no paternal effect on otolith size at hatching or egg mortality in herring. His experimental setup included two females and three males as parental fish, all of the parental fish was caught from the same population (Norwegian spring spawners). The difference between the males in that experiment, were probably smaller than in the current experiment, where the males originated from genetically and phenotypically different populations.

The experimental design was suitable for the hypotheses investigated in this thesis. Nevertheless, in regards to otolith chemistry and otolith shape it would have been ideal to keep the replications of the salinity treatments throughout the experimental period. At 122 DPH (4th October 2013), the four replicated experimental fish tanks, were merged into two fish tanks at ILAB. This merging of the tanks would potentially make it harder to discover any confounding tank effects. If there were any strong tank effects this could also be seen in a possible bimodal distribution of measured characteristic, this was however not seen in any of the traits measured. The herring was kept at the same salinity and temperature during the whole experiment, and the effect of these parameters were probably stronger than any potential confounding tank effects.

To be able to tell more about the relationship between the water chemistry and the otolith chemistry, a chemical analysis of the water used in the individual fish tanks would have been

useful (Coghlan Jr. et al., 2007). The sea water used was collected from deep water (105m) in the nearby fjord, while the freshwater used to mix the 16 psu water was collected from a nearby mountain lake (Svartediket). Before the water was pumped into the fish tanks it was filtrated through a 20 µm drum filter and UV-treated (pers.comm. Sindre Grimen). The water chemistry was however not measured during this experiment. The regulation of incorporation of elements in the otoliths could only be interpreted by differences between the different salinities and genetic groups, as the water chemistry was unknown.

The herring are very sensitive to handling due to the loose scales in the skin, and therefore can risk infections if not handled carefully. The use of anaesthesia (clove oil) always includes a risk of mortality (overdose). To avoid unnecessary stress for the fish, and the risk of unplanned mortality, the numbers of sampling dates were kept low. Luckily, no unplanned mortality took place due to handling and sampling.

4.2 Field studies vs experimental studies

It has been done many field studies on herring, e.g. Eggers et al. (2014), Johannessen et al. (2014), Langård et al. (2014) and Pampoulie et al. (2015). Field studies can give insight into how the "reality" looks like and give information about trends and changes over time. Several observations of different phenotypes and genotypes have been made of herring in the field e.g Runnström (1941) and Jørgensen et al. (2008). In a natural environment, many different biotic and abiotic factors will influence the fish at the same time. It can therefore be difficult to decide causative relationships between observed phenotypic variation, environment and genetic adaptation.

To understand variation and plasticity in phenotypic traits, experimental studies offer an opportunity to control the biotic and abiotic factors, which potentially influences the phenotypic traits in fish. Many experimental studies have been done on herring to investigate phenotypic variation, many involving different aspects of growth, e.g. growth rates between spring and autumn spawned herring larvae (Johannessen et al., 2000), and effects of light regime and prey concentration, on growth and survival in herring larvae (Folkvord et al., 2009).

4.3 Deformations, mortality, disease and injuries

The first generation experimental fish had low mortality in general, but some jaw deformations were observed. In addition, some wounds on the snout of the herring were common, especially in the 35 psu group. The reasons for this are unknown, but the density of fish in the 35 psu

group was higher than in the 16 psu group, this may have caused the fish to easier swim into the tank walls. At 589 DPH, the herring in the 35 psu tank in ILAB were diagnosed with a bacterial infection caused by *Tenacibaculum* spp., and was treated with antibiotics. This delayed the last sampling from this group. The infection may have influenced growth of the surviving fish. The fish that died or became weakened from the infection seemed to have a smaller mean body length. This can be an indication that the smaller fish somehow were more vulnerable to tenacibaculosis, or that the fish had been sick for a longer period before it were discovered and that it decreased the fish growth. The purebreds seemed to have better resistance to the infection than the hybrids. Ratio of hybrid and purebreds in the tank changed during this infection, indicating a selective mortality. This will be discussed in more detail in the fish growth, survival and selection chapter.

4.4 Measurements and analyses

To count vertebrae in fish is a job that requires precision, patience and practice. The method is vulnerable for human error. Failure to identify the first vertebrae or to recognize fused vertebrae would be the most likely source of count errors. In this study the first vertebrae dissections were done with guidance from an experienced research technician. There were also performed several control tests to ensure that the counting was done correctly. This was done during the dissection of the adult parental fish. To minimize error in counting, each fish was counted at least twice. There was no systematic difference in vertebrae nr of the herring counted from each samples.

The right otolith was chosen for both shape analysis and chemical analysis. This was done to produce the most standardized otolith images, and get more information from the same otolith. No systematic differences have, however, been observed between left/right otoliths size in herring (Libungan et al., 2015).

The otolith shape analysis were dependent on good quality standardized images for the measurements to be as correct as possible. Calibration pictures were taken in the beginning of every photo session. Due to rather large size differences in the otoliths, especially between the Atlantic and Baltic parental population, different magnifications were used when photographing the otoliths. Using different magnification could potentially cause noise in the measurements. No such noise were detected in the measurements of the otolith pictures. Broken and dirty otoliths could also create unreliable measurements. Very few otoliths were broken, and if they were discovered, they were excluded from the statistical analysis. Otoliths with dirt

on them were either washed in ethanol before photographing. If dirty otoliths were discovered after the images were taken these were excluded from the statistical analysis.

