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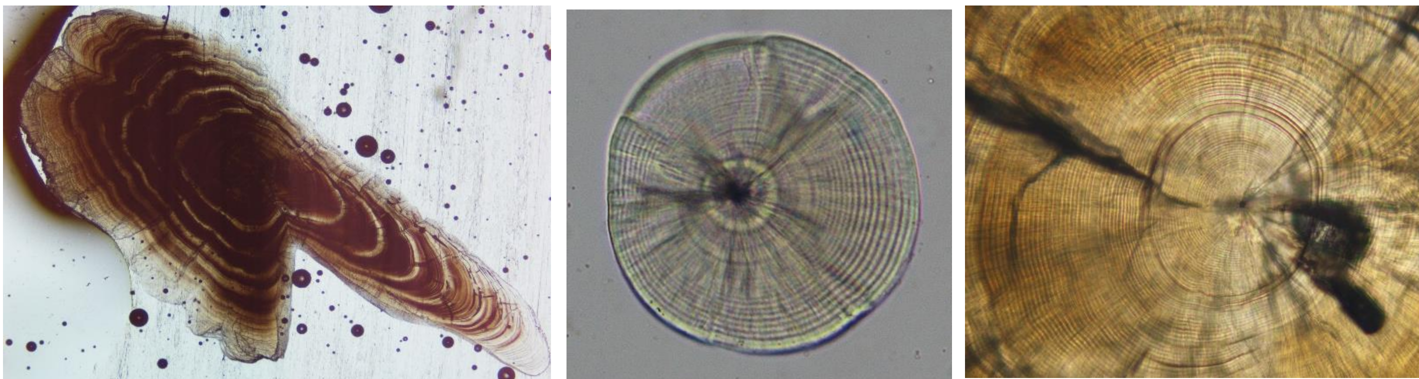
MASTER OF SCIENCE IN MARINE BIOLOGY

Otolith growth across two generations in Atlantic herring *(Clupea harengus)*



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June 2018

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ABSTRACT

The Atlantic herring (*Clupea harengus*) has a wide distribution and a complex metapopulation structure with interactions between the sub-populations. The sub-populations show different adaptations, both genetic and non-genetic, to their environments, and clear differences are found between Atlantic and Baltic herring. Offspring (F1 generation) from Atlantic and Baltic parents (Atlantic purebreds and Atlantic-Baltic hybrids) were co-reared in a common garden experimental set-up. Initially there were three salinity regimes (6 psu, 16 psu and 35 psu), but the 6 psu regime was terminated at larval life stages. Repetitive samples were taken from each group throughout the whole experiment providing a unique collection of sibling samples from larval to adult life stages. Otoliths from all life stages were available for further otolith microstructure analysis and corresponding microstructure analysis was also available from the two parental populations. The Atlantic parental herring were larger than the Baltic parental herring, but the larval otolith increments indicated an opposite trend where the Baltic herring had better larval growth. The F1 larval somatic parameters indicated a trend where the 35 psu group was larger than the 16 psu group, indicating an impact from environmental factors. The adult purebreds were longer than the adult hybrids, which could indicate a genetic impact. An interaction effect including both salinity and genetics as factors was present for the adult weights and for the otolith larval increment widths of both larval and adult life stages. The results from the present study indicate that these traits were affected by both the environment (salinity) and the underlying genetics. These findings are of high importance in sustainable management when characterising herring stocks, but other environmental factors, like temperature, should also be given more attention and included in further research in addition to having a broader genetic baseline for analysis.

Keywords: Atlantic herring, *Clupea harengus*, otolith microstructure, otolith, common garden, growth, environmental influence, genetic influence, adaptations.

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1 INTRODUCTION

1.1 ATLANTIC HERRING

1.1.1 Important species

Atlantic herring (*Clupea harengus,* L. 1758) is a pelagic, shoal forming fish species in family Clupeidae. It is a native fish in the North-Atlantic Ocean and have a broad distribution, reaching from the North European side to the East North-American side (McQuinn, 1997; ICES, 2007). According to ICES and Hay et al. (2001) the Atlantic herring in the Norwegian Sea (Norwegian spring-spawning herring, NSSH) can reach a maximum length of 40 cm, get close to 20 years old and reach sexual maturity when the fish are 5-9 years old. These characteristics vary between herring stocks in close geographic regions as the North Sea herring is ca. 10-20 cm shorter than the NSSH and younger than 7 years of age (Hay et al., 2001; ICES, 2007). The herring plays a key role in the North-Atlantic ecosystem as it is an important prey species for other species at different trophic levels as well as predator on lower trophic levels (ICES, 2007). Herring has a long history as an important food resource for people in Northern Europe and specially for people along the coast of Norway as it is economically important and a numerous species in these ecosystems. The herring stocks are being assessed by the International Council for the Exploration of the Sea (ICES), which also gives advices to secure sustainable harvest of the important species. Large amounts of herring are being caught every year; in 2016, 383 174 tons of NSSH were caught in the Northeast Atlantic and Arctic Ocean, whereas the recommended quotas in the same area for 2017 were set to a total of 437 364 tons and for 2018 a total of 384 197 tons (ICES 2018). This puts needs and requirements for knowledge and research at all life stages in order to sustainably manage the highly important species.

1.2 SUB-POPULATIONS AND GENETICS

1.2.1 Distribution

The broad distribution of the Atlantic herring has impacted its population structure. The species consists of several subpopulations where some are spatially and temporally migratory while others are more stationary, and only in the northeast Atlantic, including Arctic waters and the Baltic Sea, the presence of several subpopulations has been documented (Hay et al., 2001; ICES, 2007; Johannessen et al., 2009). The broad distribution make the herring face a

wide range of salinities, but the herring can cope with this issue as it has broad salinity tolerance and is one of few marine species which can tolerate and reproduce at such low salinities as in the Baltic Sea, where the salinity in the innermost part, Gulf of Bothnia, can be < 5 psu (Blaxter & Holliday, 1962; Hay et al., 2001; Lamichhaney et al., 2012). These subpopulations contribute to several exploited herring stocks and make its structure and dynamics quite complex, as they might mix and produce viable offspring (McQuinn, 1997; Johannessen et al., 2009).

1.2.2 Differences among sub-populations

The sub-populations may experience different environmental conditions spatially and temporally and although the sub-populations all belong to the same species they can appear different and exhibit different phenotypes and adaptations in traits which reflect the ambient environmental differences to obtain high fitness. Traits that are found to vary among the sub-populations are spawning time and ground, migratory routes, otolith shape and microstructure, vertebrae counts and meristic morphological features like length etc. (Runnström, 1941; Hay et al., 2001; Burke et al., 2008; Jørgensen et al., 2008; Almeland, 2015). These traits are to some extent influenced by the surrounding environment but it is assumed that the traits are also being influenced by genetics although the strength of influence from each of the contributors is unknown (Swain & Foote, 1999; Jørgensen et al., 2008). The genetics of an individual are a result of the genetic contribution from the parents. If the parents' genetics are adapted to their ambient environment it is assumed that the adaptations will increase their offspring fitness. If the offspring from these parents is experiencing the same environment as the parents, the offspring will potentially also have increased fitness (Via et al., 1995). But what will happen if the two parents are adapted to different environments? How will that affect the growth and fitness of the offspring?

1.2.3 Phenotypic plasticity

Phenotypic plasticity is an aspect underlying the concept of metapopulations of herring where the subpopulations interact with each other, and there are observed differences in traits among the subpopulations. This means that the subpopulations share much of the same genetic material with only small genetic differences. Lamichhaney et al. (2012) and Barrio et

al. (2016) have performed a thoroughly amount of work in sequencing the whole population genome of Atlantic and Baltic herring. They found among other things, that most of the SNPs (single nucleotide polymorphism) showed little differences among the populations. Some other (several thousand) SNPs, for example many independent loci associated with adaptations to water salinity, showed clear differences between the populations. The subpopulations could appear different as they express their genes differently through different phenotypes and these morphological differences. According to Smith and Smith (2009) phenotypic plasticity is: “the ability of a genotype to give rise to different phenotypic expressions under different environmental conditions”. For Atlantic herring, as well as for other species, this aspect has been a subject for research through many experiments e.g. in (Swain & Foote, 1999; Jørgensen et al., 2008; Geffen, 2009; Johannessen et al., 2009). Swain and Foote (1999) used the term “chameleonlike genotype” to explain that one genotype can appear in different phenotypes to match different environmental conditions.

1.3 DIFFERENCES BETWEEN ATLANTIC AND BALTIC MARINE ENVIRONMENTS

1.3.1 Environmental differences

Atlantic herring subpopulations are found both in the Baltic Sea and along the Norwegian coast. Although the two major water bodies are connected through a narrow strait separating the West coast of Sweden and the East coast of Norway from Denmark, the environments are quite contrasting. The Atlantic water masses in the southern Norwegian Sea has a salinity around 35 practical salinity units (psu) and a mean annual temperature of ca. 8.5°C (González-Pola et al., 2018) (Appendices A11 and A12). The water body in the Baltic Sea is stratified and more brackish due to freshwater runoffs and the salinity varies from almost freshwater in the innermost parts and mean annual temperatures of around 4°C to more Atlantic salinities and temperatures around central Skagerrak (ICES, 2008; González-Pola et al., 2018) (Appendix A10).

1.3.2 Impact on somatic growth

The haline and thermal differences, including other environmental factors, between Atlantic water and water from the central Baltic Sea are influencing the local herring populations. It is

found that Baltic herring differs from the herring in the Atlantic Ocean in some phenotypic traits such as vertebral count, size-at-age, lower fat content, some genetic difference, longevity and age at first maturity etc. (Hay et al., 2001; Lamichhaney et al., 2012; Almeland, 2015; Berg et al., 2018a). For example, the age at first maturity for the herring stocks in the North Sea and in the Baltic Sea is 2 – 3 years of age whereas it is 5 – 9 years of age for the NSSH (Hay et al., 2001). The fish lengths also vary among the populations: as the Atlantic herring in the Norwegian Sea stocks can get up to 40 cm long, the lengths among the Baltic herring stocks normally vary between 20 – 30 cm. To what extent these documented differences between the Atlantic (Norwegian Sea) herring and the Baltic herring are due to environmental differences or genetic differences remains unclear.

1.4 COMMON GARDEN EXPERIMENTAL SET-UP

1.4.1 Isolation of genetic influence on experimental responses

A way to test for genetic influence is to conduct an experiment with a common garden experimental set-up. Common garden experimental set-up is a popular method, known from a number of studies: Berg et al. (2018a) performed their study on the same F1 herring generation as this current study with two different genetic groups reared in the same respective tanks under two different salinity regimes. Johannessen et al. (2000) reared herring larvae from various parental crossings under the same environmental condition, but under two different feeding regimes. Marcil et al. (2006) conducted an experiment with Atlantic cod (*Gadus morhua*) larvae to test for genetic differences in body shape among different Atlantic cod population and reared the experimental groups under two temperatures and two food levels with different prey densities. Folkvord et al. (2015) reared herring and cod larvae, both together and in separate tanks, to investigate how growth eventually got affected by the presence of the other species. A common garden experimental set-up allows the researcher for example to control the environmental factors and food supply, and thereby discover and compare any eventually differences in responses among different experimental groups. This set-up also provides scientists a possibility to investigate how various groups, e.g. from distant genetic populations or species, life stages etc., respond to the same, identical environmental conditions by isolation of the influencing factors. It enables separation and investigation of

the environmental and genetic components of variation by separating each of the factors. In this current study the main differences in influencing factors between the Atlantic and Baltic herring, which will be isolated and tested, are genetics and salinity regime. Each of the latter mentioned factors has two levels: Purebred Atlantic or Atlantic-Baltic hybrid genetic combination, or 16 psu or 35 psu salinity regime. The genetic groups are co-reared under the same regime of environmental parameters, so any misleading results due to differences in external factors are excluded.

1.4.2 Validation of methodology OR unique opportunity

The use of a common garden experimental set-up combined with the assumption of non-changing otolith structure after deposition gives an additional unique opportunity to check if this methodology and sampling have been performed in a proper and correct manner. It is based on the assumption that the samples give a true representation of the true conditions in the tanks at all sampling times. This can be done by comparing otolith microstructure from larvae and adult siblings from the same generation. Since the larvae and adults are reared in the same, common environment as larvae, the ones belonging to the same genetic groups should exhibit the same larval otolith microstructure in absence of selection or any other occasion that might make an impact on the size distribution in the tanks.

1.5 FISH OTOLITHS

1.5.1 Otolith growth

Otoliths are being used to study growth and for the early growth history in fish one can look into the otolith's microstructure. Fish have 3 pairs of otoliths in their inner ear, the sagittae, lapilli and asterisci, serving a role in the auditory and vestibular system due to their heavier density than the rest of the fish's body (Popper & Lu, 2000). An otolith grows throughout the whole life of the fish and can be looked upon at two levels: macro and micro. The micro growth happens at a daily basis as the otolith is constantly depositing mainly various morphs of calcium carbonite ($CaCO_3$) and proteins (Campana & Neilson, 1985). The deposition rate can vary with time, as for example with photoperiod. This means during each photoperiodic cycle, a new growth band will typically be deposited throughout the early larval life of a fish. The

new increment is being deposited at the outer edge on the otolith, following the growth of the previous days. This makes a traceable growth trajectory which can also be followed as a function of days from the oldest part of an otolith near the core, and to the newest and outermost increments. These growth trajectories will be important for this current study. Pannella (1971) suggested that all the pattern of smaller daily growth bands collectively make annual growth rings. The formation of annual growth rings is considered as the macro growth of an otolith and is a good trait to use as a tool suitable for estimating annual age of fish. It is found in teleost taxa inhabiting various habitats, both marine and in freshwater (Pannella, 1971; Brothers et al., 1976; Mugiya et al., 1981; Campana & Neilson, 1985).

A study by Moksness (1992b) validated that Norwegian spring-spawning herring have a daily increment formation, which is necessary for estimating the age (in days) of herring larvae and Moksness (1992a) and Campana and Moksness (1991) further found that the accuracy of ageing increased with age. This could be because the increments close to the core are narrow and can thereby be overseen or misread, or that increments are being formed from a certain life stage rather than from a certain age. Fox et al. (2003) performed a validation experiment to investigate if herring actually has a daily increment formation. Their results suggest that they do, but some increments could be too narrow to spot and thereby leading to a false conclusion that herring does not have daily increments. Another experiment found that the average deposition rate among 5 groups of Atlantic herring larvae varied from 0.34 to 0.92 rings per day (Geffen, 1982). Geffen also found that for herring larvae growing less than 0.40 mm per day did not have an initial deposition rate of one ring per day. She concluded that all larvae cannot be assumed to have a deposition rate of one ring per day, but individual conditions, growth rate must be taken into consideration when using otolith microstructure to age fish larvae.

1.5.2 External and internal effects

The growth of an otolith and thereby the width of an increment, is influenced by external environmental factors as well as the fish's own physiological state. Differences in these factors will influence otolith growth differently as optimal conditions will promote otolith growth and give a wide increment. Such factors can for instance be food availability and thereby growth

and feeding, photoperiod, temperature, endogenous circadian rhythm and metabolic rate (Campana & Neilson, 1985; Folkvord et al., 1997; Bang et al., 2006). For wild caught fish the influence and exposure from these factors are unknown due to changing conditions in time and space (Campana & Neilson, 1985). In the laboratory on the other hand, these factors can be controlled and investigated. If the environmental conditions promote normal to optimal somatic growth, the somatic and otolith growth seem to be coupled, giving both positive somatic growth rate and positive otolith growth rate. But this is not always the case as the somatic and otolith growth can be uncoupled. Studies have documented that otolith deposition continues throughout periods of starvation when somatic growth decreases or ceases (Campana, 1983; Mosegaard et al., 1988; Moksness et al., 1995). Some even claim that the metabolic rate is influencing the otolith growth more than somatic growth (Bang et al., 2006). Despite the influence from external factors, the otoliths growth is continual throughout the whole life of the fish and is not being reabsorbed.

1.5.3 Otolith analysis

Otolith investigations are used as methodology in many occasions and otoliths are a good tool due to the fact that they are not being reabsorbed and holds a daily growth trajectory. This trait makes the otolith a better indicator and representative for growth than e.g. fish scales where their growth may ceases when the fish is exposed to stress (Campana & Neilson, 1985; Campana & Thorrold, 2001).

Otoliths can be used to obtain much information. Counting annual increments can reveal the age of fish. The micro growth in an otolith can give an indicator of the growth from day to day and if the otolith growth is positively correlated to the somatic growth, the otoliths can tell something about the somatic growth experienced as larvae as well. In addition, it is thought that the morphological features of an otolith are species specific (Härkönen, 1986; L'Abée-Lund, 1988; Campana, 2004). Stransky and MacLellan (2005) used for instance intraspecific otolith shape variation for species identification and separation among species in the species-rich genus *Sebastes*. The species-specificity has also been applied to fisheries for stock identification and assessment (Tracey et al., 2006).

Campana and Neilson (1985) also mention that the influence from these factors can be used as a method to artificially induce checks in the otoliths by introducing the fish to stress, e.g. lowering the temperature for some time. The increment deposited during the stress period will differ from the other increments and thereby be a visual mark in the otolith. Also, settlement marks are found in some species as the pelagic larvae shift their habitat and become demersal juveniles. This comes most likely from a rapid change in environment and thereby also condition, and similarities in these marks can reveal similarities in ecology among species (Wilson & McCormick, 1999).

Experienced differences due to different environment can give distinct microstructures in the various environment. By tracing and interpreting the patterns found in the microstructure, one can obtain knowledge about nursing grounds and size-at-age through back-calculations etc. Moksness (1992b), Campana and Casselman (1993), Clausen et al., (2007) and Stenevik et al. (1996) all used otolith methodology to investigate spawning time, hatching season, and stock and home area identification. The formation of a growth trajectory in the microstructure gives an incredible possibility to trace growth and age back to a specific date when back calculated from the date of catch. This is more applicable and precise for young life stages, e.g. larval- or juvenile stages, as the daily increments may be difficult to observe in older fish (Campana and Neilson, 1985).

1.6 AIM OF STUDY

1.6.1 Overall aims

Atlantic-Baltic hybrids and purebred Atlantic herring were reared in a common garden experimental set-up to test for salinity effects and to see how the effects were affecting the otolith microstructure of the offspring as the findings from this current study could be implicated in stock assessment and management of Atlantic herring. With this in mind, the main objective of the study is to compare otolith growth patterns across generations through contrasting individual otolith microstructure of genetically related fish (parental and offspring) from both larval and adult stages. An additional objective is to contrast otolith microstructure from sibling fish at both larval and adult stages. These objectives can be divided into sub-objectives:

- Contrast growth of Atlantic and Baltic herring in natural systems.
- Contrast otolith growth in common salinity regimes and compare against the growth in their natural systems.
- Investigate the relative contribution of environment and genetics to otolith growth.
- Check the methodology and contrast the microstructure in the otoliths of larvae and their adult siblings.