Chemical analysis of very small pieces of material can introduces several possibilities of errors in the measurements. The otoliths analysed were quite small (mean weight 0.36 mg) and could potentially be contaminated by dust and other small particles, this could have influence the results. Several precautions were made to avoid possible contamination of the samples. An auto pipette were used to add accurate amounts of acid and milliQ water into the centrifuge tubes where the otoliths were dissolved. Auto pipetting are can be influenced by the technique of the handler, this is especially important when dealing with small volumes. An auto pipetting test, were water was pipetted and weighted in a series of 10 times per volume was done, to determine the precision of the pipetting, before the preparations to the chemical analysis was performed. There were also some potential for interference in the mass-to-charge-ratio of ions in the ICP-MS machine. This could also have influenced the results. A few measurements were very different from the other measurements and were unlikely the real values. These measurements were excluded from the statistical analysis. The cause of these outliers were not detected.

5 DISCUSSION OF RESULTS

In this thesis, the aim was to investigate phenotypic plasticity in herring. Vertebrae counts, otolith shape, otolith chemistry and size-at-age was measured and analysed. The results from this experiment showed that mean differences in phenotypic traits of the first generation herring was more dependent on the genetic background of the herring than the salinity, with some exceptions. Both genetic and salinity effects will be discussed in more detail. Finally, the implications of the findings will be discussed in relation to herring population structure and fishery management.

5.1 NUMBER OF VERTEBRAE IN HERRING

There could not be found any difference in mean vertebrae number between the herring that where reared in 35 psu and 16 psu salinity. This result is in agreement with the study of, and other studies mentioned in Fahy and Ohara (1977). A different result was found by Hempel and Blaxter (1961) who described a correlation between salinity and myotome counts in herring larvae, reared at salinities ranging from 5 and 50 psu. The eggs in these experiments were however fertilized in 15 psu seawater. When they tried fertilizing the eggs in the respective salinities, they only got surviving offspring in 5 psu to 35 psu and no significant difference in myotome count. In the current experiment, the eggs were fertilized in the respective salinities. The 6 psu salinity group did however, not have good enough survival to be brought through to the juvenile experiment. The difference in myotome count in Hempel and Blaxter (1961) could be a result of shock from abrupt change in salinity instead of a pure salinity effect. Eggers et al. (2014) used the salinity, 10-15 psu, at the supposed spawning depth in Landvikvannet to explain the observed lower vertebrae count in Landvikvannet herring (compared to local coastal herring and Norwegian spring spawning herring). In the light of the results from the current study, this might be less probable, and other environmental effects or genetic effect should be explored. Hence, the first part of the null hypothesis in this study, which claims that the number of vertebrae is not influenced by ambient salinity, cannot be rejected.

However, a difference in mean vertebra number in the offspring of the two different male parental fish was found. The hybrid group had a mean ± SD vertebrae count of 55.9 ± 0.4, while the purebred group had a mean vertebrae count of 56.4 ± 0.6. Løken and Pedersen (1996) found a similar result when investigating the effect of temperature and genetic influence on vertebrae number in cod. They also found that the vertebrae count was more affected by paternal genetic contribution than maternal. This could not be seen in the data from the current experiment, as

there only was one female parental fish. The results found in the current study indicate that the vertebrae number of herring to some extent reflects the genetics of herring. It is however difficult to say anything about how this trait is inherited, as only three parental fish were used. The results from this study does however not exclude effect of other environmental factors on mean vertebrae count experienced by herring in the field. Surface water temperature has been shown to be opposite related to the mean number of vertebrae in cod (Brander, 1979, Løken and Pedersen, 1996), where low temperature led to increased number of vertebrae. The second part of the null hypothesis, which states that the mean vertebrae number is not influenced by the male genetic origin, can thus be rejected.

The Atlantic parental population group had a mean ±SD vertebrae count of 57.3 ± 0.8 with a maximum observed total length of 38 cm. The Baltic parental population on the other hand, had a mean vertebrae count of 55.2 ± 0.6 with a maximum observed total length of 22 cm. Based on mean vertebrae number, the Atlantic parental population could belong to the Norwegian spring spawning population which have a mean vertebrae count of approx. 57.2 or above (Runnström, 1941). The Baltic parental population mean vertebrae count are similar to Baltic spring spawning herring collected by Ryman et al. (1984) in the Vaxholm area (~59°N). The migratory behaviour of the parental populations have however not been explored in the current study, but the vertebrae counts and the maximum observed length can give some ideas of why there is a genetic difference in mean vertebrae count between the populations. Herring that undertake long migrations, like the Norwegian spring spawning herring, does in general, have a higher mean vertebrae count than the coastal more stationary populations, they also grow faster and have a longer maximum length. This phenomenon is also true for Atlantic cod that have populations with different migratory behaviour (Løken and Pedersen, 1996).

The maximum observed length in the population has been proposed to be determining of vertebrae count in herring (Lindsey, 1975). Maximum length have been correlated with vertebrae counts across species and between populations (Lindsey, 1975). There is a size-dependent cost of spawning migration, where the smaller individuals have a higher relative energy loss compared with larger herring (Slotte, 1999). This might partly explain why migrating herring populations tend to grow larger and faster than non-migrating herring populations. The vertebrae number may be a side-effect from the selection for larger size. The larger fish in the Norwegian spring spawning population could also get to the most suiting spawning ground at Møre to spawn (Vikebø et al., 2012), and their offspring would potentially

have more food available than the smaller herring. This would increase the survival of this group and increase the selection for being bigger.