1.6.2 Expectations

There are some expectations to the aims described above: considering the differences between the environments the Atlantic and Baltic herring experience, is it assumed that the morphology and otolith microstructure differ between Atlantic and Baltic herring. It is also expected that the purebred Atlantic offspring will perform well under an Atlantic salinity regime and that Atlantic-Baltic hybrids might exhibit growth histories that are in between the purebred Atlantic and purebred Baltic growth histories. The environmental influence and genetics are both believed to contribute to the growth of the otolith microstructure. If the method is valid for this kind of study, it is expected that the otolith microstructure in samples of sibling larvae and adults should exhibit the same growth histories when reared under the same salinity regime and if no selective mortality has occurred.

2 MATERIAL AND METHODS

2.1 PARENTAL FISH AND PRODUCTION OF FILIAL GENERATIONS OF HERRING

Wild caught herring were used as parental fish for further crossings to produce first (F1) generation filial herring in this study. The wild caught herring were caught at two different regions: the south-western Norwegian coast (hereafter termed the Atlantic) and the Baltic Sea. The Atlantic wild caught parental herring were caught 21st of May 2013 at Herdla, Askøy, which is located on the west coast of Norway not far from Bergen (60°34’11.2’’N 5°0’18.9’’E).

The Baltic wild caught parental fish were also caught 21st of May 2013 at Hästskär, North-East of Uppsala, Sweden (60°38'52.0''N 17°48'44.2''E) (Figure 1).

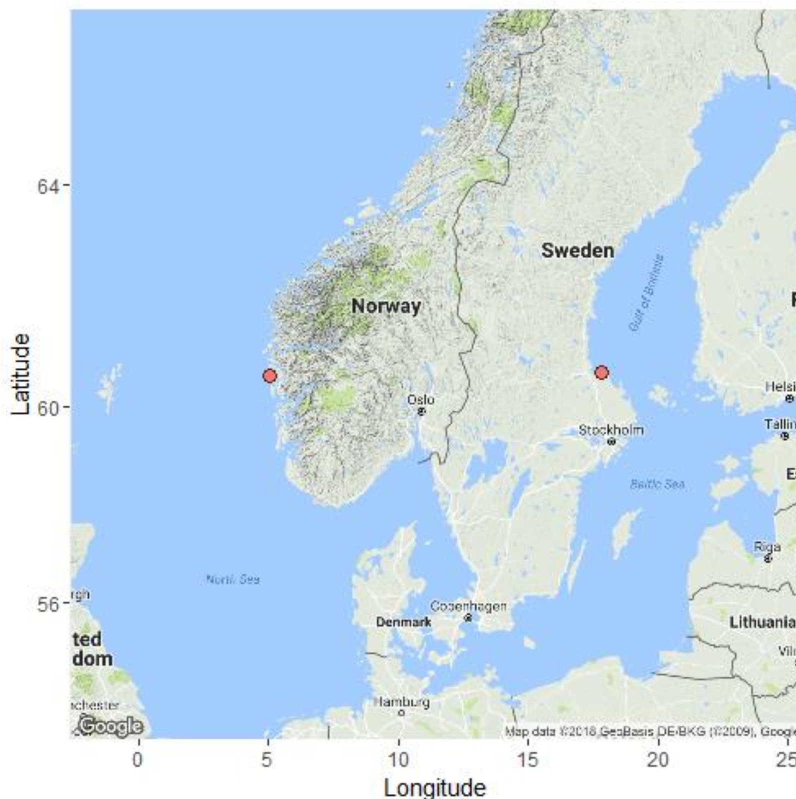

Figure 1: Locations for collecting wild caught parental fish. Herdla and Hästkär are represented by red dots on the map, respectively on the West coast of Norway (point to the left) and East coast of Sweden (point to the right).

These fish samples will be considered as purebreds of respective populations and will in the following be termed as Atlantic parental fish ($P0_{AA}$) and Baltic parental fish ($P0_{BB}$), respectively. The herring were caught by gillnets and the Baltic herring were transported to Bergen on ice by airplane shortly after retrieval, while the Atlantic herring were transported by car to the lab. One single female of Atlantic origin was crossed with 2 males of different origins, one Atlantic and one Baltic (see Table 1), to produce first generation herring (F1) (see Figure 2). These crosses produced respectively first-generation purebred Atlantic herring and F1 Atlantic-Baltic hybrid herring (hereafter termed hybrid herring). Another cross was conducted to produce an additional limited number of F1 offspring in the 6 psu group. In addition, a crossing between a Baltic female and a Baltic male of the parental generation was conducted

to follow the development of a purebred Baltic F1 group. These purebred Baltic F1 offspring were reared in a separate tank. Age, total length and weight measurements for the individuals from the parental populations used in crossings to produce the F1 generation offspring can be found in Table 1 and comparisons against their respective parental populations can be found in Appendix A1.

The age of each of the individual fish from the parental generations (Atlantic and Baltic) was estimated from overview pictures showing the whole otolith after some grinding and polishing, but the estimations were uncertain due to unclear annual increments at the outer part of the otoliths. The visible annual increments were read and counted and based on the growth in the outermost visible annual increments, total age was estimated by extrapolating similarly sized increments to the outer otolith edge. Each annual increment was counted according to guidelines in Mjanger et al. (2008). All parental fish used for crossings to produce the F1 generation were all in maturity stage 6 (Mjanger et al., 2008) (Table 1).

*Table 1: Information, including somatic measurements and age estimates for parental fish used for fertilisation to produce the F1 generation. * indicates the main three fish used for fertilisation to produce the F1 generation (Atlantic purebreds and hybrids). ** indicates fish that only contributed with a limited number of offspring to the F1 generation reared at 6 psu. *** indicates fish used to produce purebred Baltic offspring. The purebred Baltic offspring were reared at 16 psu.*

		Age (years)			
Origin and sex of parental fish used for fertilization	**Fish code**	Visible annual increments	Estimated age after extrapolation	**Total length (cm)**	**Weight (g)**
Atlantic female*	AF31	5	7	29	295
Atlantic male*	AM33	4	5	31	320
Atlantic female**	AF29	7	10	36	343
Atlantic male**	AM27	5	6	32	270
Baltic female**	BF21	4	7	21	67.3
Baltic male*/***	BM19	4	7	20	61.8

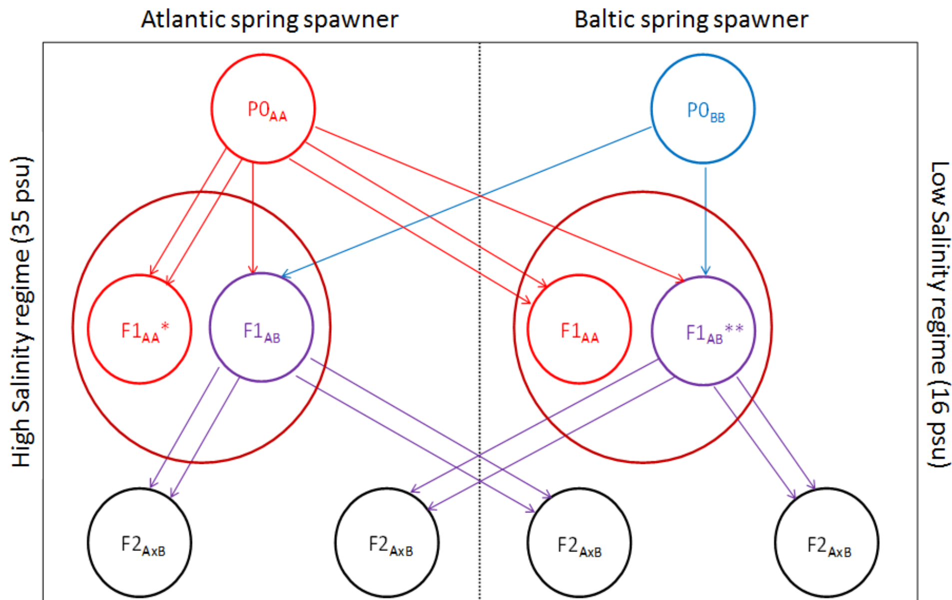

Figure 2: Common garden experimental design, indicated by the two large, circular circles where offspring from different crossings are exposed to the same environmental regimes. Tthe $P0_{AA}$ (purebred Atlantic) contribution to $F1_{AB}$ was only eggs from an Atlantic female (single arrow). Double arrows indicate contribution from both male and female of same genetic origin. The $P0_{BB}$ contribution to both the high salinity and low salinity regime was from a Baltic male. Not included in this figure is the 6 psu salinity regime and the rearing of purebred Baltic F1 fish originating from another cross in 16 psu. The F2 generation was not included in this study.

2.2 Experimental design: common garden

The F1 generation juvenile herring from 16 psu and 35 psu were co-reared in a common garden experimental set-up (Figure 2). The F1 generation herring from the 6 psu salinity regime were also part of the experimental set-up but had poor survival rate during the larval part of the experiment and were therefore from the 15th of August 2013 not included further into the experiment (Almeland, 2015). Offspring (F1 generation) of known parents but different crossings, were exposed to the similar environmental parameters and treatments

but salinity, which varied across the tanks (Figure 3 and Table 2). Salinity of 34-35 psu (hereafter termed 35 psu) was chosen to simulate Atlantic water, whereas salinities of 16 psu and 6 psu were chosen to simulate the natural salinity of Baltic water (Zettler et al., 2007; ICES, 2008), which varies geographically within the Baltic Sea.

Table 2: Overview over the different tanks, their salinities and which crosses (F1) were present in each tank. AxB indicate hybrid (crossing between an Atlantic female and a Baltic male), AxA indicate purebred Atlantic (crossing between an Atlantic female and an Atlantic male), while BxB indicate purebred Baltic (crossing between a Baltic female and a Baltic male).

Tank nr.	**Transferred to larger tanks 16th of August and part of main design (see Figure 3)**	**Crosses**	**Salinity**
1		A x B + A x A	6 psu
2	X	A x B + A x A	35 psu
3	X	A x B + A x A	16 psu
6	X	A x B + A x A	16 psu
7	X	A x B + A x A	35 psu
9		B x B	16 psu
10		A x B + A x A	6 psu

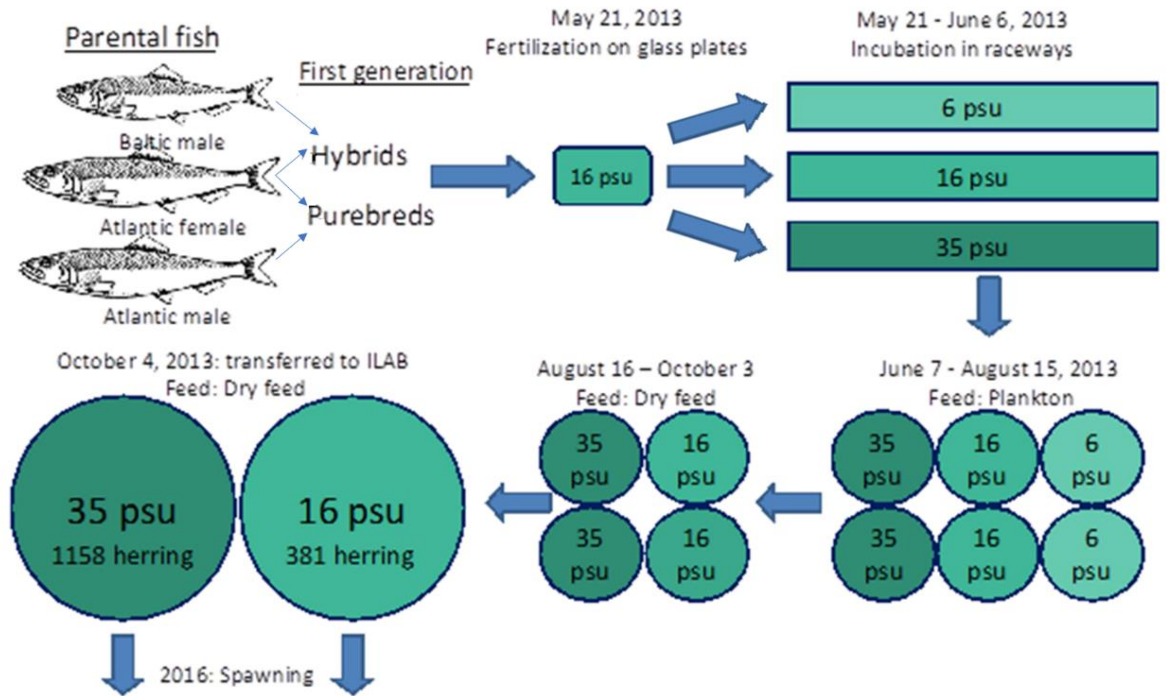

Figure 3: An overview over the most important events in the experiment: the fertilization in 16 psu and production, and incubation of F1 larvae. The introduction of different food regimes and the transfer to ILAB. Not included in this figure is the 16 psu tank (tank 9) containing purebred Baltic larvae, which was an additional crossing, not included in the main design. Number of larvae in each tank is given for the large tanks at ILAB; 1158 juvenile herring in the 35 psu tank and 381 juvenile herring in the 16 psu tank. Modified from Almeland, 2015.

The crossings between the three parental fish to produce the F1 generation herring were conducted on May 21st 2013. The fertilisation was performed by placing and evenly distributing the eggs on glass plates which were placed in plastic trays containing enough sea water with a temperature of 8.0-8.5°C and salinity of 16 psu to cover the eggs (Arild Folkvord pers. com). Due to the natural adhesive properties of herring eggs, they attached to the glass plates (Runnström, 1941; Bone & Moore, 2008; Almeland, 2015). Sperm from the male gonads were then added to the plastic trays containing the eggs and the water gently stirred to ensure fertilisation. After fertilisation the trays with the fertilised eggs were transferred to separate raceway systems for further incubation, each with their specific salinity of 6 psu, 16 psu and

35 psu respectively, and kept there until the 6th of June 2013 (Almeland, 2015). The incubation temperatures were for 8.0±0.5°C, 8.3±0.5°C and 8.3±0.2°C for the 6, 16 and 35 psu water, respectively. The herring were exposed to a natural light regime corresponding to a latitude of 60° N, which is close to the current light regime for the two sampling locations for the parental herring. After hatching (5th of June, 50% hatching) the larvae were transferred to larger fish tanks, where the water volume was gradually increased over the two first weeks from 200L to 400L and exposed to the same salinity as before; 2 replicate tanks holding a salinity of 6 psu, 2 tanks of 16 psu and 2 tanks of 35 psu, while the pure Baltic larvae were reared in only one separate tank (tank 9, Table 2) with a salinity of 16 psu. After transfer, each tank contained 1000 individuals, including both purebreds and hybrids larvae (Figure 3) in a 2:1 hybrid to purebred ratio. From this time the larvae were fed live feed. The live feed consisted of algae (*Rhodomonas* and *Isochrysis*), rotifers (*Brachionus* spp.) and natural zooplankton and later *Artemia* spp. were added to the diet (Almeland, 2015). A continuously flow of running water was connected to each tank on the 24th of July. The running water supply kept the respective salinities in the different tanks (see Figure 3). From the 16th of August until 3rd October the remaining herring larvae in 16 psu and 35 psu salinity regimes were given dry feed. At this time in the experiment the 6 psu salinity regime groups were terminated. On 4th of October the juvenile herring from both two replicates were mixed within the respective salinities and transferred into two larger fish tanks (3 meters in diameter, one tank per salinity) at ILAB at the High Technology Centre in Bergen. The tanks had corresponding salinities to the herring's previous tanks; 16 psu and 35 psu respectively (see Figure 3). A total of 1158 juvenile herring were transferred to the 35 psu tank, while 381 herring were transferred to the 16 psu tank. The herring were reared here until they reached maturity in June 2016.

At 590 DPH (15th of January 2015) a bacterial infection was discovered in the 35 psu salinity tank. The infection was caused by *Tenacibaculum* spp. and the herring were put under an antibiotic treatment. Sick and weakened fish were specially selected for and sampled in the 589 DPH sample (not included in this current study), making this sample not a random sample.

Having two salinity regimes and to genetic groups in the F1 generation gave four experimental F1 groups: hybrids reared at 16 psu, purebreds reared at 16 psu, hybrids reared at 35 psu and purebreds reared at 35 psu. These groups will hereafter be called respectively 16H, 16P, 35H and 35P, experimental groups collectively.

2.3 OTOLITH EXTRACTION AND ANALYSIS

2.3.1 Larval F1 herring otolith extraction

Samples of 10 larvae from each of the different tanks were taken on a weekly basis throughout the larval period from 07th of June 2013 to 08th of August 2013. On the 15th of August 2013 (Table 2 and Figure 3), all of the remaining tanks but tanks 2, 3, 6 and 7, were terminated. Between 12 and 20 larvae were further sampled and measured from the remaining tanks. Shortly after sampling, measurements for length and mass were taken before the whole larvae were dried in a desiccator and stored in NUNC™ multi dishes. The selected larvae used in this study (Table 3) were rehydrated before dissection. The otolith extraction followed much of the same procedure as described in Folkvord et al. (2004) and Burke et al. (2008); both sagittal otoliths were extracted under a Leica MZ9,5 dissection microscope equipped with a polarisation filter attached, and mounted on microscope glass slides using QuickStick 135 Mounting Wax, with the proximal (convex side) facing up (Beamish et al., 1987). Multiple images were taken with 40x magnification with a Nikon camera (camera: DS-Fi2, control unit: DS-U3) attached to an Olympus BX microscope for each otolith for further microstructure analysis.

In total, 62 and 86 pairs of otoliths from the two main F1 larval sampling dates, 01st of August 2013 and 15th of August 2013 respectively, where available for otolith microstructure analysis. Of these, 57 and 84 larval otoliths were analysed from the respective dates. Thus, more than 90% of the available sampled larvae at given dates were measured. Additional 7 individual otoliths from extra sampling dates (25th of July and 8th of August) were specifically chosen to ensure significant numbers of purebred Atlantic larvae for statistical analysis (Table 3).