5.2 DEVELOPMENT OF OTOLITH SHAPE

As the herring grew the otolith shape, changed in several aspects. In general, the otoliths in both groups grew more elongated and relationship between otolith perimeter and otolith area decreased with age. This confirms the results of Bird et al. (1986) that found that otolith shape in juveniles are different from the otolith shape in adult herring. It also corresponds to the results of Hüssy (2008), who found that otolith shape formation in cod is an ontogenetic process which can be influenced by the environment. In this study, the shape was found to differ significantly in regards to circularity and aspect ratio in all the main samples, collected at 187, 297 and 614 DPH. This suggests that otolith shape is changing throughout the juvenile period in herring.

The hybrids had a significant higher otolith circularity than the purebreds in all the main samples. This means that the hybrids had relatively wider otoliths than the purebreds. Hüssy (2008) found that otolith shape is influenced by feeding levels in cod, and not by temperature. Higher feeding levels resulted in a wider otolith shape due to more lobes formed in the cod otolith (Hüssy, 2008). In the current study, the wider otoliths were found in the fish with lowest growth rate (hybrids) and lowest length at age. The food availability was however the same in both salinity treatments. Campana and Casselman (1993) revealed that growth rate contributed more to the variation in cod otolith shape than the origin of the stock, when they compared cod population from the coasts of Canada, USA and Iceland. Hüssy (2008) also found covariance between growth rate and otolith shape parameters in cod. This implies a genetic basis for otolith shape that is closely related to growth rate in fish. There was some uncertainty in the otolith shape data concerning the purebreds reared in 16 psu salinity at 297 and 614 DPH. Only 2 otoliths from this group were analysed from each sample date. Based on the otolith circularity and aspect ratio results, the last part of the null hypothesis, which claims that otolith shape does not reflect male genetic origin, can be rejected. The first part concerning the salinity cannot, as there was no effect of salinity on otolith circularity and aspect ratio.

The perimeter divided on the area of the otolith can give an indication of how lobed the otolith edge is, as an edge with many lobes would give a longer perimeter, compared to the area of the otolith. This shape parameter was variable between the main samples. At 187 DPH there was a significant interaction between salinity and genetics regarding this parameter. The purebreds had potentially more lobed otoliths in the 16 psu salinity treatment, while the other groups

where similar. This was the only otolith sample where the purebreds were well represented (12 otoliths) in the 16 psu salinity. At 297 DPH the purebreds had more lobed otolith edges than the hybrids. At 614 DPH this effect was no longer apparent; in this sample the herring reared in the 16 psu salinity had more lobed otolith edges. The amount of lobes in the otolith edge was affected of both genetic background and salinity, and the null hypothesis, which states that the amount of lobes in the otolith edge is not influenced by the salinity or the male genetic origin, can therefore be rejected.

Otolith weight was only measured for the otoliths used in the otolith chemistry analysis. A significant interaction between salinity and genetic background was found on otolith weight. The otolith weight was influenced by the salinity in the purebreds, but not in the hybrids. The heaviest otoliths was found in the purebreds reared in 35 psu salinity, while the lightest otoliths were found in the purebreds reared in 16 psu salinity. An intermediate mean otolith weight was found in the hybrids, in both salinities. The cause for this is unknown.

The otoliths of the Baltic parental population showed a higher circularity, than the otoliths of the Atlantic parental population. The otoliths of the Atlantic parental population were more elongated, than the otoliths of the Baltic parental population. This corresponds well with the findings of more circular otoliths in the hybrids. In the parental fish groups, however, the Baltic parental population had a significantly more lobed edge than the Atlantic parental population. This was not apparent in the first generation hybrids before 614 DPH. The otolith length compared with body length was significant higher in the Baltic parental population. Larger otoliths compared to fish length have previously been seen as a sign of lower growth rate in the population (Hare and Cowen, 1995). This relationship was not different between the first generation herring groups. The offspring seems to reflect the otolith shapes of parental population in some aspects, at least late in the juvenile stage. This supports the influence of genetic effects on otolith shape in herring.

5.3 OTOLITH MICROCHEMISTRY

Strontium levels were significantly higher in the first generation herring reared in 35 psu salinity, and interestingly also significant higher in the purebreds within both salinity levels. The Sr levels in the otoliths have been proposed to reflect the salinity of the ambient water (Secor, 1992, Campana and Thorrold, 2001). Different Sr levels between purebreds and hybrids in in this study suggest that there is a difference in regulation and incorporation of strontium in the otoliths of the two genetic groups. If the first generation herring is representative for the

physiology of the parental populations, this could indicate that the degree of regulation of Sr is different in the two parental populations. This cannot be concluded from the data from this experiment, as there was only three parental fish used to produce the first generation herring. The salinity in the natural environment experienced by the two parental populations is very different in regards of the level and stability of salinity, and some kind of regulatory adaptation to their own environment is expected. The Sr levels in juvenile herring otoliths have been shown to be high at temperatures less than 5 °C, possible caused by physiological changes in response to low temperatures (Townsend et al., 1992). In the current experiment, the temperature was kept constant between 8-9 °C and similar between the two salinity treatments. The temperature has most likely not influenced the Sr otolith levels in this study. The null hypothesis, otolith microchemistry is not influenced by ambient salinity or by male genetic origin, can be rejected for otolith strontium levels. This means that both the environment and fish physiology influence the otolith strontium level in herring. Otolith barium levels in fish have also been related to salinity (Tabouret et al., 2010). Very little Ba was found in the otoliths analysed in the current study, this can be a result of little Ba in the water or strict regulation of Ba in the herring. However, based on the data in this study the null hypothesis for barium cannot be rejected.

Otolith magnesium was found to be higher in the 35 psu salinity than in the 16 psu salinity. There was no difference between genetic groups. This indicates a strong effect of the ambient environment and might be a reflection of the relative high concentration of Mg in seawater (Stewart, 2004). In this experiment, seawater was mixed with freshwater to create the 16 psu salinity and would probably contain the same ions in a lower concentration. The null hypothesis, otolith levels of Mg is not influenced by ambient salinity or by male genetic origin, can thus not be rejected for genetic origin but can be rejected when it comes to salinity.

Otolith potassium levels were only higher in the purebred genetic group reared at 35 psu, than the other genetic and salinity groups. This means that under some circumstances, the K levels will be higher in this genetic group. This was peculiar since potassium are under physiological regulation and are less likely to reflect the environmental chemical composition than other elements (Elsdon et al., 2008). It is however interesting that only one group had higher levels of this element in the otoliths. The null hypothesis can be rejected for otolith potassium levels.

An interaction effect between salinity and genetic background was found in otolith Ca levels. Hybrids in 16 psu, and purebreds in 35 psu, had higher levels than the other groups. As otolith chemistry usually is described in relation to Ca, this difference is needed to be accounted for when comparing the result of this study with other studies that uses Ca ratios.

5.4 FISH GROWTH, SURVIVAL AND SELECTION

At 187 DPH the first generation herring had reached a mean length of over 9 cm. Length and weight was influenced by a significant interaction between genetic effect and salinity effect. The hybrids were larger in the 16 psu salinity, while the purebreds were larger in the 35 psu salinity. This was expected since they probably would grow better in salinities that were similar to the salinities in their natural nursery areas (Ern et al., 2014). A salinity that is different from the natural salinity range of the fish could potentially cause reduced growth, due to increased effort in maintaining the homeostasis by osmoregulation (Ern et al., 2014). The results from the current study suggest that ambient salinity similar to the natural salinity in the nursery ground, enhances the growth in the juvenile period, from hatching to 187 DPH. An alternative cause could also be that environmental stress caused by the salinity reduced the growth in the purebreds reared in 16 psu and the hybrids reared in 35 psu salinity (Sampaio and Bianchini, 2002).

A mean total length of 13 cm or above was reached at 297 DPH in the first generation herring. There mean length was not different between the groups, nevertheless the purebred genetic group and the fish reared in the 35 psu salinity, were significantly heavier than the other groups. The small difference in size between the groups indicate that the purebreds grew a little faster than the hybrids in the period between 187-297 DPH.

At 614 DPH the mean total length was above 20 cm in the first generation herring, and both length and weight were significantly different between the groups. The herring reared in 35 psu were larger than the herring reared at 16 psu. The purebreds was also larger than the hybrids within in each salinity treatment tank. This suggest that the purebreds grew slightly faster than the hybrids in both salinity treatments, and that both genetic groups grew better in the 35 psu salinity in this part of the juvenile period, 297- 614 DPH. This indicates that the 35 psu salinity was advantageous for both purebreds and hybrids at this stage of the juvenile growth period, when the fish were between 14 - 21 cm in total length. Faster growth in full salinity water have also been observed in e.g. the euryhaline flounder, *Paralichthys orbignyanu* (Sampaio and Bianchini, 2002) and Tilpia, *Oreochromis mossambicus* (Borski et al., 1994). Other studies, concerning fish growth in different salinities, have showed unlike responses to various salinities between species (Ern et al., 2014). In theory, intermediate salinities that are nearly isosmotic with the blood (11-12 psu) would be energy saving, as the fish could use less energy on osmoregulation and more on e.g. growth (Sampaio and Bianchini, 2002). Different studies have however found that this not to the rule. Intermediate rearing salinities results in higher growth

in some species e.g. sea bream (Woo and Kelly, 1995) and Atlantic cod (Imsland et al., 2011), while not in others e.g. Southern flounder (Daniels et al., 1996) and bluefish (Buckel et al., 1995). The results of the current study show, however, that the growth in herring juveniles from 297 to 614 DPH was better in 35 psu than in 16 psu salinity. In a natural environment, however, other factors like temperature, light conditions and food availability will also affect the growth of the fish (Folkvord et al., 1997, Boeuf and Payan, 2001). When compared with the parental size-at-age there is expected to be a different growth rate between hybrids and purebreds, as the Baltic parental male were 22 cm in total length and 8 years old. The Atlantic parental fish on the other hand, was both longer and younger, approx. 31 cm in total length and 5 years old.

The original ratio of hybrids and purebreds in each fish tanks was 2:1. There was a change in ratio to approx. 9:1 in the 16 psu salinity group before the first sample was collected for this study at 187 DPH. In an earlier stage of development, the purebreds have not been able to survive as well as the hybrids in the 16 psu salinity. In the 35 psu salinity treatment the ratio between hybrids and purebreds was more stable at a 2:1 ratio. Which suggests that the hybrids had a wider salinity tolerance range than the purebreds. One potential cause for the selective mortality on purebreds in the 16 psu, is poorer osmoregulation capacity during the embryonic, larval or early juvenile stage. Fish tend to be more vulnerable to osmotic stress from the environment during the early developmental stages (Varsamos et al., 2005). If the salinity was the cause of the selective mortality observed, this could be explained by relating it to the salinity in the natural spawning areas of the Atlantic and the Baltic parental fish groups. The Atlantic parental population group spawns in an area of approx. 31- 34 psu while the Baltic parental population group was caught at salinities of approx. 6 psu (A. Folkvord pers. com.). The hybrids would then genetically be more adapted to handle both salinities from the start of their life, while the purebreds would be more adapted to 35 psu salinity. This also indicates that the wide salinity tolerance observed in herring (Blaxter and Holliday, 1962) may be different for different herring populations spawning in environments with different salinities.