*Table 3: Overview over the number of larvae used for otolith microstructure analysis from various dates and genetic groups. Samples from 01.08.2013 and 15.08.2013 were the main samples where intentionally all otoliths were analysed. Dates marked with * indicate extra samples taken from the respective dates to ensure significant numbers of purebred Atlantic larvae for the analysis.*

Sampling date/ days post hatching (DPH)	**Number of analysed larvae**						
	Hybrids			Purebreds			Purebred Baltic
	6 psu	16 psu	35 psu	6 psu	16 psu	35 psu	16 psu
25.07.2013*/ 50					3		
01.08.2013/ 57	6	19	11	2	1	8	10
08.08.2013*/ 64					4		
15.08.2013/ 71		26	30		3	10	15

2.3.2 Adult herring otolith extraction

All the otoliths from the adult herring samples had been extracted and stored from time of sampling until the microstructure analysis took place. Samples were taken throughout the whole period from the juvenile to adult stage. Standard somatic measurements, like sex, total length and weight etc., were taken alongside with the otoliths for each individual fish. A subsample from each of the different groups of adult herring (Table 4) were randomly chosen for microstructure analysis. In total otoliths of 111 adult fish were used, including both parental herring and F1 adult herring. One otolith from each individual of the subsample was randomly selected (left or right otolith) and mounted on glass slides in thermos plastic glue

(Crystalbond) with the sulcus acusticus (Beamish et al., 1987) facing up. The otoliths were then grinded (grit 600 grinding paper) and polished (grit 1200 grinding paper). The slides were thereafter reheated and the otoliths flipped around, so the distal side was facing up (Beamish et al., 1987). The process of grinding and polishing was repeated on the distal side of the otolith until hitting the core and the microstructure of the otolith appeared clear and visible when looking at it in a Leica DMLB light microscope with a 20x magnification. A series of pictures was taken for each otolith with a Nikon camera (camera: DS-Fi2, control unit: DS-U3) attached to the light microscope for microstructure analysis.

A total of 97 Atlantic wild caught herring and 48 Baltic wild caught herring were sampled 21th of May 2013. In the three samples of adult F1 generation individuals from 7th of June 2013, 15th of June 2013 and 29th of June 2013 (Table 4), there were a total of 90, 61 and 51 individuals respectively. Only 17 individuals from the 16 psu purebred group were present in the F1-samples for the respective days. Therefore, this group was the constraining group, setting the size of the least number of individuals in each subsample. Ratio calculations for F1 adults were performed on the total number of sampled fish in the F1-samples. The individuals in the subsamples from the F1 generation (Table 4) were specifically selected based on their genetics and rearing salinity.

Table 4: Sample sizes of subsamples and dates used for microstructure analysis of adult otoliths. Note that adult F1 fish from three sampling dates were combined due to biological interpretations.

Experimental group		Subsample size (individuals)	Sampling days
Parental generation	Atlantic	17	21st of May 2013
	Baltic	17	
F1 generation	35 psu purebreds	20	07th of June 2016 (1098 DPH)
	35 psu hybrids	20	
	16 psu purebreds	17	15th of June 2016 (1106 DPH)
	16 psu hybrids	20	29th of June 2016 (1120 DPH)

2.3.3 Otolith microstructure analysis

The microstructure analysis was carried out along the longest possible radius of the otolith. For processing the larval otoliths, this was measured from the core to the outer edge using an open source image processing program; ImageJ version 1.46r (U.S. National Institutes of Health, USA). An increment was defined as one D-zone (dark zone) plus one L-zone (light zone) (Figure 4) (Beamish et al., 1987). One increment was measured from the middle of one D-zone to the middle of the next D-zone (see magnified square in Figure 4). The core of the otolith was marked as the starting point and the first visible and thereby marked D-zone (check) outside the core was termed the "first check" (sometimes referred to as hatch check) (Folkvord et al., 2004), while the next annotation was set to where the first pronounced and (presumed) daily increment was visible around the whole circumference and termed "first visible increment". All the increments, from the "first visible increment" towards the outer margin, were marked, numbered, counted and their widths measured along the longest possible radius of the otolith (see Figure 4). By assigning the outer increment of a larval otolith to the day of sampling, a measurement of increment width-at-age was made. The penultimate increment was then assigned to the previous day and so on (Folkvord et al., 2004). Incomplete daily increment in the outermost margin of the otolith could appear due to ongoing formation

of the next daily increment at the time of sampling. The penultimate whole daily increment was annotated as normal and the incomplete daily increment was measured from the end of the penultimate one and to the edge of the otolith.

To determine the appropriate microstructure reading axis from an adult otolith, an increment more than at least 150 µm from the core was chosen from an image of the otolith's microstructure. This increment had to be visible throughout the whole circumference. The longest measured radius from the core and out to the chosen increment was set as the longest larval otolith radius and the microstructure analysis were performed along this radius in the same manner as Clausen et al. (2007), using an image-analysis package for Windows™: Image-Pro Plus® version 7.0 (Media Cybernetics, USA) . In Image-Pro Plus increments were marked along a line drawn in the programme along the longest radius, but these could be adjusted, and points added or removed manually using the Caliper tool in Image Pro.

To enable comparisons of increment widths between larval and adult life stages, days post hatching or age, was excluded as a factor as age based back-calculation was difficult to perform due to uncertainties in age estimates as the daily increments get harder to observe in older fish. This could lead to mis-matching of age- and date-specific increments between the life stages and end in wrong comparisons. Therefore, distance from otolith core was chosen for this comparison.

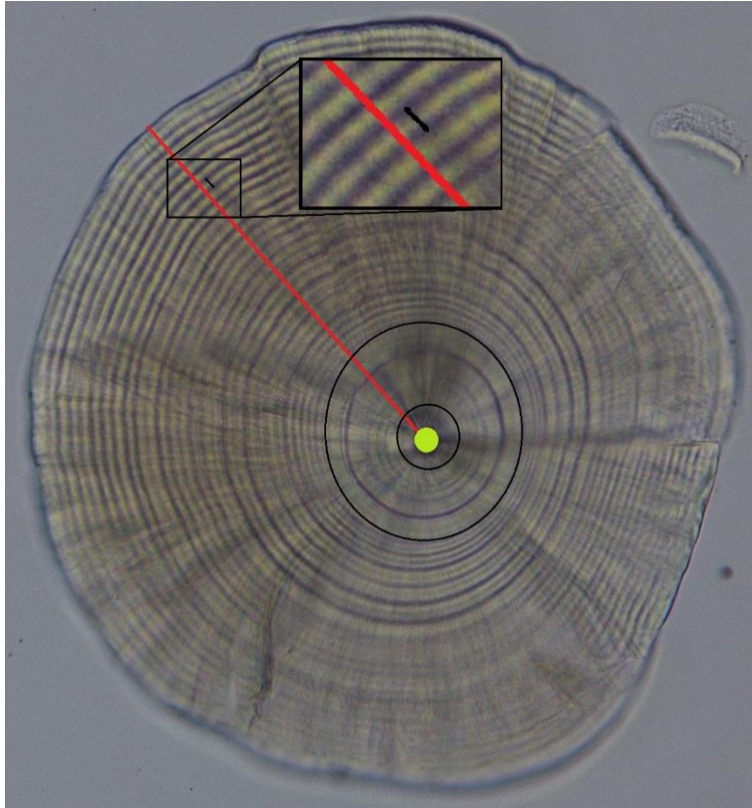

Figure 4: Overview over the different marks annotated in the otoliths. The core is marked in light green and the increment along the red radius were annotated, measured and counted. The innermost circle close to the core is the "first check" while the outer circle is the "second check". The magnified square shows one increment (black line). The increment is measured from the middle of one D-zone to the middle of the next D-zone. An L-zone is visible between the two D-zones. The photograph was taken under a light microscope with 20x magnification. The radius indicated with a red line was 120.8 µm long.

Calibration photographs of a measuring scale were taken to ensure equal calibration for all measurements, and between the different image processing programs used. A global scale was made for each type of magnification by measuring a length (for example 10 or 100 µm) on a calibration picture of a scale and thereby manually type in the measured length and magnification used on the calibration picture. In this way measurements from the different generations and life stages can be compared and analysed against each other. In ImageJ the photographs were calibrated to the magnification used by converting pixels into micrometres

(µm). Image-Pro Plus® version 7.0 had a fixed calibration coupled to the microscope and magnification used for taking the photographs.

2.4 IDENTIFICATION OF GENETIC ORIGIN OF CO-REARED INDIVIDUALS

All the adult individuals were fin clipped for DNA analysis. A piece from each of the individual larva bodies containing body tissue was used for the same purpose. One population specific allele was selected in each of the wild caught parental fish populations (Atlantic and Baltic). These alleles were chosen as markers due to differences between the populations and not due to the function of the allele. This made it possible to identify the genetic origin of the F1 generation and thereby categorise them as purebreds (Atlantic x Atlantic), or hybrids (Atlantic x Baltic), using a Custom TaqMan® Assay Design Tool. The allele differed at a specific single nucleotide on a locus: the Atlantic fish were homozygous T (thymine), while the Baltic fish were homozygous C (cytosine) (Berg et al., 2018a). The population specific alleles will be referred to as Allele 1 and Allele 2 for purebred Baltic and purebred Atlantic, respectively.

2.5 STATISTICAL ANALYSIS

2.5.1 Statistical software and R packages

RStudio, version 1.1.423 (RStudio Team, 2016) was used for performing statistical analysis and making figures. RStudio is an open source software for the statistical software R, version 3.1.1 (R Development Core Team, 2008) and various R packages were used for making figures and perform statistics (Appendix A2).

2.5.2 Data and modifications

The increment measurements were divided into three main groups for statistical analysis within each group: parental populations group, F1 larvae group and F1 adult group. For statistical analysis, the daily increments in each individual herring were grouped in intervals of 10 µm, for example from 20 – 30 µm from core, and would appear in figures as one measurement mark at 20 µm from core. Each mark represented mean widths of daily increments which will hereafter be called increment width. Only increments within the area

20 – 100 µm from the otolith core were used for visualisation of data and statistics. In addition, the 6 psu group was excluded from the dataset when performing statistical analysis due to presence in only one of the sampling days. They were still visualised in figures for comparative purposes. Further, measurements were occasionally log-transformed (log10) to achieve linearity of the data prior to testing. One individual (running number 57, 35H group) was excluded from the dataset and treated as an outlier for the F1 adult somatic parameters due to unrealistic biological interpretation.

When calculating hybrid to purebred ratio for the F1 generation, the calculations were performed on the whole samples and not only the subsamples containing individuals chosen for further microstructure analysis.

Total length and weight measurements for F1 adults from 1098 DPH, 1106 DPH and 1120 DPH were pooled based on biological and practical reasons as the herring does not grow noticeably in length over a 22 days period.

2.5.3 Choice of statistical model

For each group a backward selection procedure was performed to select the best fitted effect model to the data for the tested variable. The backward selection started with a full and complex model where all predictor variables (V) interact and affect the dependent variable (Y). Any interaction effect will only be mentioned if significant. The initial full model had an outline as seen in Equation 1 where Y represented the dependent variable and V1, V2 and V3 represented the predictor variables. Which, and the number of predictor variables, could vary regarding the dependent variable. Further, the procedure was to remove predictor variables which were not significant and continue to remove non-significant predictor variables until all remaining variables were significant. The effect by single predictor variables were not considered if an interaction effect including several predictor variables was significant. Then only the interaction was given attention.

Equation 1 $$Y \sim V1*V2*V3$$

When the best fitted model was found, an ANOVA test (analysis of variance) could be performed on the model to test if there are any significant difference in Y with regard to the variables in the model. If one variable was categorical and had more than two levels a Tukey-HSD test was applied to identify levels being significantly different from the others. For all statistical analysis and test a significance level (alpha, α) of 0.05 was used.

The choice of model was dependent on whether the tested variable had one or several values for each individual fish. If the tested variable had one measurement for each individual, as for weight, an ordinary regression model was chosen. If the tested variable had several measurements for each individual, as for increment measurements, a linear mixed effect model was chosen and individual measurements of each individual fish as random effect. Distance from core, genetics and salinity were the main variables in the initial full model for increment measurements which were being tested if significantly affecting the tested variable. Genetic effects were analysed on a per tank basis by comparing the genetic groups of hybrids and purebreds, but due to lack of true replication, testing for salinity effects by comparisons between salinities should be performed with cautiousness.

When checking for validation of this methodology for contrasting otolith growth histories over two F1 life stages and between sibling fish, the comparisons between F1 larval and F1 adult measurements were being performed within each combination of salinity and genetic group. This means that the i.e. hybrid F1 larvae reared at 16 psu are being contrasted against the hybrid F1 adults also reared at 16 psu. It was noted that the 16P group caused the generation variable to interact with other variables and contributed to significant interaction effects. The 16P group was then further excluded from the statistical analysis. Further analysis were then carried out without the 16P group (Appendix A13).

3 RESULTS

3.1 PARENTAL POPULATIONS

3.1.1 Somatic parameters

All individuals in the Atlantic and Baltic parental populations were in either maturity stage 5 or 6 (late maturing or spawning, respectively), regardless of origin. They were also similar in number of read annual increments, both between the Atlantic and Baltic population and between the sexes (ANOVA, p=0.37 and 0.57, respectively) (Table 5).

Table 5: Mean age ± standard deviation of the two parental herring populations; Atlantic and Baltic. The ages are based on number of read annual increments and estimated age of annual otolith increments. n represents the number of individuals in each group.

Parental population	Number of read annual increments	Age (estimated, years)	n
Atlantic females	4.8 ± 1.1	6.0 ± 1.9	9
Atlantic males	5.0 ± 1.1	6.0 ± 2.0	8
Whole Atlantic population	4.8 ± 1.1	5.7 ± 1.8	17
Baltic females	5.1 ± 1.1	7.0 ± 1.0	10
Baltic males	5.1 ± 1.1	7.3 ± 0.9	7
Whole Baltic population	5.1 ± 1.1	7.5 ±1.0	17

The Atlantic parental herring population had generally much higher weight-at-length than the Baltic parental herring population (ANOVA, p<<0.001). The mean total length and weight of Atlantic herring was 31.9 ± 2.0 cm and 277.6 ± 36.3 g, respectively, while it was 19.9 ± 0.6 cm and 51.3 ± 8.4 g for Baltic herring (Figure 5).

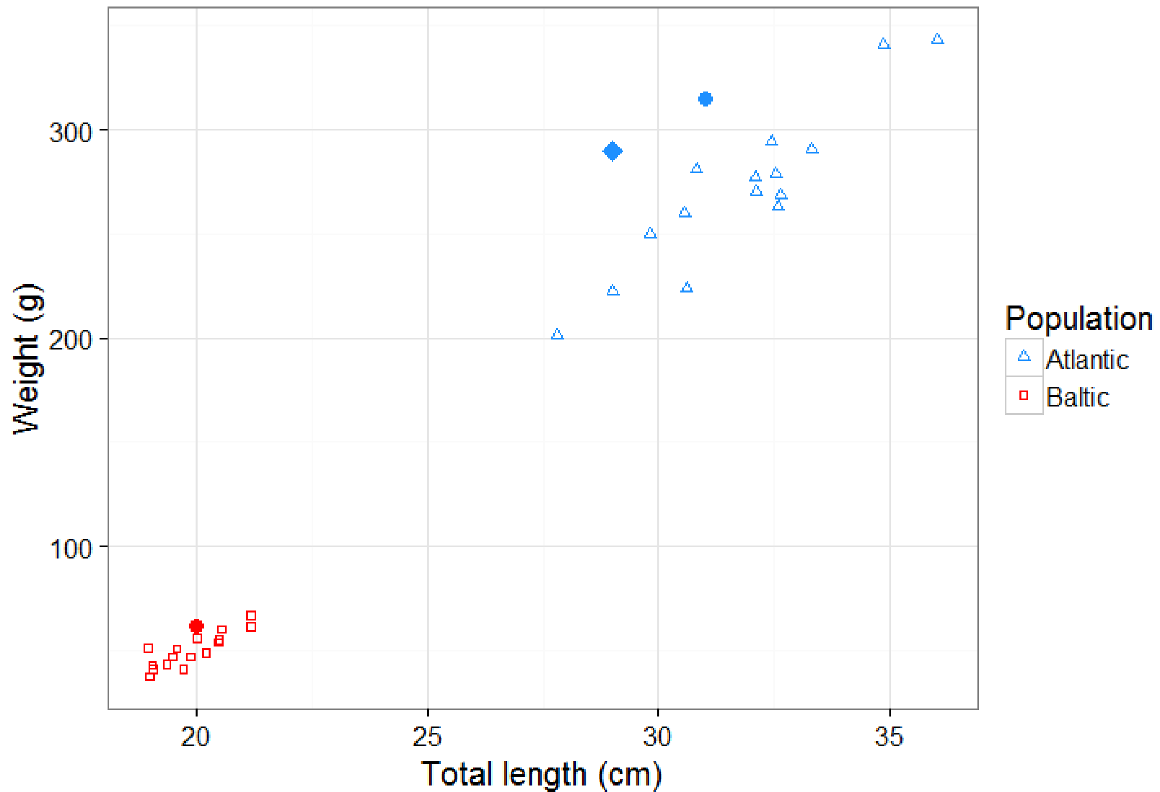

Figure 5: Total length (cm) and weight (g) measurements for the 17 Atlantic and 17 Baltic individuals in the parental populations. The Atlantic male and female used for crossings to produce the F1 generation is represented by a filled blue circle and a filled blue diamond, respectively. The Baltic male used for crossings to produce the F1 generation is represented by a filled red circle.

3.1.2 Otolith measurements

Shortly after hatching, until approx. 70 µm from the core, the Baltic parental population had wider increments than the Atlantic parental population (ANOVA, p=0.012; Figure 6). From 70 µm from the core and further, no difference in increment widths was found between the two populations (ANOVA, p=0.63). Both populations had a steady increase in increment widths with increasing distance from the core until approx. 70 µm from the core.

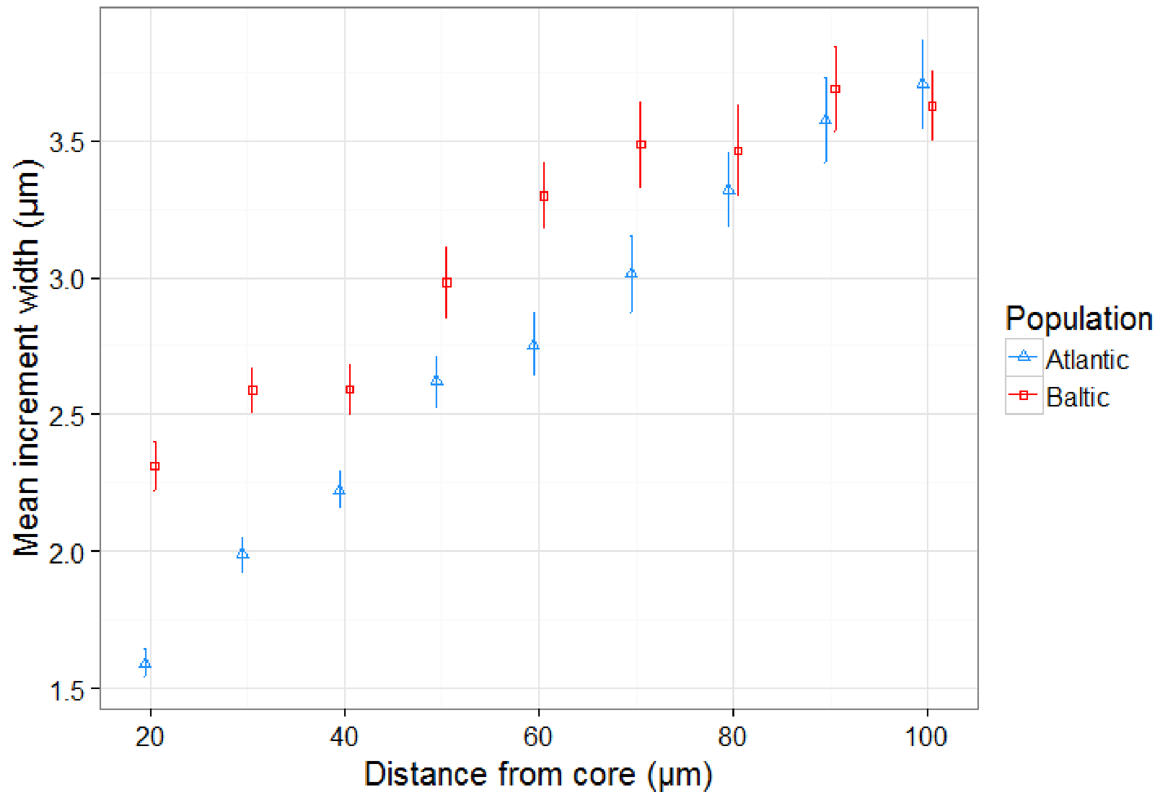

Figure 6: Mean increment widths (µm) for Atlantic and Baltic parental populations. Each point represents grouped mean increment widths with standard error bars in 10 µm intervals for all individual in each population. The points are slightly jittered to prevent overlapping points and error bars.