A second event of selective mortality took place in the end of the sampling period, approx. 589 DPH. An outbreak of tenacibaculosis which is an infection caused by the bacteria *Tenacibaculum spp.,* occurred in the 35 psu salinity fish tank. The infection affected more hybrids than purebreds, 83 % of the 93 fish taken out due to sick and weakened condition were hybrids. This was different from the previous samples were the hybrid part of the samples ranged from 62 - 67 %. This indicated that the hybrids had less resistance against this infection. As this was not studied in detail this cannot be fully explained, but some speculations can be

made based on the biology of the pathogen. One of the possible treatments for this infection is a bath in water with low salinity (Avendaño-Herrera et al., 2006). In the Baltic Sea there is a lower salinity than in the Atlantic Ocean, and *Tenacibaculum spp*. may not be able to survive in this region. If the pathogen is not present in the area this could potentially lead to lower resistance in the hybrids, this can however not be concluded from the data in the current study. In the 614 DPH sample, the ratio of hybrids and purebreds were close to 1:1 in the 35 psu salinity, this was possible a result of the selective mortality caused by the tenacibaulosis infection.

5.5 PHENOTYPIC PLASTICITY

Phenotypic plasticity was observed in some of the phenotypic traits investigated. The salinity influenced the size-at-age in both genetic groups. At 187 DPH the size-at-age was highest in the more "natural" salinity. This corresponds to 16 psu for hybrids and 35 psu for purebreds. The trends in size-at-age changed during the experiment, at 614 DPH the highest size-at-age was found in the 35 psu salinity treatment for both the genetic groups. From 297 – 614 DPH of the juvenile period both genetic groups grew better in the 35 psu salinity. Increasing salinity have been coupled with increased growth in some tilapia species and a coupling between the production of growth hormone and osmoregulation in fish (Borski et al., 1994). These results could also be explained by an ontogenetic development of osmoregulation capacity in herring juveniles (Varsamos et al., 2005).

At 614 DPH, the herring otoliths originating from fish reared in 16 psu salinity, had a more lobed edge compared to the otoliths from fish in 35 psu salinity. This could also be described as a phenotypic plasticity influenced by salinity. The other otolith shaped parameters were more influenced by the genetic background of the fish. Otolith weight were plastic between the two salinities in the purebreds, but not in the hybrids at 187 DPH. This indicates that phenotypic plasticity might be different in the different herring populations.

The phenotypic plasticity observed in herring in the wild might also be a result of genetic adaptation and not necessarily the effect of the environment, at least not the salinity. Other environmental effects in addition to salinity will be present in the natural environment. Herring might show more plasticity in the traits measured in this study when exposed to other environmental effects. Limborg et al. (2012) found genetic markers that were correlated with salinity and temperature within the Baltic Sea, and suggested that this was an indication of local adaptations between different spawning populations. The results from the current study suggest

that the genotype is important when it comes to variation in morphometric and meristic traits in herring populations.

5.6 MANAGEMENT IMPLICATIONS

The salinity in the nursing areas have previously been assumed to influence the vertebrae count in herring, possibly based on the research done by Hempel and Blaxter (1961). The current study suggests that salinity in the nursery area of herring may not be important in developing the number of vertebrae. These finding indicate that at least intermediate and oceanic salinity seems to have little effect on this trait. This can influence the way we look at herring populations and why they are different. The genetic comparisons between suspected populations have shown some genetic difference (Teacher et al., 2013, Pampoulie et al., 2015), this support the results from this study as most of the differences between the offspring were genetic and not a result of different salinities. The genetic influence on vertebrae number supports the use of mean vertebrae number to separate herring populations. As this would not change between year classes with different environmental condition in the nursery areas. However, other environmental effects like temperature is most likely important in developing this trait, either directly or indirectly as it influences the growth rate in herring.

Research on otolith shape has become increasingly popular, as new methods have made the analysis more sensitive for shape differences than earlier. The results of this supports findings of Vignon (2012) and Hüssy (2008) that found that the otolith shape is changing with fish size, in young fish. Otolith shape analysis may therefore, be influenced by ontogenetic effects when comparing young herring of different size and age. This can be critical when looking for population specific differences in shape. The time when the adult otolith shape stabilizes should be determined, to avoid ontogenetic effects when comparing shape between populations. There was an effect of male parental fish on shape, which indicates that the shape to some degree is inherited. The environments effect on otolith shape should be investigated further, to better understand the development of otolith shape. Other environmental factor like temperature, light conditions, food availability and pollution may also influence the shape in a field setting.

Otolith chemistry has also become popular in investigations of population dynamics and life history in fish e.g. Geffen et al. (2011) and Quinn et al. (2014). In this study it has been shown that the strontium levels in the otoliths reflects the ambient water chemistry and the inherited physiology of herring. This difference in physiology may be important to account for when comparing different populations from different areas. Estimations of migration from otolith

chemistry can therefore not be compared directly between populations using otolith chemistry without background knowledge of the physiologic trends in the populations.

6 CONCLUSIONS

The number of vertebrae seems to be more influenced by the number of vertebrae in the parental fish than the salinity in the ambient environment.