3.2 CHANGE IN F1 HYBRID TO PUREBRED RATIO

Initially, the hybrid to purebred ratio of F1 herring was 2:1 in both salinity regimes. The larvae from the 6 psu salinity regime were only present in one samples for this current study. No change in the ratio was found from the initial 2:1 hybrid to purebred ratio at 6 psu. The ratio in the 35 psu tank did not change much over the course of sampling days (Table 6a and 6b). The ratio varied from 1:1 at the lowest to close to 3:1 at the highest. None of these were statistically dissimilar to the initial ratio of 2:1 (Berg et al., 2018b). The hybrid to purebred ratio had a more marked change in the 16 psu tank. The ratio had an extreme high value in the earliest sample, but it decreased throughout the experiment (Table 6a and 6b).

In total, the ratio in the 35 psu regime did not change much from larval stages (57 and 71 DPH

samples) to adult stages (1098, 1106 and 1120 DPH samples). In the 16 psu regime the purebred to hybrid ratio changed from around 11:1 at larval stages to around 4:1 at adult stages.

Table 6a: Hybrid to purebred ratios for larval F1 samples from 57 – 71 DPH.

Salinity		**16 psu**			**35 psu**			**6 psu**		
Genetic group		**Hybrid**	**Purebred**	**Total**	**Hybrid**	**Purebred**	**Total**	**Hybrid**	**Purebred**	**Total**
57 DPH	Number of individuals	19	1	20	11	9	20	6	3	9
	Percentage of total	95%	5%	100%	55%	45%	100%	67%	33%	100%
71 DPH	Number of individuals	27	3	30	31	10	41			
	Percentage of total	90%	10%	100%	76%	24%	100%			
Total F1 larvae	Number of individuals	46	4	50	42	19	61	6	3	9
	Percentage of total	92%	8%	100%	69%	31%	100%	67%	33%	100%

Table 6b: Hybrid to purebred ratios for adult F1 samples from 1098 DPH - 1120 DPH.

Salinity		**16 psu**			**35 psu**		
Genetic group		**Hybrid**	**Purebred**	**Total**	**Hybrid**	**Purebred**	**Total**
1098 DPH	Number of individuals	33	5	38	38	14	52
	Percentage of total	87%	13%	100%	73%	27%	100%
1106 DPH	Number of individuals	23	8	31	18	12	30
	Percentage of total	74%	26%	100%	60%	40%	100%
1120 DPH	Number of individuals	17	4	21	16	14	30
	Percentage of total	81%	19%	100%	53%	47%	100%
Total F1 adult	Number of individuals	73	17	90	72	40	112
	Percentage of total	81%	19%	100%	64%	36%	100%

3.3 F1 LARVAE

3.3.1 Somatic parameters

The standard length trajectories at early stages for the F1 larvae from 8 days post hatching (DPH) to 57 DPH showed an expected increase in standard length with increasing age at all three salinity regimes (6 psu, 16 psu and 35 psu; Figure 7). No difference in log-transformed standard lengths was found between the 3 salinity groups in the 8 – 57 DPH time period (ANOVA, p>0.05).

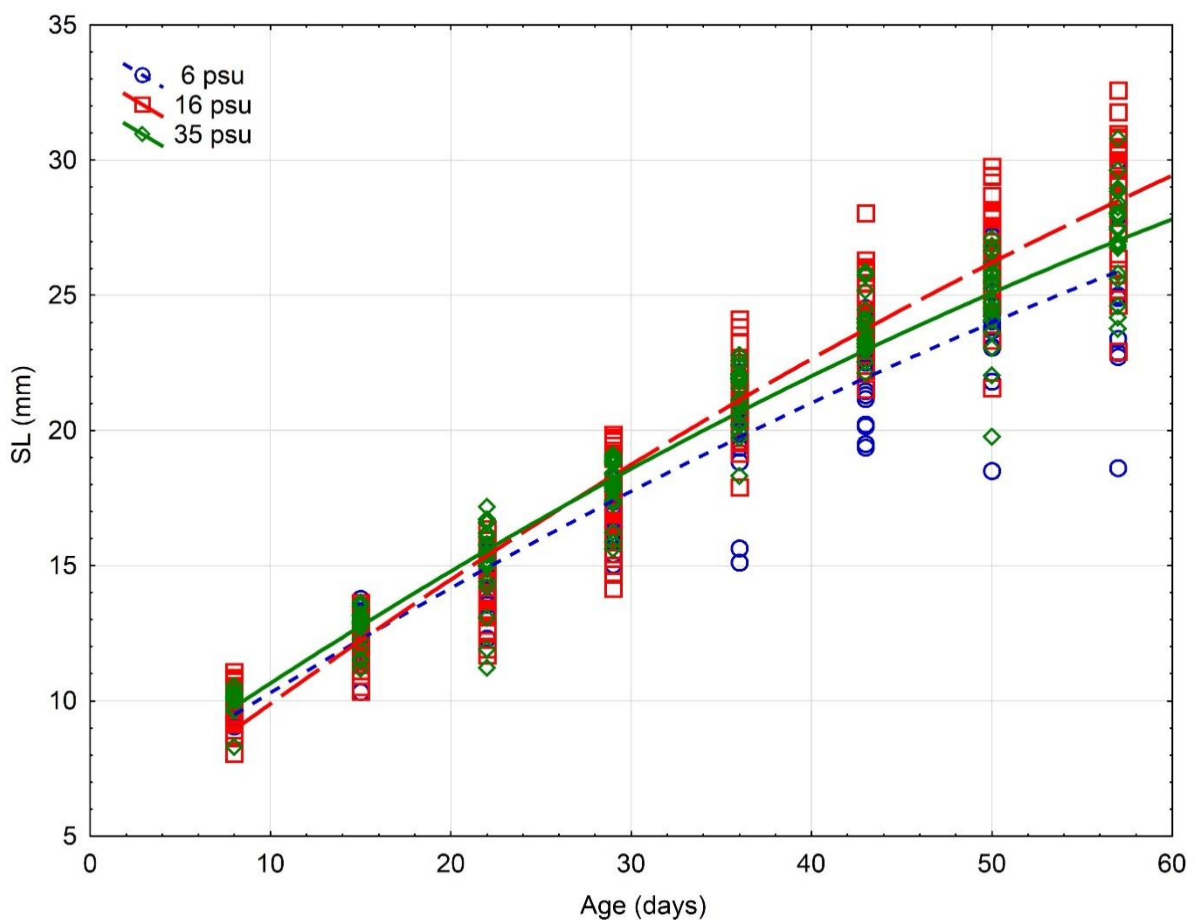

Figure 7: Standard length (mm) for early stages of F1 larvae. The blue open circles dashed line represents the 6 psu salinity group, the red squares and long-dashed line represents the 16 psu salinity group and the green diamonds and solid line represents the 35 psu salinity group. Each point represents individual measurements (Arild Folkvord 2018, pers. com.).

Dry weights increased linearly with standard length (ANOVA, p<<0.001) with the herring reared at 35 psu being heavier than the herring reared at 16 psu (Figure 8). No significant difference was found in between the genetic groups (ANOVA, p= 0.46). The dry weights of hybrids increased faster with increasing standard length than purebreds (ANOVA, p<0.001).

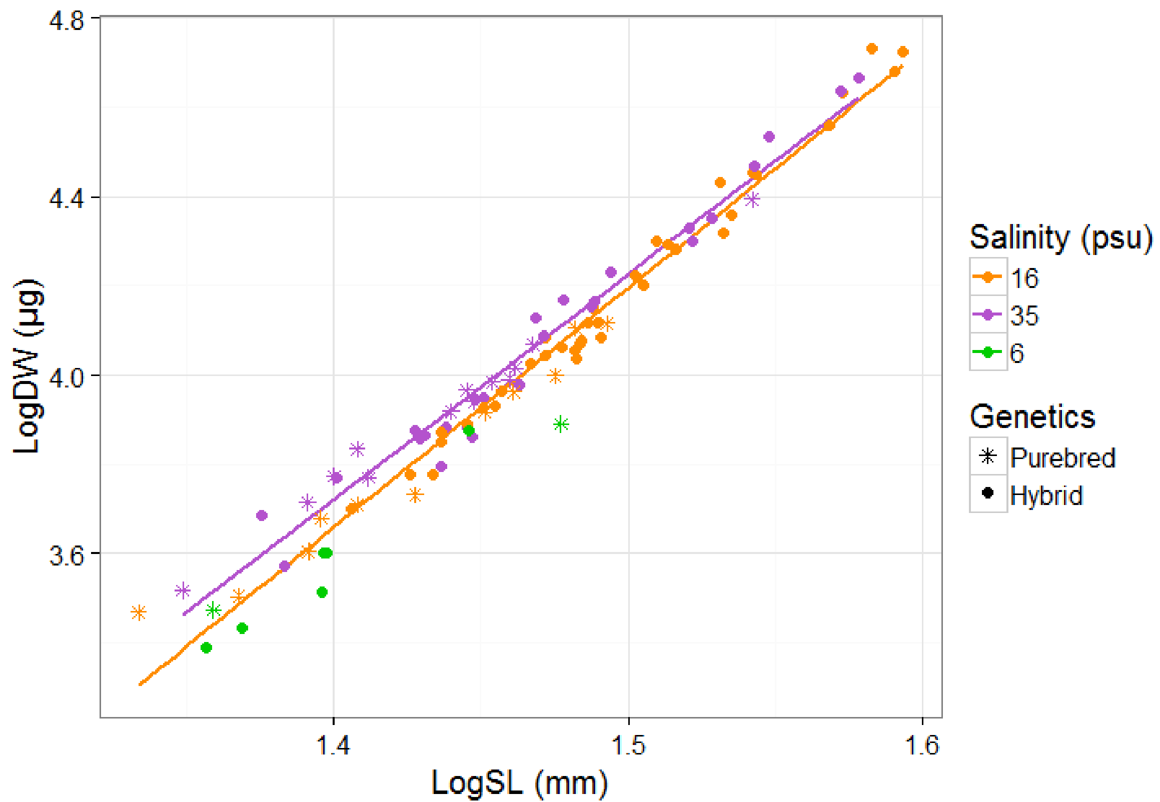

Figure 8: Relationship between log transformed standard lengths and log transformed dry weights for F1 larvae. Lines represent trendlines for 16 psu (orange) and 35 psu (purple) salinity groups. The 6 psu groups is included and visualised in the figure with green colour but excluded in statistical analysis. Asterix symbol represents purebred genetic group and filled circle represents hybrid genetic group.

3.3.2 Otolith measurements

A comparison between the two salinity regimes and two genetic groups was performed as the dry weights were compared against the otolith radii for the F1 larvae used for microstructure analysis in this study. All groups showed generally a positive linear relationship where the

otolith radii increase with increasing dry weights (ANOVA, p<<0.001; Figure 9). The herring reared at 35 psu had significant larger otolith radii than the herring reared at 16 psu (ANOVA, p<0.001). In addition, the otolith radii of purebreds increased faster with increasing dry weight (ANOVA, p=0.037).

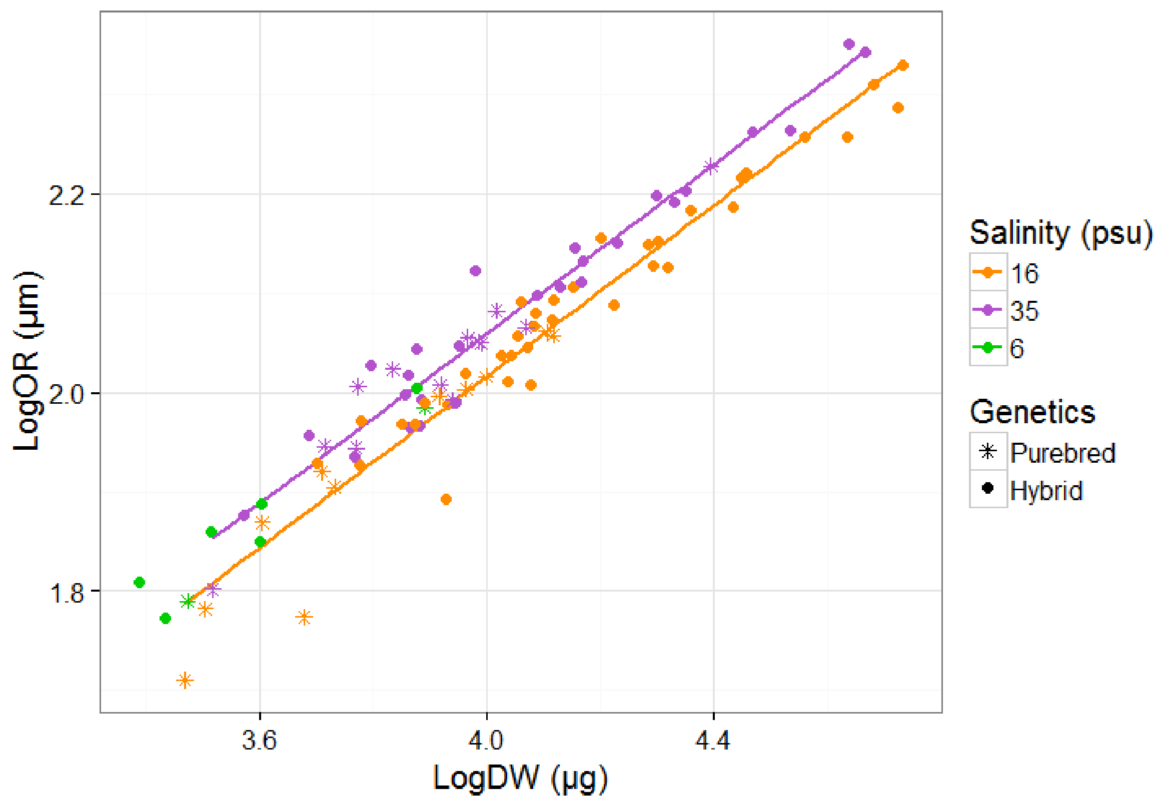

Figure 9: Relationship between log transformed dry weight (µg) and log transformed otolith radii (µm) for F1 larvae. Lines represent trendlines for 16 psu (orange) and 35 psu (purple) salinity group. The 6 psu groups is included and visualised in the figure but excluded in statistical analysis. Orange colour represents 16 psu salinity, purple colour represents 35 psu salinity and green colour represents 6 psu. Asterix symbol represents purebred genetic group and filled circle represents hybrid genetic group.

The otolith microstructure analysis of both larval salinity groups (16 psu and 35 psu) showed generally a positive linear relationship where the mean increment widths increase with

increasing distance from core. No difference was found between the 16 psu group and the 35 psu group (ANOVA, p>0.05), but the increment widths of the 16 psu salinity group increased in size faster than the 35 psu salinity group (ANOVA, p<.0001; Figure 10).

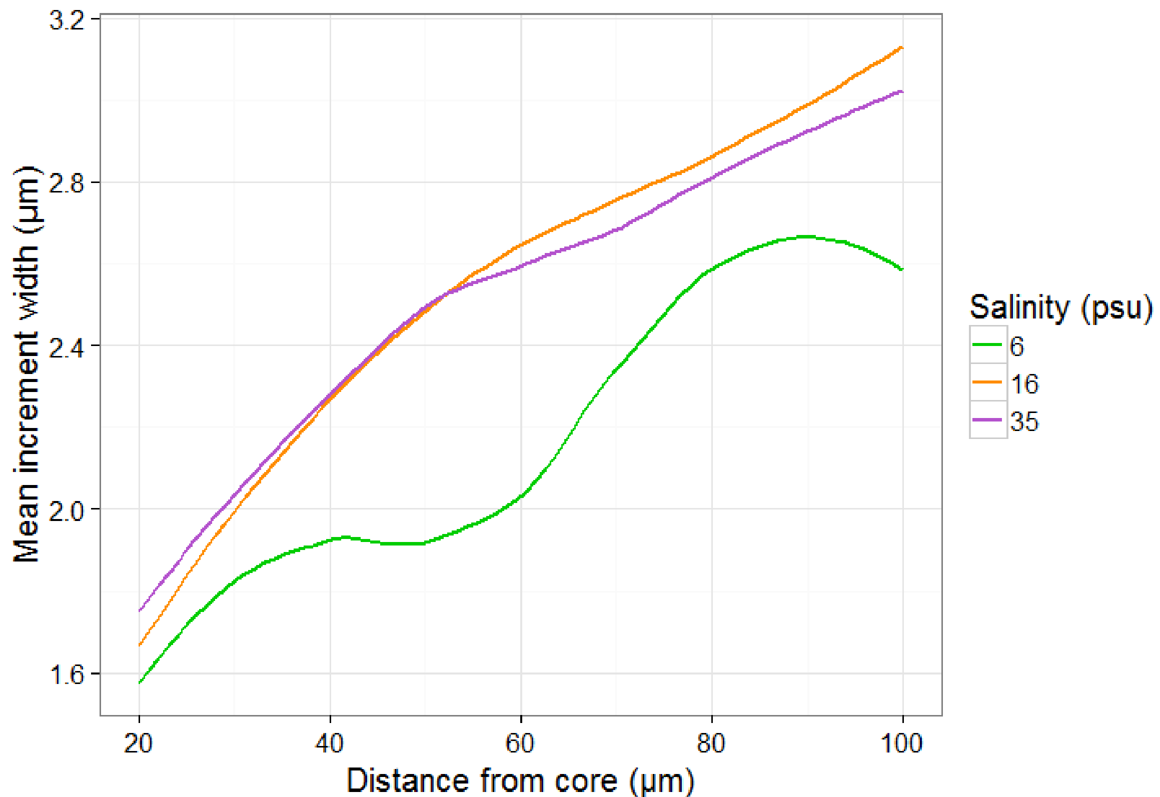

Figure 6: Salinity trendlines for mean increment widths (μm) F1 larvae. The 6 psu groups is included and visualised in the figure with green colour but excluded in statistical analysis. Orange colour represents 16 psu salinity and purple colour represents 35 psu salinity.

A significant full interaction effect between distance from core, salinity regime and genetics was found for the mean increment widths for all the F1 larval experimental groups (ANOVA, p=0.001). A trend was observed where the hybrids had generally larger increments than the purebreds, but only in the 16 psu group the hybrids had larger increments than the purebreds (ANOVA, p<.0001). The genetic groups reared at 6 psu were among the groups having generally the narrowest increments (Figure 11; Appendix A6).

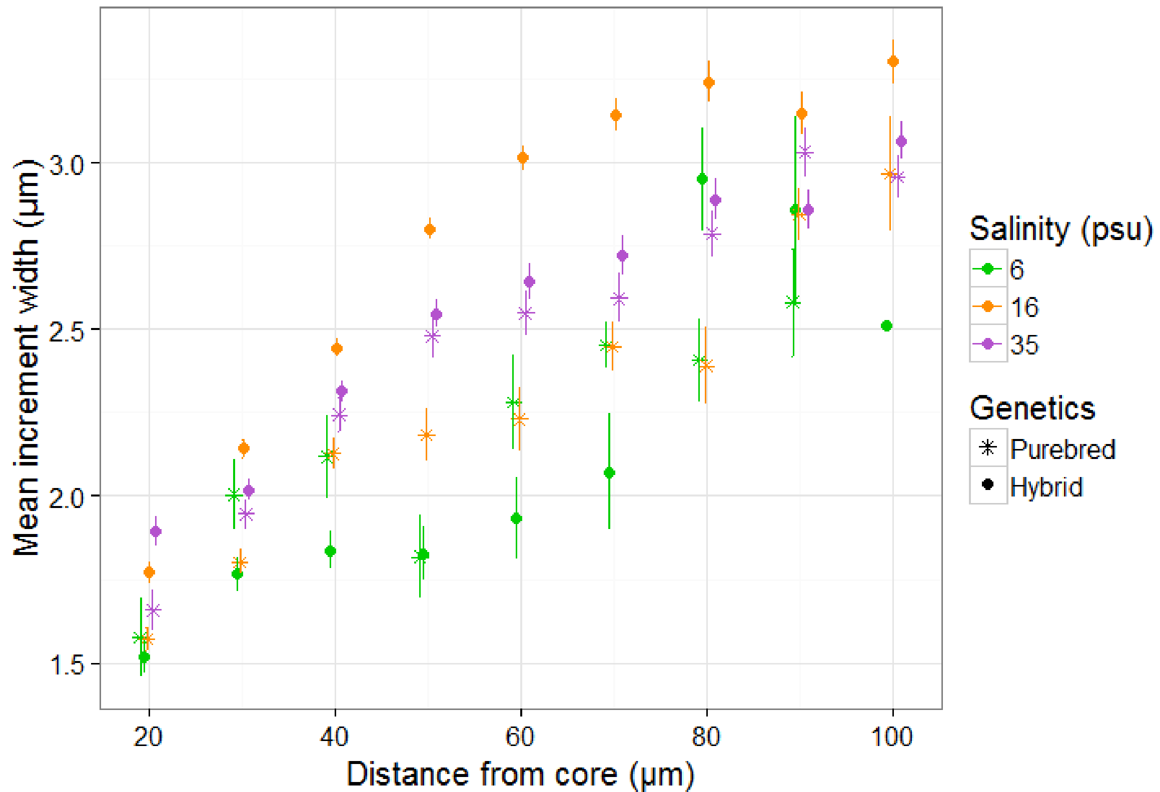

Figure 7: Mean otolith increment widths (µm) with standard error bars for F1 larvae. The 6 psu groups is included and visualised in the figure with green colour but excluded in statistical analysis. Orange colour represents 16 psu salinity and purple colour represents 35 psu. Asterix symbol represents purebred genetic group and filled circle represents hybrid genetic group.

3.4 F1 ADULTS

3.4.1 Somatic parameters

The total lengths of purebred F1 adults were significantly longer than the hybrid F1 adults (ANOVA, p<<0.001; Figure 12). The 35P group was the largest group, having a mean total length of 24.8 cm, the second largest group was the 16P group (mean TL = 24.3 cm), followed by the 16H group (mean TL = 23.6 cm) and the 35H group (mean TL = 23.2 cm).

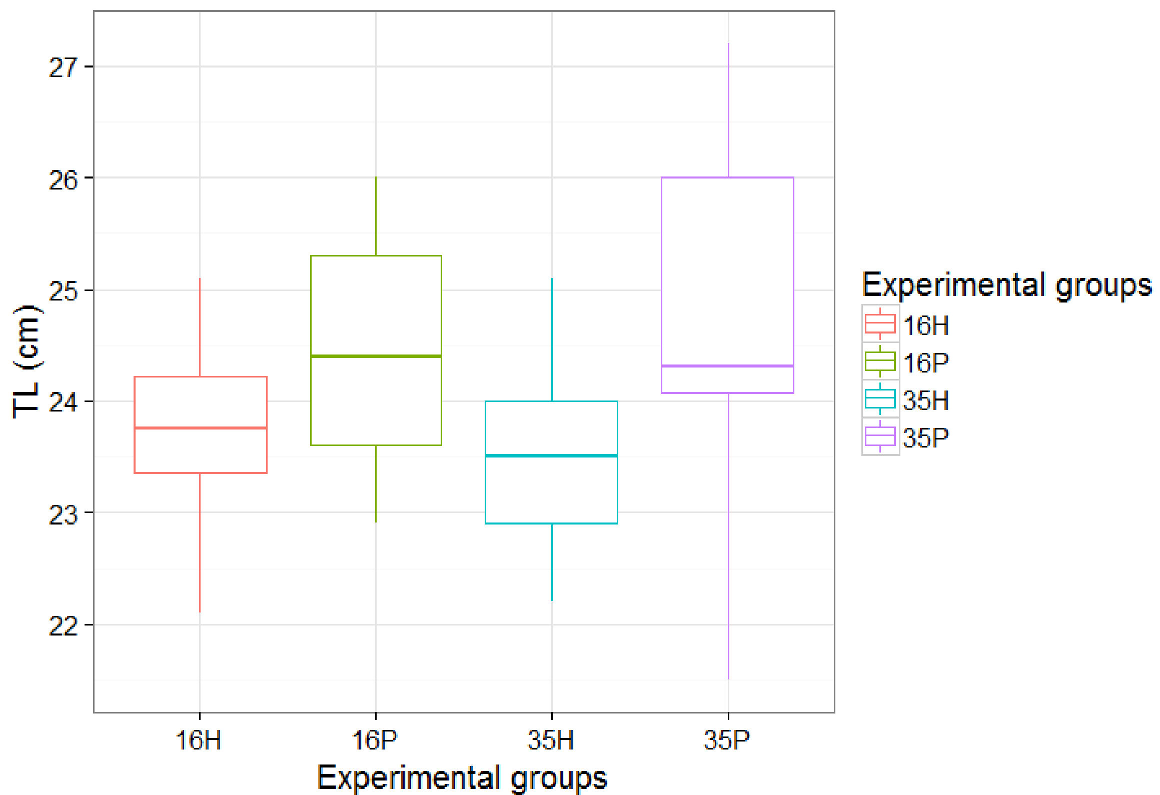

Figure 8: Total length measurements for F1 hybrid (H) and purebred (P) adults reared at either 16 or 35 psu salinity. Each group consists of 17 individuals. The length measurements represent measurements taken at three different sampling days coinciding with the sampling days for the adult F1 herring: 1098 DPH, 1106 DPH and 1120 DPH. The horizontal bar in each box represents the median value. The lower and upper end of a box represent the lower and upper quartile, respectively. Ends of whiskers represent extreme values.

The fish weights excluding gonad weight exhibited a similar trend as the total length where the 35P group was the heaviest group with a mean weight of 125.5 g. The 16P group was the second heaviest group (mean weight = 109.3 g), third heaviest group was the 16H (mean weight = 92.9 g) and the 35H group was the lightest group (mean weight = 83.9 g). However, a full interaction effect between total fish length, salinity and genetics was found for fish weight excluding gonad weight (ANOVA, p=0.029) (Figure 13). In addition, the weights of the 35P group became increasingly higher than the other experimental groups with increasing fish length (ANOVA, p=0.029).

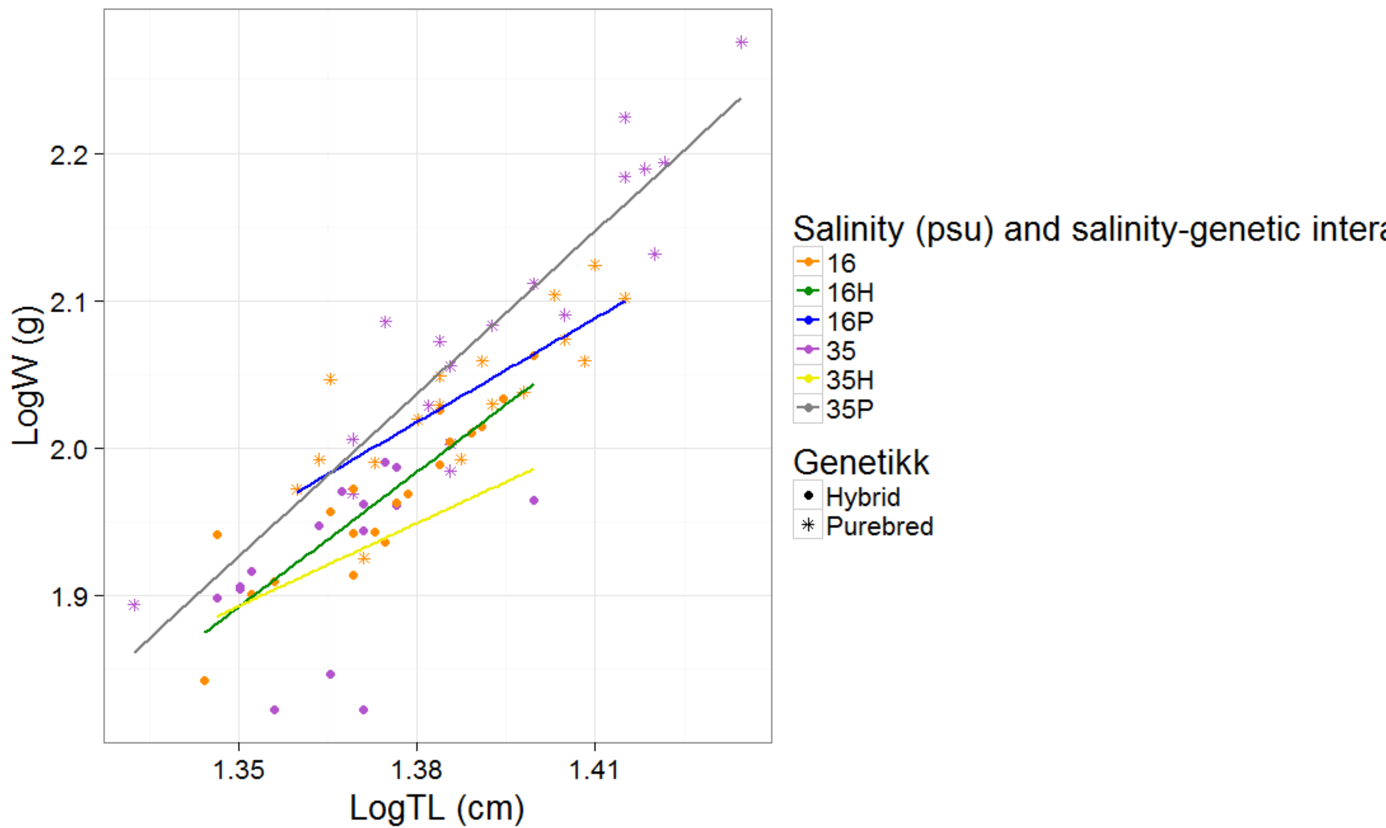

Figure 9: Relationship between log transformed fish total length (cm) and log transformed weights excluding gonad weight (g) for F1 adults. Each group consists of 17 individuals. Orange colour represents 16 psu salinity and purple colour represents 35 psu salinity. Asterix symbol represents purebred genetic group and filled circle represents hybrid genetic group. Lines represent significant interactions between genetics and salinity. Green line represents hybrids reared at 16 psu salinity, blue line represents purebreds reared at 16 psu salinity, yellow line represents hybrids reared at 35 psu salinity, grey line represents purebreds reared at 35 psu salinity.

3.4.2 Otolith measurements

The otolith microstructure analysis of the F1 adult individuals showed generally a positive linear trend for both salinity groups where the increment widths in the 16 psu salinity group increased faster in size than the increment widths in the 35 psu salinity group due to an interaction effect between distance from core and salinity (ANOVA, $p<.0001$; Figure 14).

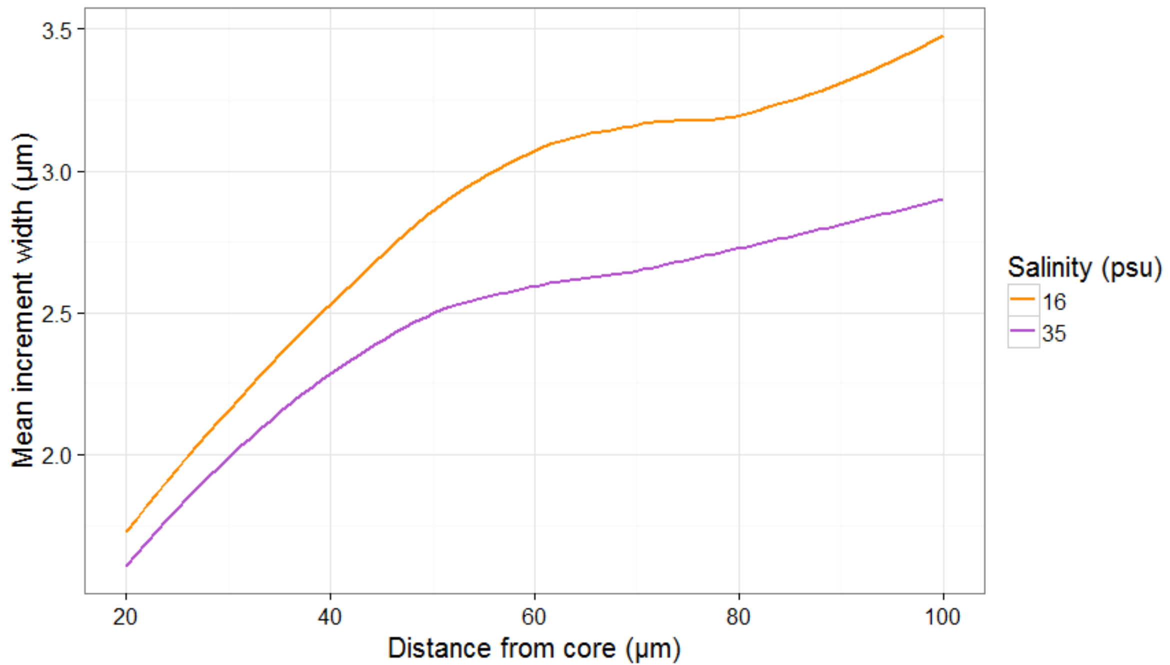

Figure 10: Salinity trendlines for mean increment widths (µm) F1 adults. Orange colour represents 16 psu salinity, purple colour represents 35 psu.

A full interaction effect was found on the increment widths of the F1 adult experimental groups between distance from core, salinity regime and genetics for the mean increment widths for all the F1 adult experimental groups (ANOVA, p= 0.008; Figure 15; Appendix A7) The increment widths in the purebred group increased faster than the hybrid group (ANOVA, p=0.008).

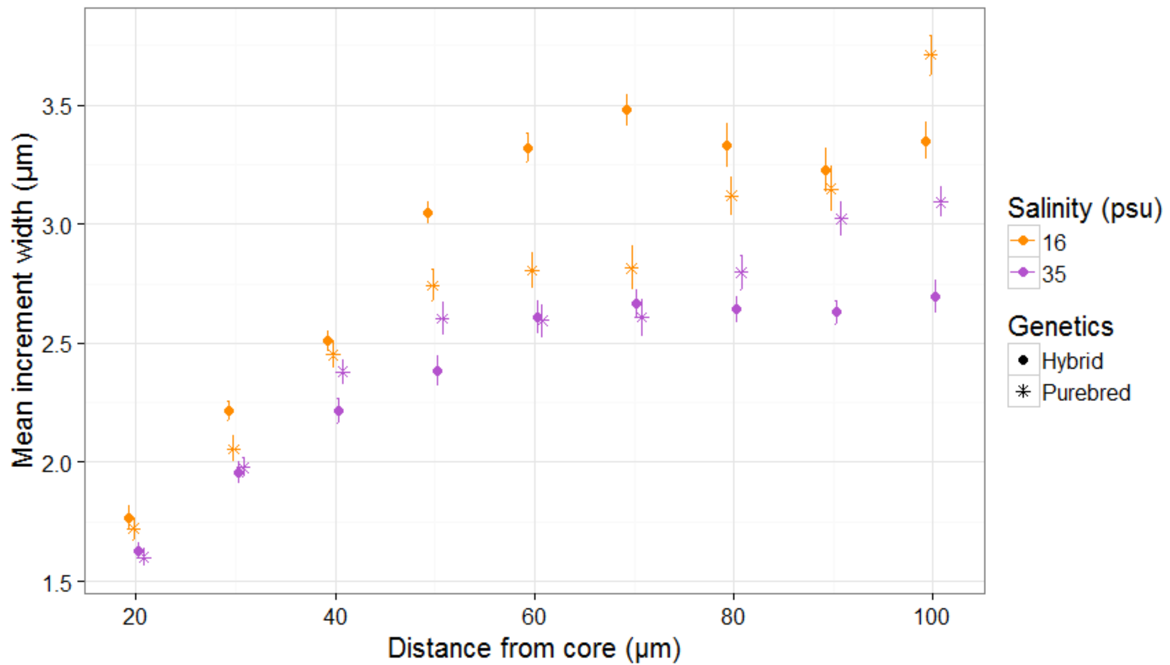

Figure 11: Mean otolith increment widths with (µm) standard error bars for F1 adult group. All experimental groups had 20 individuals except 16P which had 17 individuals. Orange colour represents 16 psu salinity, purple colour represents 35 psu salinity. Asterix symbol represents purebred genetic group and filled circle represents hybrid genetic group.