The otolith shape grows from a more circular to more oblong shape as the fish gets older. The shape in the offspring also seems to be affected by otolith shape in the parental fish. The circularity of the otoliths decreased with age and the aspect ratio increased. The hybrids had a more circular otolith shape than the purebred herring. The amount of lobes in the otolith edge changed in the different groups as the fish grew. At 614 DPH, the herring in the 16 psu had more lobed otolith edge than the herring in the 35 psu. The Baltic parental fish had also more lobed otoliths than the Atlantic parental fish. This indicates that the salinity might create more lobed otolith shape in the late juvenile stage.

Both physiology (genetics) and the ambient environment (salinity) influenced the otolith chemistry. The strontium levels were higher in the high salinity and in the purebred group, which indicate a difference physiology between the genetic groups and an influence of the salinity of the water.

The purebred group had the largest size at age at 614 DPH and they also had the highest specific growth rate. Both hybrids and purebreds had a larger size in 35 psu salinity. This indicates that growth in juvenile herring is influenced by both salinity and genetic background.

The ratio of hybrids and purebreds in each tank changed before the main samples was collected, possibly due to selective mortality caused by salinity. The ratio of purebreds in the 16 psu treatment was lower than expected. During the sampling period, an outbreak of an infection also changed the ratio of hybrid and purebreds in the 35 psu salinity, and the hybrids seemed to be most susceptible to the infection.

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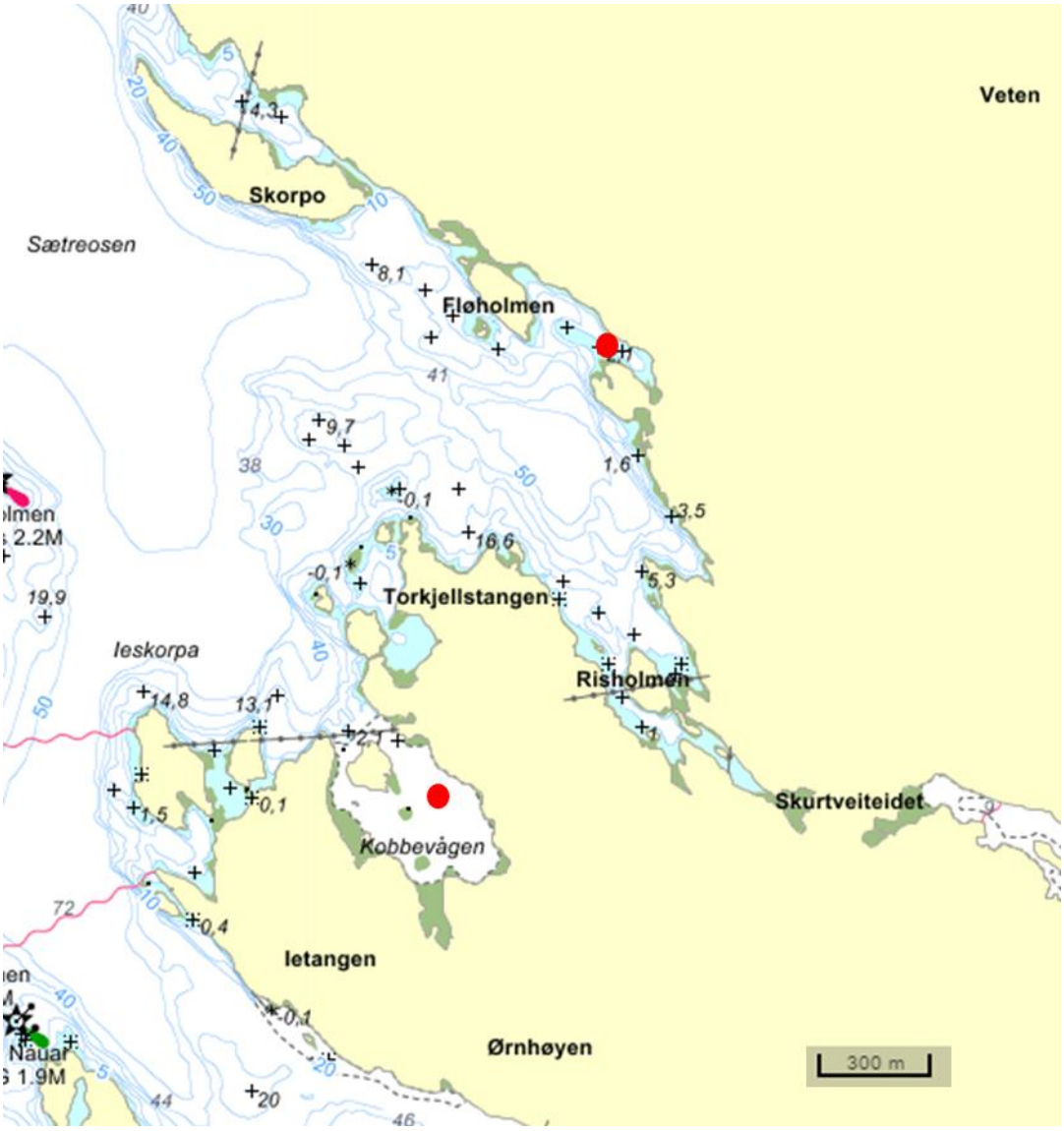
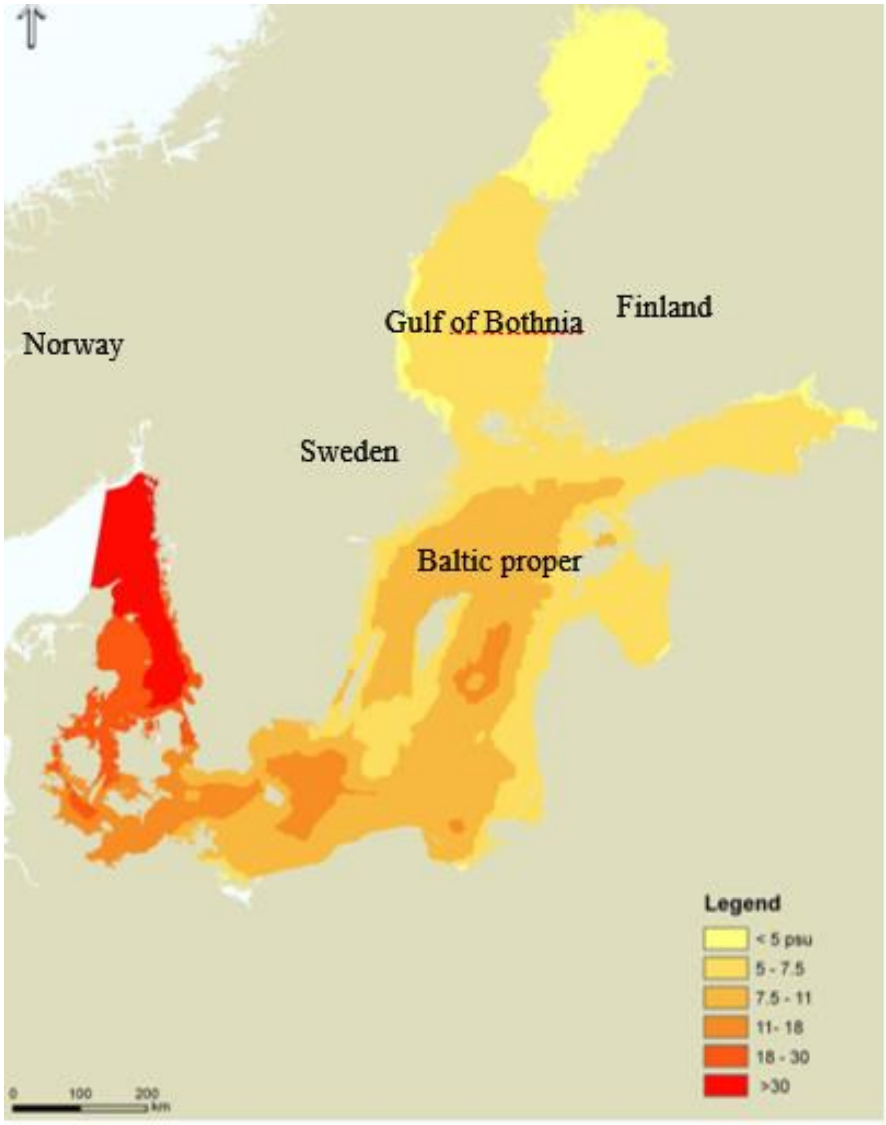
A. PARENTAL POPULATION GROUPS