3.5 Comparisons of increment widths between two F1 life stages; larva and adult

Otolith growth histories were contrasted over two life stages, the F1 larval stage and adult stage. Several interaction effects affecting mean increment width were found (Appendix A3).

When comparing larval mean increment widths to their sibling adults, with corresponding salinity and genetics (comparisons within each salinity-genetics combination), no difference in mean increment widths was found between the generations for the 35P group, 35H group and 16H group (ANOVA, p= 0.99, 0.13 and 0.75, respectively; Figure 16). In the 16P group the increments of adults were wider than for larvae (ANOVA, p= 0.001). If the 16P group was removed from the statistical analysis, no difference was found between the generations in any of the salinity-genetics combinations (ANOVA, p=0.30).

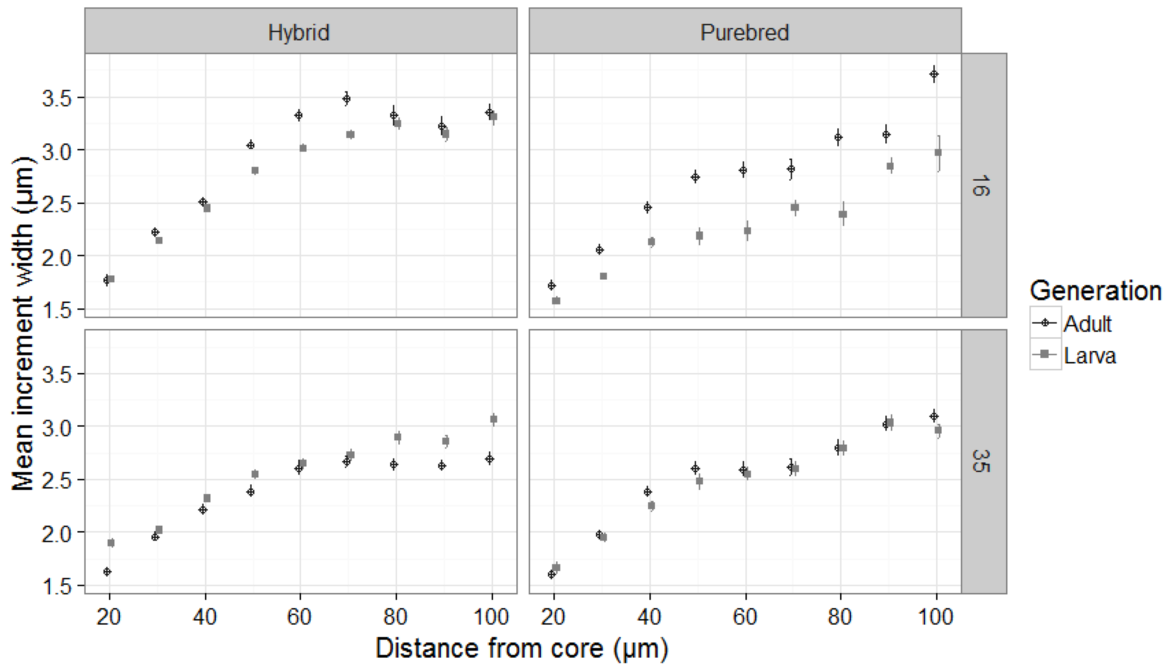

Figure 16: Mean increment widths (µm) with standard error bars for both F1 larvae and adult, separated into the two salinity regimes: 16 psu and 35 psu, and genetic groups: hybrid and purebred. Asterix symbol in dark grey colour represent purebred genetic group and filled circle in light grey represent hybrid genetic group.

4 DISCUSSION

4.1 DISCUSSION OF METHODOLOGY AND LIMITATIONS

4.1.1 Methodology

The common garden experimental set-up appeared to be appropriate and useful tool to isolate and vary salinity as a single factor to see how the groups, in this study with different genetics, performed in terms of somatic and otolith growth. This enabled investigating the genetic basis of the observed phenotypic differences between the experimental groups. A stable temperature and ad lib food supply were present during the whole experiment, so any variation in the results should mainly be due to different responses to the two salinity regimes. The fact that the experimental set-up only contained one tank holding each salinity after transfer to ILAB, lead to some constraints when it comes to comparing results. The absence of replicate tanks for each salinity makes comparisons within each tank stronger than between tanks of different salinities. Therefore, such comparisons between salinities should be interpreted with some caution. Tank effects could potentially occur in this study, as it did in a study on juvenile rainbow trout (*Oncorhynchus mykiss*) where the researcher wanted to investigate tank effects regarding placement of tanks in a research facility (Speare et al*.,* 1995). They studied if growth indices including body weight, feed intake, feed conversion index, and specific growth rate got affected by the location of the tanks, and their findings suggest there was an effect regarding placement of the tanks which could affect the growth indices. Co-rearing hybrids and purebreds together in each tank helped to control for any eventual tank effects between the genetic groups, as all the individuals in the tank would experience the same tank effect. Despite this, as only one tank was used per salinity group, a potentially tank affect would have been hard to discover. Almeland (2015), who worked with the same generation of F1 herring as in this current study, highlighted stable temperature and keeping the same salinity in each tank throughout the whole experiment as more important for the herring than any eventually tank effect.

The F1 generation was a result of crossings between one Atlantic female and one Atlantic male to produce the F1 purebred Atlantic herring, and between the same Atlantic female and a Baltic male to produce the F1 hybrid herring. Using only three parental fish limits the genetic variance which will be further discussed later. Much of the variation found in the early life history among fish larvae can be coupled to heritage and contribution from the parents. Parental effects consist of both genetic and non-genetic effects. Maternal effects are typically defined as only non-genetic parental contribution from the mother to her offspring as traits like egg size, yolk sac volume etc. which are largely affected by non-genetic factors, but rather affected by the condition and nutritional contributions of the female. Paternal effects on the other hand are almost always defined as genetic effects, since the paternal contribution is mainly of genetic origin (Bang et al., 2006). They further suggest that the maternal and paternal effects rather should be termed respectively female and male effects as they both contribute with genetic and non-genetic effects to their offspring. For example, they found that female effects in Atlantic herring are influencing for example larval length and weight, in addition to the larval length and RNA:DNA ratio which are influenced by male effects. The latter mentioned ratio could be an indicator of a cell's metabolic rate, which is linked to otolith size and growth. Also Høie et al. (1999) found that parental effects influence otolith size at hatching. Therefore, one can assume that the metabolic rate and otolith growth are influenced by both female and male effects and the genetic contribution will lay a foundation for further growth and metabolic capacity, although these traits are influenced by environmental factors. This means that the experienced differences in otolith growth between the F1 genetic groups are closely connected to the underlying contribution, both genetic and non-genetic, from the different males and on average the same contribution from the female. This gives a clearer result of actual genetic differences and the genetic contribution on otolith growth is more clearly detectable among the F1 genetic groups than among the parental population which could be affected by different environmental conditions.

Using only one female fish to produce the whole F1 generation, and the fact that the F1 generation was kept in a common garden experimental set-up, any female effects were minimized. Differences within the F1 generation due to different female effect contributions from different females were excluded, but there could still be some differences even when

using only one female fish. Egg size and quality are examples of traits that can vary at a within-female level and Chambers and Waiwood (1996) found in their work on Atlantic cod, that the egg sizes of one female varied throughout the spawning season. Even though cod is a batch spawner and herring is not, it is assumed that some within-female differences in the eggs could also be found among herring. As only three parental fish were used, this naturally constrains the genetic variability and thereby also lessen the strength of the interpretation to what is actually going on in the wild (see below).

This experimental design was also applied for comparisons over life stages by repeated sampling of the same population. This comparison was also a good possibility to test whether the sampling throughout the experiment was done in an appropriate way and that the samples gave the correct impression of the population at given times. As long as no selection or any other major incident affecting the dynamics differently in the groups, one would assume that the larval and adult increment measurements should be similar within each experimental group (16H, 16P, 35H and 35P). If so, this methodology appeared to be suitable for this study where the objectives were to extract and contrast individual otolith growth histories, both between parental and offspring (F1) herring, and between two life stages of F1 fish. No differences were found between the F1 larval and F1 adult increment widths in the 16H, 35H and 35P groups, which indicated that the sampling throughout the experiment has been performed in an acceptable manner and that the samples reflected the true status in the tanks. An additional point is that the variation in the initial measurements (in this study the larval measurements) should be reflected in the variation of the end set of measurements (in this study the adult measurements). The larval and adult increment widths in the 16P group on the other hand, were significantly different from each other where the adult measurements were larger than the larval ones. Since all other groups had no difference, and especially not the other 16 psu group the 16P group was sampled with, it is reasonable to assume that something unexpected had happened in this group rather than an error in the methodology. This will be discussed more in detail in the discussion of results section.

To use otoliths with their daily increment resolution as a tool for comparing growth trajectories appears to be suitable for the current study. The daily increment formation, traceable larval growth in adult otoliths and the fact that they are not reabsorbed were important and valuable traits for this kind of study. The fact that the adult otoliths were hard to back-calculate to a specific day, distance from core was chosen as a reference measurement rather than days-post-hatching. This prevents directly comparisons of increment widths at specific dates, but since the F1 herring were reared in a common garden experimental set-up and all the herring within each tank experienced the same environment comparisons between increment widths at a distance from core should be appropriate. The otoliths can also hold some traits that can be misinterpreted and in turn lead to wrong results (Campana & Moksness, 1991). These will be further discussed in the limitations of the study section.

4.1.2 Limitations of the study

There are some limitations that could have been affecting the results in the current study, such as human errors. There are some features that could occur in the otolith that can cause misinterpretation of the daily increment pattern and weaken the validity of the microstructure analysis. One of these features is sub-daily increments which appear as additional smaller increments in between the sequence of daily increments. This can cause smaller increment measurements than the actual width of a daily increment if they are interpreted as daily and not sub-daily increments. Such errors can give more variability and higher estimates of age (days) than the actual daily increments (Campana & Neilson, 1985). In addition to apparent decreases in increment width which initially should increase with increasing distance from core when there are good growth conditions as in a spring situation with a high abundance of prey organisms (Fossum & Moksness, 1993). Some studies also indicate that the formation of sub-daily increments could be affected by feeding frequency in some fish species (Campana, 1983) as well as temperature fluctuations (Campana & Neilson, 1985). By counting sub-daily increments as daily increments, one overestimates the age which will affect the validity of calculations and comparisons between day-specific growth. In this current study with a common garden experimental set-up any eventually changes in temperature or feeding regime that could cause sub-daily increments would most likely apply for all individuals in a tank and not only some individuals, assuming all fish had the same availability of food. In this

case the sub-daily increments could be a systematic error in each tank and can thereby be taken into consideration when interpreting the sequence of increments. Some of the results in this current study indicate that some of the experimental groups, including the parental populations, had mean increment widths as wide as 4 µm. According to findings in (Berg et al., 2017), the widest mean increment width in Norwegian spring spawning herring was around 2.5 µm. This is in line with Clausen et al. (2007) earlier findings from Fossum and Moksness (1993). Clausen et al. (2007) found that the increment widths of spring hatched herring had increments wider than 2 µm early in the measurement transect in the otoliths (from core and out towards the otolith edge) and that the widths continued to increase at increasing distances from core. Fossum and Moksness (1993) found that spring-spawned herring larvae captured off western Norway had a monthly increase in increment widths of 0.7 µm per month after April. The spring-spawners they analysed had already by May 1990 an increment width over 2 µm. Thus, I cannot exclude the possibility that some increments might have been missed during analysis.

Another feature that could possible cause misinterpretations in the increment readings is the “edge effect”. The curved otolith edge can cause the light from the microscope to be refracted at obtuse angles which makes the increment narrower than what they actually are. This can cause the increment measurements from the outer edge of the otoliths to be narrower than what they actually are. In this current study this possible error was accounted for by limiting the section of the otolith where the measurements were taken from. The measurements used in the statistical analysis were between 20 µm and 100 µm from the core. To not include the innermost 20 µm of the otolith radius confined another possible error; the unclarity of increments near the otolith core. The unclarity in this section can influence the measurements in becoming either too large or small compared to the actual size. There is also a risk of overseeing narrow increments. Fox et al. (2003) found that slow-growing herring larvae appear to deposit daily increments that are too narrow to detect under the optical resolution limit for optical light microscopy. Even scanning electron microscopy (SEM) did not manage to detect all increments. Overseeing increments will give an underestimation of the number of increments and make increments appear wider than they actually are. This will in turn

influence the results and give a wrong impression of back-calculated hatch date, which in turn will influence any eventual estimates in population structure and recruitment studies.

An advice found in literature to get more consistent and reliable data is to have several people performing the same analysis. This could lead to a higher precision in the age estimate, but it is important to remember that the precision does not have to be correlated with the accuracy of the age estimate as all the people performing the analysis could all potentially perform the same error. Campana and Moksness (1991) mentioned four factor which can lead to differences in accuracy and precision among individual otolith readers: type of viewing equipment, the magnification used, whether or not the otoliths were polished and how experienced the otolith readers were. Having more people performing the analysis will in term be more costly than if only one person performed the analysis in addition to the presence of any errors due to the former mentioned factors influencing accuracy and precision of age estimates. If only one person is doing the analysis on the other hand, any errors the reader makes will most likely be made for all the readings, so the error becomes a systematic error. Campana and Moksness (1991) also put up some recommendations for conducting otolith microstructure studies which can be useful for further studies and further improve age estimations in fish.

There are also some limitations when it comes to contrasting the results from an experimental laboratory study to what actually is going on in the natural environments. Compared to the natural habitat in the open oceanic waters, the larval density in the rearing tanks, as well as access to food are much higher. In addition to other potential tank effects, this can potentially influence the results in the study and give an inaccurate impression of what is going on in the wild where the larvae and adult fish occur at lower densities. In a common garden experimental set-up, one can control all the environmental factors and feeding regime to specifically find out how specific factors affect the fish. This is quite contrasting to the temporary and spatially varying (favourable) conditions present in the wild where temperature, hydrographic conditions, food availability, salinity, competition and predation etc. make an impact on the life histories for the different fish (Sætre et al., 2002; Fox et al.,

2008; Munk et al., 2009; Stenevik et al., 2012; Higuchi et al., 2018). Based on these variating conditions, it is expected that the wild samples will exhibit more variability than the F1 generation of experimental herring.

4.2 Discussion of results

4.2.1 Parental effects and genetic adaptations; differences between the parental populations

The parental populations have experienced and are genetically adapted to quite different environmental conditions from each other compared to the F1 generation. They come from very dissimilar environments with a salinity difference of almost 30 practical salinity units, are experiencing different annual temperature fluctuations and appear different in somatic characteristics such as length- and weight-at-age where the Atlantic parental herring were longer and heavier than the Baltic parental herring. It may be reasonable to assume that the Atlantic herring have had better growth conditions as many prey species thrive in the warmer and saltier Atlantic water (Bachiller et al., 2016; Fanjul et al., 2018; Illing et al., 2018). The adult Atlantic parental herring were larger than the Baltic parental herring and it is therefore easy to assume that the parental Atlantic larvae were larger than the parental Baltic larvae. Another fact that also builds up under the latter assumption is change in prey community in the Baltic Sea. There has since the late 1980s been a change in oceanographic conditions in the Baltic Sea resulting in an increase in water temperature and a decrease in salinity (Möllmann et al., 2005). This has had an impact on the mesozooplankton community, leading to a shift in prey species for the herring and resulting in density-dependent competition for food resources and thereby a decrease in herring growth and condition. Looking into the larval microstructure gave an opposite impression as the Baltic parental population had wider increments than the Atlantic population, indicating the Baltic population had better growth conditions as larvae. The otolith growth is dependent on both external environmental factors and internal factors in the fish. Since the current study has not investigated the abiotic and biotic conditions at the time the parental fish were hatched, and they come from different year classes, it is hard to draw any clear conclusion as to why the parental populations' somatic parameters and otolith growth do not indicate the same trend. One possibility could be that the Baltic somatic growth is more constraining at a later stage of life than what can be read in the microstructure in the

first 100 µm from the otolith core. Another possible explanation is natural selection of the best fit individuals. Assuming the Baltic conditions are marginal, only the strongest and best adapted individuals actually live through the larval period, become adult herring and transfer their genes to the next generations. The larvae which have their first exogenous feeding at the same time as an increase in biomass from lower trophic levels can have an opportunistic approach and become advantaged by having a sufficient access to food through the critical period (Fossum, 1996). Growth, swimming performance, nutritional condition and metabolic rate etc. of larvae and adult herring will improve when high fish abundance is matched with high prey abundance. These traits will all decline if there is a mismatch between high fish abundance and high prey abundance. Illing et al. (2018) reported that this decline happened faster in spring-spawning Baltic herring than autumn/winter-spawning herring from the North Sea and it is assumed that large larvae will survive such a mismatch better than shorter larvae (Illing et al., 2018). Strong selection for the best adaptations can also be the case in the Atlantic herring as some stocks, like the Norwegian spring-spawning herring (NSS), undergo spawning migration which require a substantial amount of energy and thereby set demands on having a large body size. This can drive the selection among the NSS herring towards longer and heavier individuals (Slotte, 1999), increasing the morphological differences. Even though the Atlantic parental herring in this current study most likely is a group of local spring spawners, one can assume that size-selection also appears in this group. After all, the heterogenous environments the parental populations experience can amplify the contrasting phenotypic responses induced by the different genotypes. The observed differences can with other words be a result of a combination of adaptive phenotypic plasticity and selection, which is also suggested by results from Jørgensen et al. (2008).

4.2.2 F1 generation vs. parental populations

Comparing the mean lengths and weights of the F1 adults to the mean lengths and weights from the parental populations showed a trend where the parental groups represented extreme values and that the measurements for the F1 groups were found in between these values (Appendix A4 and A5). The purebred groups were larger than the hybrids, which can indicate that the purebred Atlantic genes in the F1 generation influence the phenotype towards that of the Atlantic parents. The adult F1 hybrids were larger than the wild caught

Baltic parental population. This could be due to the genes from the Atlantic parent potentially influencing and contributing to the better somatic growth than what was found among the Baltic parental population (Berg et al., 2018a). In addition to this, Bœuf and Payan (2001) gave some examples which indicate that salinity almost always influence growth in fish and that at intermediate salinities, the growth rate increased significantly for 'true' saltwater species. Such a 'true' saltwater species is turbot (*Scophthalamus maximus*) and juvenile turbot e.g. grew better when the larvae were reared under intermediate salinities (Imsland et al., 2001). Since 16 psu is an intermediate salinity compared to both the salinities the parental herring populations were adapted to, the latter example of increased growth in intermediate salinity could partially explain why the hybrids performed better than the Baltic parental population. Coupled with salinities, Imsland et al. (2001) also highlighted the importance of including temperature as a factor and that salinity and temperature interact. It is thus important to keep in mind that the parental populations were wild caught with many highly variable factors that could influence growth while the F1 generation was reared in a laboratory throughout their whole life under stable conditions and with excess amount of food.

There was generally less variability between the F1 generation groups than between the parental populations (Appendix A4 and A5). A common way to explain basic phenotypic variance is to look into variance due to both genetic effects and environmental effects: the total phenotypic variance in a trait is affected by and can be reduced into many other sub-variances like variance due to genotype, environment, interaction between genotype and environment and covariance between genotype and environment (Chambers, 1993). The F1 generation originated from crossings between only one Atlantic female and one Atlantic male, and between the Atlantic female and one Baltic male, thus narrowing the genetic variance, and this co-occurring with naturally genetic drift cause loss of variability. Genetic drift together with selection are two reasons that Chambers (1993) puts up as reasons for loss of variability. The environmental variance was also minimized, as the herring were reared under identical environmental conditions with salinity regime as the only factor being different for the F1 herring groups. This naturally constrains how much variability the F1 generation could display. The basis for variability is much larger in the wild whereas theoretically any male in a population can mate with any female, and the offspring generation thus will consist of

individuals from many different crossings. Additionally, the natural environments with all the influencing factors, like salinity, temperature, access to food and competition etc. vary considerably from area to area and from time to time (Möllmann et al., 2005; Munk et al., 2009; Fanjul et al., 2018). The observed difference in variance between and within each of the parental populations and within the F1 generation is as expected. All in all, it appears from the findings in this study that the growth of the F1 generation is influenced by both the genetic contribution from the parents as well as environmental adaptations linked to salinity, which may as well also be the case for the parental populations. In addition, there was less variance the F1 generation than between the parental populations. The results from the F1 generation can thus indicate how these traits are regulated in the wild as the basis of variance was already more constrained than in natural environments.

4.2.3 F1 generation

At larval stages (57 and 71 DPH), the larvae reared under a 35 psu salinity regime were larger than the larvae reared at 16 psu. A possible explanation for the trend found in the somatic parameters, could be genetically adaptation to natural salinity. The Atlantic parental fish were caught in water holding a salinity around 35 psu whereas the Baltic parental fish were caught in water holding a salinity around 6 psu, so neither the Atlantic herring nor the Baltic herring are fully adapted to 16 psu. For the purebreds, the 35 psu regime is similar to their natural salinity which they are adapted to and they could potentially spend more energy on growth. Ern et al. (2014) performed an examination on oxygen uptake and consumption and osmoregulatory costs at different salinities, including isosmotic and normal habitat salinities, in fishes. They found a trend among some fish species that the ion and osmoregulatory costs are lowest when the fish is in water with a salinity similar to their normal habitat salinity. Although the trend is not universal and opposite trends were found in other species, this could be a reason why the purebreds in 16 psu were smaller than the purebreds in 35 psu. Since they had a higher energetic cost due to maintaining internal homeostasis and osmoregulation, less energy was spent on growth which would result in reduced size-at-age.

The otolith microstructure in the F1 generation at larval stage revealed a trend where the hybrids had wider mean increment width than the purebreds, although the 35P group was

reared under their natural salinity and was supposed to have good growth. The trend showed the largest difference between the genetic groups in the 16 psu salinity regime. Another possible explanation for this could be due to a “hybrid-vigour” effect among the F1 hybrids. The “hybrid-vigour” effect was discussed in Folkvord et al. (2009) as a potential cause to why their hybrid strain performed better than a purebred strain under the purebred’s natural conditions. This is an effect which can occur after a crossing between parents with different genetics and is more commonly found among first-generation offspring in experiments. The hybrid offspring from such a crossing have often a greater positive fitness compared to either of their parents (Falconer & Mackay, 1996). This effect could might be the cause why the hybrids have wider mean increment widths than the purebreds as in the 16 psu salinity regime. The increment widths were similar in the 35 psu salinity regime which can indicate that the Atlantic genes in the F1 hybrids were the most influential genes regulating the growth in the natural Atlantic salinity. Further, 16 psu is more similar to the Baltic environment than the Atlantic and it is easy to assume that the hybrids’ Baltic genes will be more dominant here than the Atlantic ones. The increment measurements could indicate that this might be the case. Increment measurements from purebred Baltic herring reared at 16 psu revealed good growth conditions at 16 psu despite local adaptations to 6 psu (Appendix A8 and A9). This builds up under the assumption of the Baltic genes being more dominant in 16 psu. The larval Atlantic purebreds reared in 16 psu had the narrowest mean increments. This could in general be a result from reduced fitness due to adaptations to other local environments. After all, it is important to bear in mind that otolith growth is also affected by internal and external factors in addition to genetics (Campana & Neilson, 1985; Folkvord et al., 1997; Bang et al., 2006). In addition, the different trends found in somatic and otolith growth could support the notion discussed in Bang *et al.* (2006) that metabolic rate is linked to otolith growth more than the somatic growth. Given these points, our findings from the F1 generation larvae also suggest that both the somatic and otolith growth are influenced by both genetic and environmental induced factors.

It is assumed that the same reasons as previously mentioned for why the purebred F1 larvae in 35 psu did well, can also be applied to the F1 adults; they are adapted to this salinity and did not have to spend energy on osmoregulation and maintaining homeostasis (Ern et al.,

2014). In addition, the purebreds have a good adaptation potential to become larger as the Atlantic parental population were larger as adults than the herring from the Baltic parental population as seen in this study. Contrary to the findings among the purebred larvae, the adult purebreds reared at 16 psu did surprisingly well compared to the hybrids and what was expected based on the findings in the F1 larval otolith readings. A possible explanation to this could be better growth at intermediate salinities (Imsland et al., 2001), or another incident had happened, such as selection for the strongest individuals. This will be shortly discussed.

4.2.4 Selection and mortality

Many findings in this current study indicate that there has been a selection against purebreds in the 16 psu salinity regime. Selection is one of the most fundamental processes when it comes to driving evolution of species and natural selection occurs in nature between individuals in a population where diseases, competition for resources etc. are operating (Smith & Smith, 2009). The selection eliminates the less well adapted individuals and giving 'only' the individuals with the highest fitness the opportunity to reproduce and pass on their high-fitness inducing genes to future generations. A number of studies have documented natural selection among fish; one example showed a decrease in number of White seabream *(Diplodus sargus)* when moving into adult habitat (Planes & Romans, 2004). This decrease was coupled to a decrease in appearance of a specific allele in the remaining population. Later, it was demonstrated that individuals carrying this allele showed significantly lower growth than the ones which did not. This indicated that smaller individuals carrying this allele were under a higher size-selective mortality pressure than those without the allele. Some experiments are not showing any direct genetic selection, but rather direct size-selection, such as an experiment performed on groups of Atlantic cod revealed by otolith analysis size-selective mortality in some groups during a change of diet (Folkvord et al., 2010). Size-selective mortality has also been documented among larval turbot, as the surviving larvae after a starvation period were generally larger individuals estimated from otolith back calculation (Rosenberg & Haugen, 1982). A possible driving force for the clear morphological differences between the Atlantic and Baltic parental populations is selection for the best adaptations for the respective environments or aspects in their life histories e.g. migration.

The increment widths of the 16P group indicate that a selection has been going on in that group. At larval stages the 16P group had the narrowest mean increment width while it had changed at adult stages where the 16P group had the second widest mean increment widths. A possible explanation to this could be selection for the strongest individuals with the highest growth rate as 16 psu is not the purebred Atlantic herring native salinity. The hybrid to purebred ratios in the 16 psu tanks changed essentially throughout the experiment, which suggests the presence of a high mortality selection in the 16 psu tanks. The initial hybrid to purebred ratio in the tanks was 2:1, but the total larval ratio was on average 11:1 in the larval 16 psu samples and 4:1 in the adult 16 psu samples. The higher mortality of purebreds could be because the smallest and poorest individuals were selectively removed, leaving the bigger and stronger ones left to become adults. A trait that support this hypothesis is the otolith microstructure of the purebreds reared in 16 psu when comparing the larval and adult life stages. The larval microstructure contained relatively small increments at distances from core compared to the widths present in the adults. This means that in the earliest larval samples, the purebred group included more of the weaker and slower growing individuals which later would have died off due to selection leaving only the best and fastest growing larvae to become adults. The sampling in the 35 psu tank was not always random as sick and weakened fish from the bacterial infection were selectively sampled. This could be the reason for the observed small change in ratio in the 35 psu tank. Almeland (2015) reported that 83 % of the sampled sick and weakened fish were hybrids and that the hybrid to purebred ratio later changed as a result of the infection. This could indicate that the hybrids were more prone for this infection possibly due to lack of immune resistance as the infecting bacteria does not thrive in lower salinity, but it is highly emphasized that this hypothesis cannot firmly be concluded from the data in the study. Regardless of this, this selection was not as prominent as in the 16 psu tank. It is highly possible that such a selection can occur in the wild which will in turn make a local impact on the genetic dynamics in a population.

5 CONCLUSIONS

The findings in this present study suggest that despite the Atlantic parental herring being larger than the Baltic parental herring, the larval otolith increments of the Baltic herring indicated better larval growth than the Atlantic herring. This could indicate that the differences between the populations are not large during larval stages, and that the environment at later stages highly influences the terminal somatic size.

Several adaptations could be observed in this study, such as genetic adaptations to their natural salinity. In addition, better performance at intermediate and/or natural salinities could also have an impact on the increment widths. Another influencing factor to why the hybrids performed relatively well compared to the purebreds could be the “hybrid-vigour” effect.

Generally, for both larval and adult life stages, both salinity and genetics were affecting the increment widths which indicate that both factors influence the otolith growth. There were no clear differences between the purebreds and hybrids at larval stages, indicating that the terminal somatic differences are mainly caused by environmental influences. This is supported by other studies like Barrio et al. (2016) who found both genetic (coding) and environmental (non-coding) changes contribute to adaptations. Further studies and research should include a larger spectrum of parental crossings to obtain a get a broader genetic baseline. Emphasis should also be directed towards other environmental factors also differing between the two herring stocks, like for example temperature.

The observed trends, interpretations and conclusions from this present study, including the selection, can with some cautions be transferred to the actual situation in the wild and be implemented in management of the Atlantic herring metapopulations. The underlaying genetics, selection and ecological adaptations are of high importance in order to obtain sustainable stock assessment and population management as larval drift, year class strength, year to year fluctuations in abundance and migration patterns etc. which all have impacts on the herring sub-populations. For implications of this study into management plans, one should include more environmental factors in the analysis as all different the factors the herring experience, genetic and environmental, seem to interact and affect each other.

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APPENDICES

A1: Age, total length and weight measurements for the individuals from the parental populations used in crossings to produce the F1 generation offspring and comparisons against their respective parental populations. * indicates the main three fish used for fertilisation to produce the F1 generation (Atlantic purebreds and hybrids). ** indicates fish that only contributed with a limited number of offspring to the F1 generation reared at 6 psu. *** indicates fish used to produce purebred Baltic offspring.

	Atlantic							Baltic				
	Female	Male	Whole population	AF31*	AM33*	AF29**	AM27**	Female	Male	Whole population	BF21***	BM19*
Number of read annual increments/ estimated ages in Table X1	4,8 ± 1.1/ 6.0 ± 1.9	5.0 ± 1.1/ 6.0 ± 2.0	4.8 ± 1.1/ 5.7 ± 1.8	7	5	10	6	5.1 ± 1.1/ 7.0 ± 1.0	5.1 ± 1.1/ 7.3 ± 0.9	5.1 ± 1.1/ 7.5 ± 1.0	7	7
Weight (g)	277.6 ± 36.2	283.9 ± 30.9	277.6 ± 36.3	295	320	343	270	50.3 ± 8.1	51.3 ± 8.3	51.3 ± 8.4	67.3	61.8
Total length (cm)	31.9 ± 2.1	32.2 ± 1.9	31.9 ± 2.0	29	31	36	32	19.9 ± 0.7	19.9 ± 0.6	19.9 ± 0.6	21	20

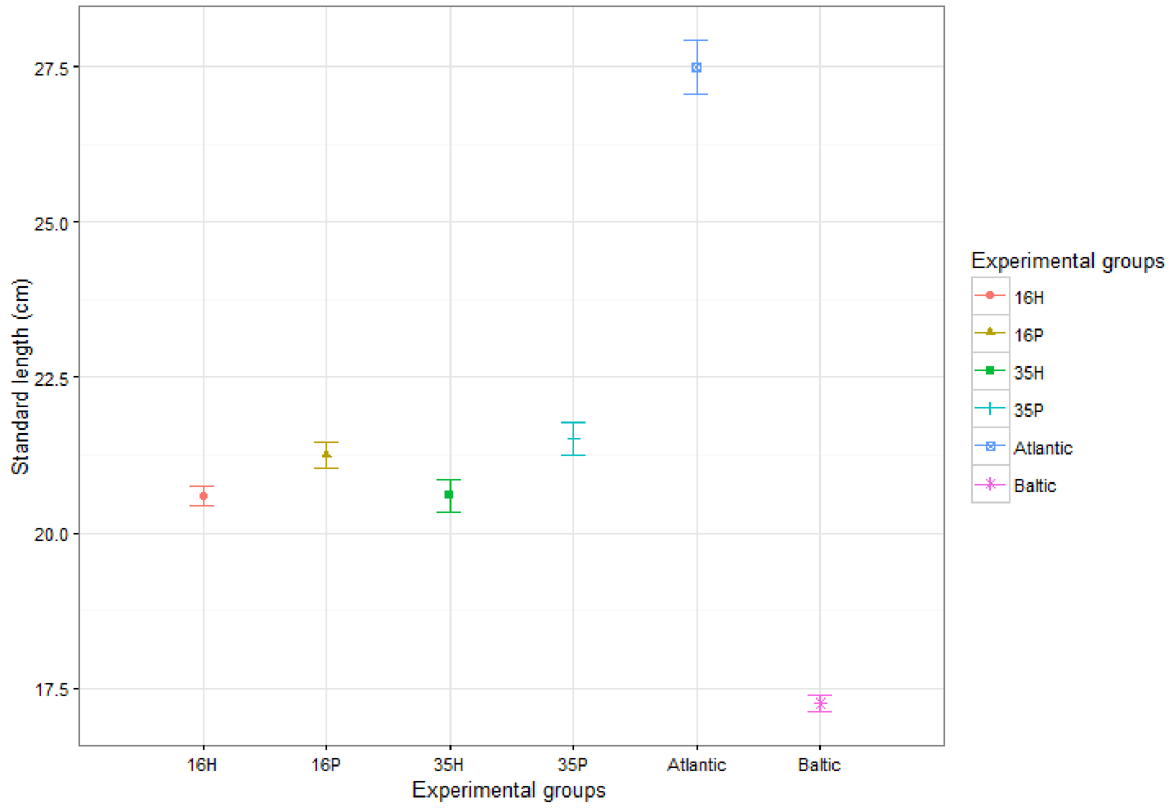
A2: Overview over R packages used for statistical analysis.

R package	**Developer and referance**
library(ggplot2)	H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2009.
library(lsmeans)	Russell V. Lenth (2016). Least-Squares Means: The R Package lsmeans. Journal of Statistical Software, 69(1), 1-33.<doi:10.18637/jss.v069.i01>
library(multcompView)	Spencer Graves, Hans-Peter Piepho and Luciano Selzer with help from Sundar Dorai-Raj (2015). multcompView: Visualizations of Paired Comparisons. R package version 0.1-7. http://CRAN.R-project.org/package=multcompView
library(ggmap)	D. Kahle and H. Wickham.(2013) ggmap: Spatial Visualization with ggplot2. The R Journal, 5(1), 144-161. URL http://journal.r-project.org/archive/2013-1/kahle-wickham.pdf
library(nlme)	Pinheiro J, Bates D, DebRoy S, Sarkar D and R Core Team (2014). _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-117, <URL: http://CRAN.R-project.org/package=nlme>

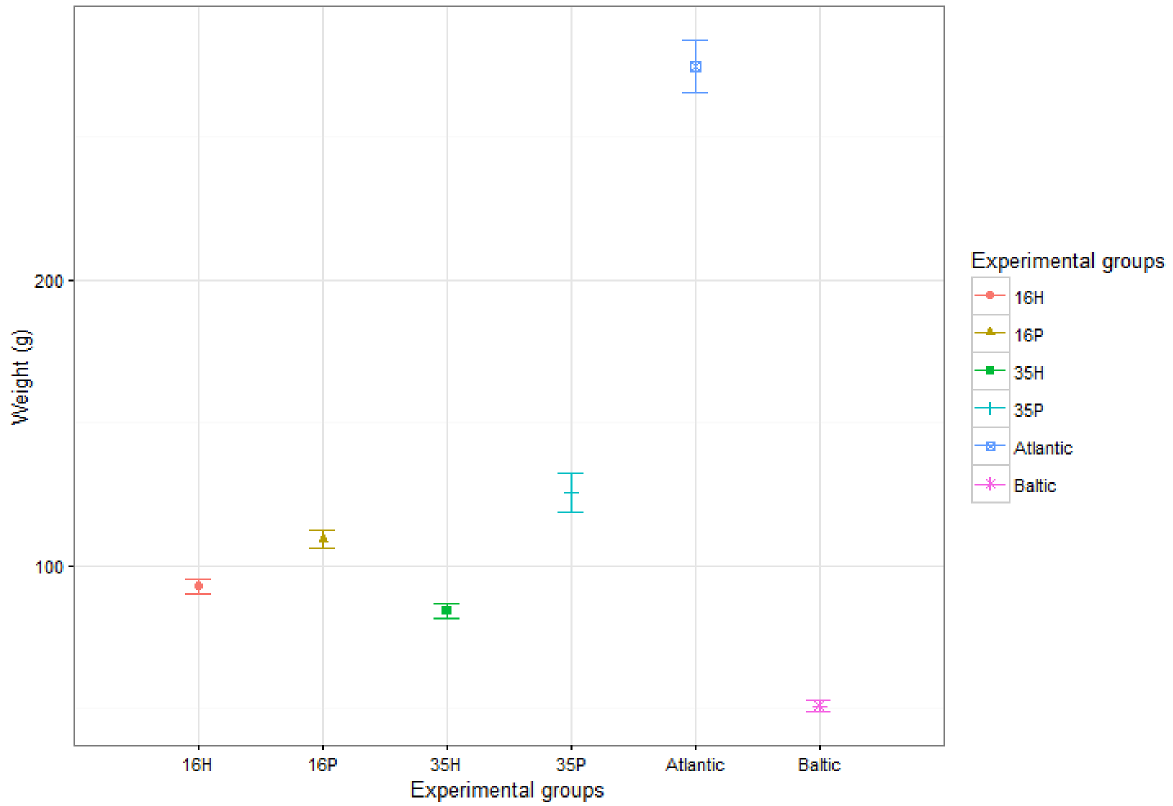
library(epicalc)	Virasakdi Chongsuvivatwong (2012). epicalc: R package vers -ion 2.15.1.0. http://CRAN.R-project.org/package=epicalc

A3: Significant interactions when comparing larval and adult otolith increment widths. Only significant interactions are presented. : represents an interaction sign, "DIST" represent distance from core (µm), "SalinityF" represents salinity (psu), "Genetics" represents genetic groups (hybrid and purebred) and "life_stage" represents life stages (larva and adult).