Appendix A, Figure 1: Map of sampling area where the Atlantic parental population were caught. The main fishing sites are marked with red dots. The coordinates for the catch site in Kobbevågen were 60°34'11.2"N 5°0'18.9"E.

Appendix A, Figure 2: Sampling site for where the Baltic parental population were caught. The main fishing area is marked with blue line. The coordinates for the middle of the blue line were 60°38'52.0"N 17°48'44.2"E.



Appendix A, Figure 3: The bottom salinity (psu) in the Baltic Sea, modified from (Al-Hamdani and Reker, 2007).

Appendix A, Table 1: Otolith mean measurements of the parental fish groups.

Otolith parameter	Catch site	mean	sd
Circularity	Atlantic	0.48	0.05
	Baltic	0.54	0.03
Perimeter/Area	Atlantic	1.89	0.14
	Baltic	2.09	0.09
Aspect ratio	Atlantic	2.18	0.12
	Baltic	1.95	0.11

B. FIRST GENERATION HERRING

Appendix B, Table 1: Mean fish length, weight and otolith measurements from the first generation herring.

			187 DPH		297 DPH		614 DPH	
	Salinity (psu)	Genetic group	Mean	sd	Mean	sd	Mean	sd
Weight (g)	16	Hybrid	4.91	0.98	15.58	1.90	61.95	12.34
	16	Purebred	4.83	1.13	15.83	3.59	73.23	7.35
	35	Hybrid	4.55	0.75	16.71	1.60	76.00	9.01
	35	Purebred	5.12	0.47	18.26	2.49	82.49	14.49
Total length (cm)	16	Hybrid	9.23	0.59	13.59	0.46	20.19	0.85
	16	Purebred	9.15	0.73	13.47	0.75	21.20	0.36
	35	Hybrid	9.13	0.48	13.42	0.39	21.15	0.79
	35	Purebred	9.46	0.30	13.56	0.59	21.83	0.89
Otolith length (mm)	16	Hybrid	1.46	0.10	2.24	0.11	3.27	0.16
	16	Purebred	1.45	0.13	2.31	0.00	3.27	0.08
	35	Hybrid	1.46	0.08	2.23	0.07	3.42	0.13
	35	Purebred	1.51	0.06	2.27	0.08	3.51	0.13

Appendix B, Table 2: Mean vertebrae counts in the first generation herring including all the sample dates. sd = standard deviation, se = standard error.

Genetic group	Salinity (psu)	N	mean	sd	se
Hybrid	16	147	55.86	0.42	0.03
Hybrid	35	286	55.91	0.38	0.02
Purebred	16	20	56.37	0.68	0.15
Purebred	35	124	56.46	0.60	0.05

Appendix B, Table 3: Mean vertebrae counts in the first generation herring per main sampling date. sd = standard deviation, se = standard error.

	187 DPH			297 DPH			614 DPH		
Genetic group	mean	sd	se	mean	sd	se	mean	sd	se
Hybrid	55.94	0.36	0.03	55.84	0.41	0.05	55.75	0.49	0.08
Purebred	56.58	0.60	0.09	56.24	0.62	0.13	56.35	0.61	0.15

Appendix B, Table 4: Mean values of otolith shape parameters in the three main samples of the first generation herring. sd = standard deviation.

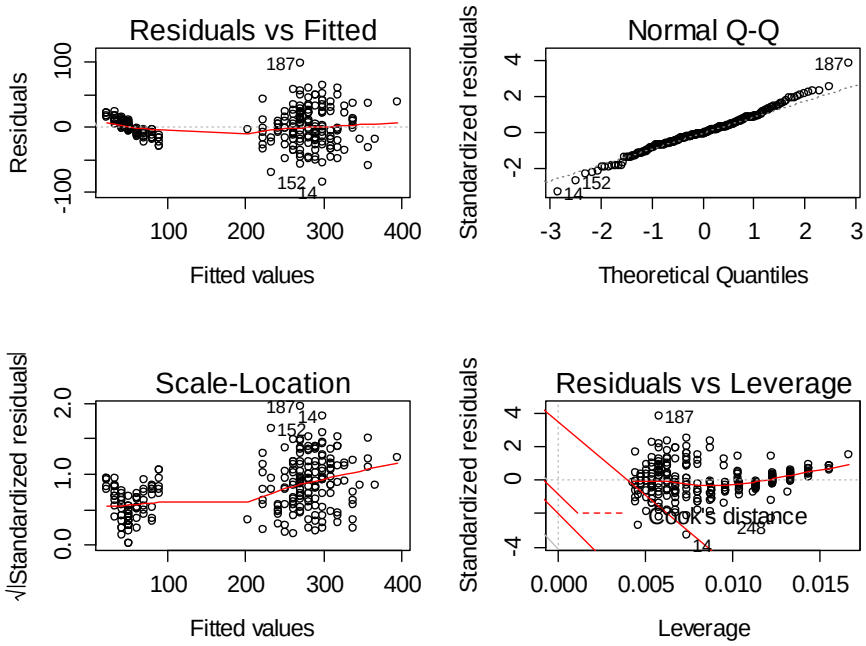
			187 DPH		297 DPH		614 DPH	
	Salinity (psu)	Genetic group	Mean	sd	Mean	sd	Mean	sd
Circularity	16	Hybrid	0.78	0.03	0.68	0.02	0.64	0.03
	16	Purebred	0.77	0.05	0.68	0.01	0.61	0.00
	35	Hybrid	0.78	0.02	0.69	0.02	0.64	0.03
	35	Purebred	0.75	0.03	0.66	0.03	0.60	0.03
AR	16	Hybrid	1.67	0.07	1.90	0.09	2.01	0.10
	16	Purebred	1.81	0.16	1.99	0.06	2.00	0.08
	35	Hybrid	1.69	0.06	1.89	0.05	2.04	0.06
	35	Purebred	1.77	0.09	2.03	0.13	2.20	0.12
Perimeter/ Area	16	Hybrid	4.24	0.19	3.17	0.12	2.31	0.14
	16	Purebred	4.46	0.25	3.12	0.05	2.34	0.02
	35	Hybrid	4.25	0.18	3.14	0.09	2.20	0.06
	35	Purebred	4.27	0.14	3.24	0.08	2.28	0.07

Appendix B, Table 5: The mean levels of element in the first generation herring otoliths, sampled 187 DPH. sd= Standard deviation and se= standard error. mg element weight/ kg otolith weight.

Element	Salinity (psu)	Genetic background	mean (mg/kg)	sd	se
Na	16	Hybrid	2737.75	99.67	22.29
		Purebred	2741.93	90.77	24.26
	35	Hybrid	2766.24	71.64	15.63
		Purebred	2829.25	104.83	25.42
Sr	16	Hybrid	792.71	55.33	12.37
		Purebred	812.51	42.91	11.47
	35	Hybrid	959.81	83.63	18.25
		Purebred	996.79	63.16	15.32
K	16	Hybrid	503.96	33.24	7.43
		Purebred	484.61	28.16	7.53
	35	Hybrid	495.58	34.64	7.56
		Purebred	536.21	52.02	12.62
Mg	16	Hybrid	32.03	4.88	1.09
		Purebred	32.37	5.99	1.60
	35	Hybrid	35.79	6.69	1.46
		Purebred	39.10	8.90	2.16
Mn	16	Hybrid	2.67	0.47	0.10
		Purebred	2.86	0.49	0.13
	35	Hybrid	2.66	0.45	0.10
		Purebred	2.68	0.37	0.09
Ca	16	Hybrid	398247	8087	1961
		Purebred	395400	8212	2195
	35	Hybrid	395460	6049	1353
		Purebred	400729	10462	2537

C. STATISTICS

Diagnostics plots like the examples in Figure 1, was used to make sure that the assumptions of ANOVA was met, when analysing the data.



Appendix C, Figure 1: Examples of diagnostic plots made from a linear model of X vs Y