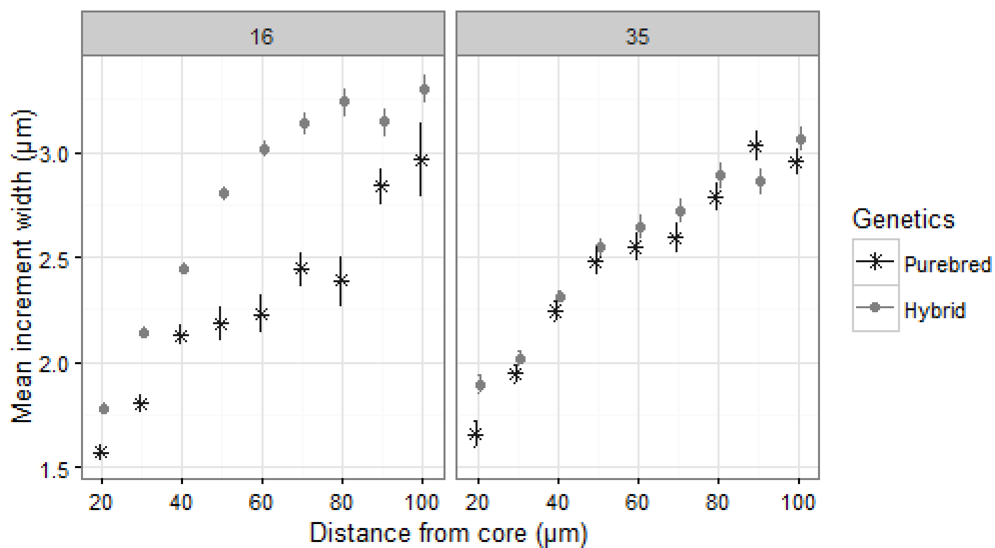
Significant interactions in comparisons between larval and adult life stages	**p-value**
DIST:SalinityF	<.0001
DIST:Genetics	0.0020
SalinityF:Genetics	0.0150
DIST: life_stage	0.0005
SalinityF: life_stage	0.0023
Genetics: life_stage	0.0079
DIST:SalinityF:Genetics	0.0012
DIST:SalinityF: life_stage	0.0127
DIST:Genetics: life_stage	<.0001



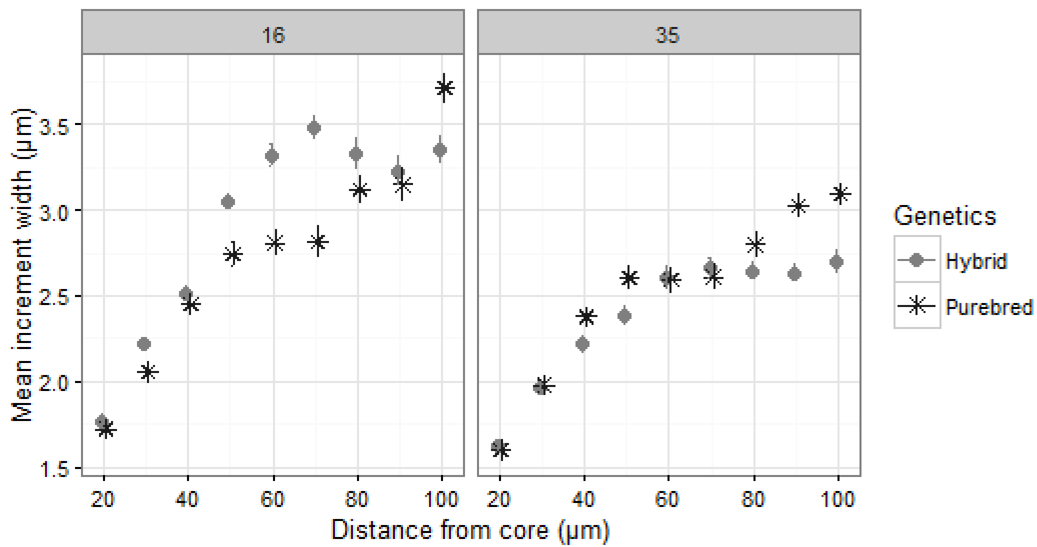
A4: Standard length (cm) comparisons between F1 adult experimental groups and Atlantic a nd Baltic parental populations.



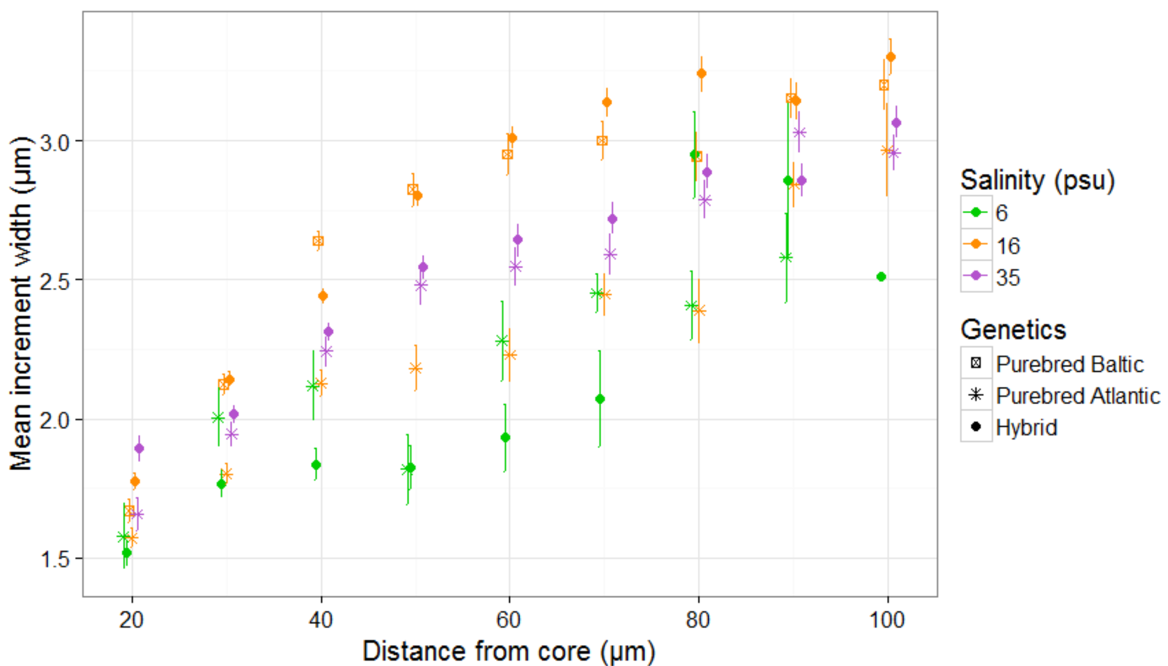
A5: Weight (g) comparisons between F1 adult experimental groups and Atlantic and Baltic pa rental populations



A6: Comparisons of otolith increment widths (µm) between larval genetic groups within each salinity regime (16 psu and 35 psu). Asterix symbol in dark grey colour represent purebred genetic group and filled circle in light grey represent hybrid genetic group.

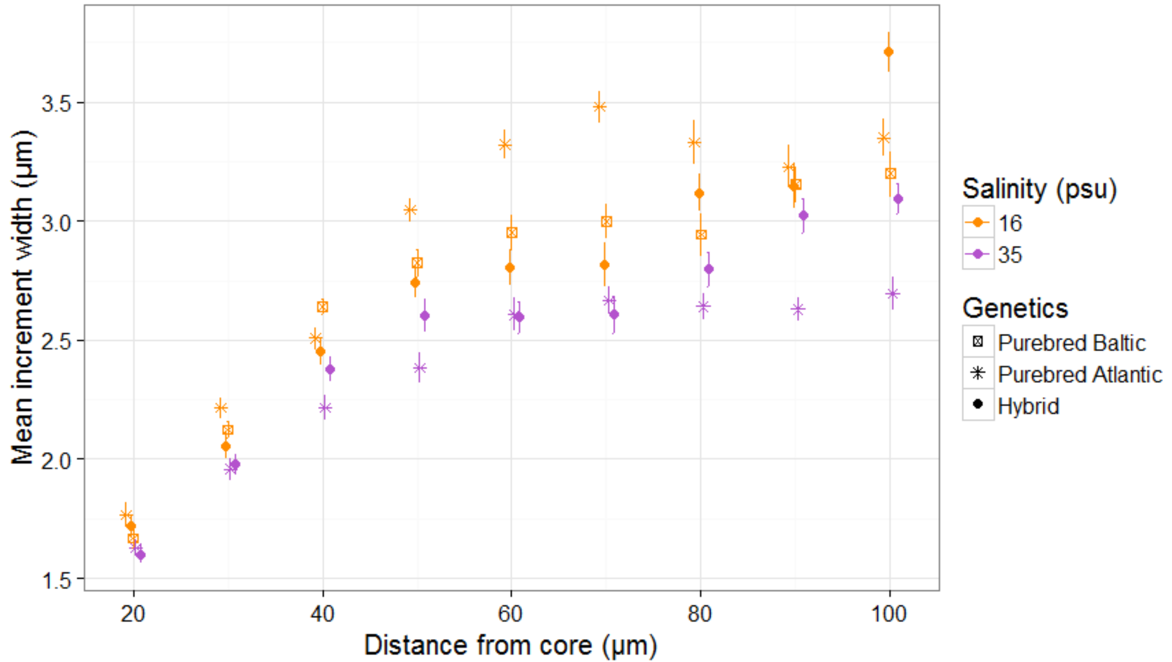


A7: Comparisons of otolith increment widths (µm) between adult genetic groups within each salinity regime (16 psu and 35 psu). Asterix symbol in dark grey colour represent purebred genetic group and filled circle in light grey represent hybrid genetic group.

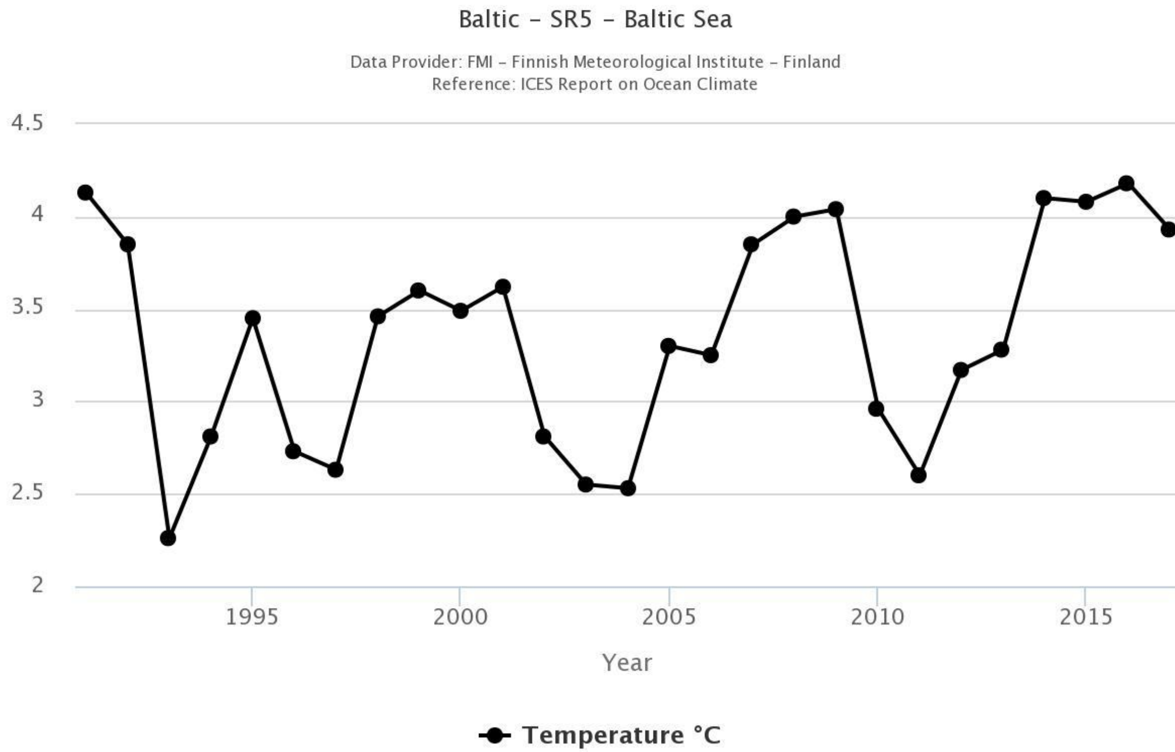


A8: Mean otolith increment widths (µm) with standard error bars for F1 larval group and purebred Baltic group. The 6 psu groups is included and visualised in the figure with green colour but excluded in statistical analysis. Orange colour represents 16 psu salinity and

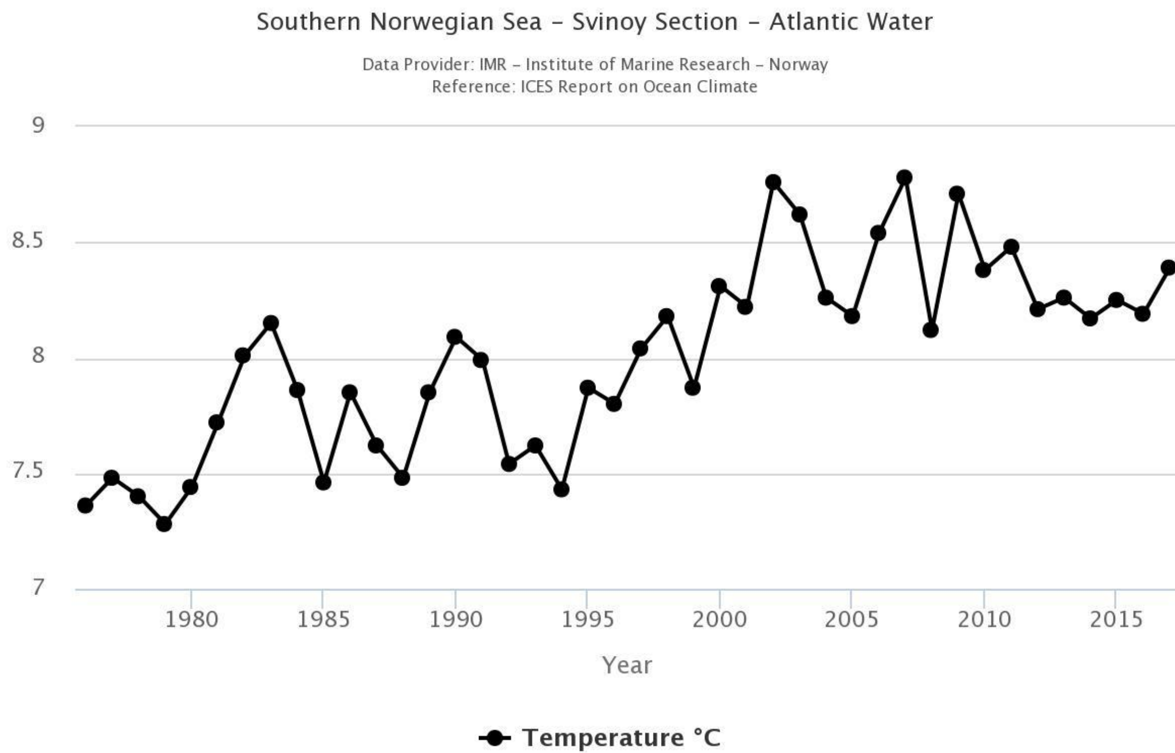
purple colour represents 35 psu. Asterix symbol represents purebred genetic group, filled circle represents hybrid genetic group and crossed square represents purebred Baltic group.



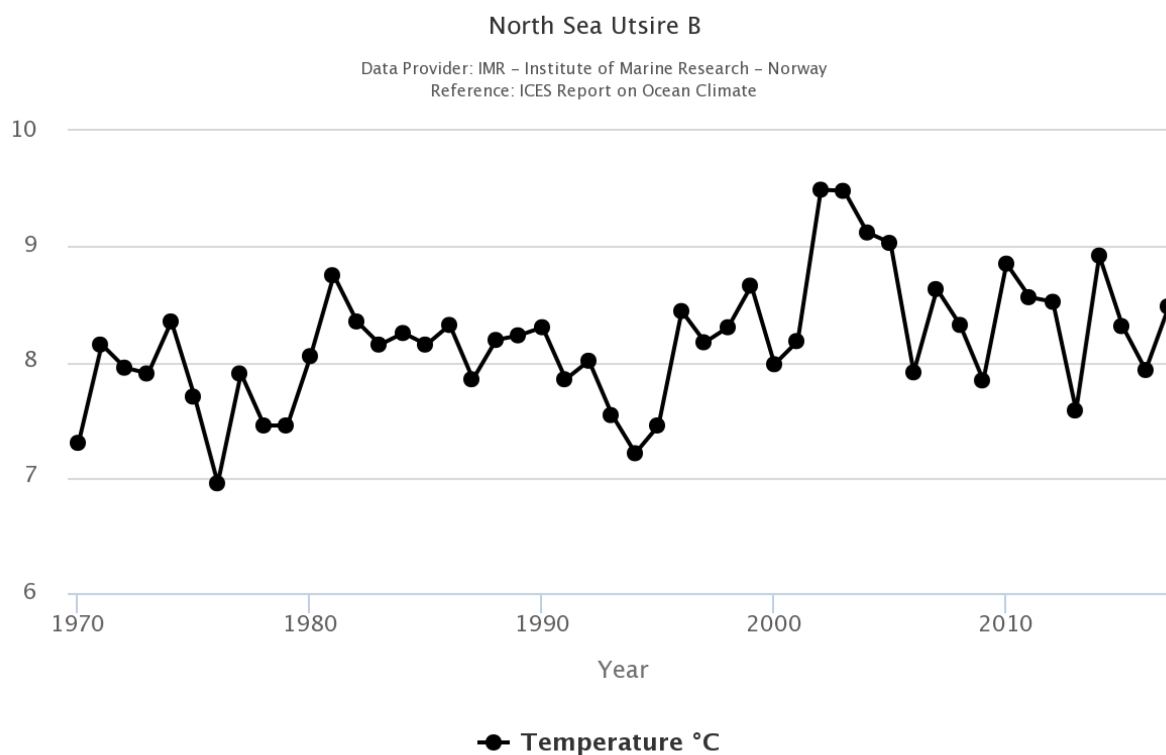
A9: Mean otolith increment widths with (µm) standard error bars for F1 adult group and purebred Baltic group. Orange colour represents 16 psu salinity, purple colour represents 35 psu salinity. Asterix symbol represents purebred genetic group, filled circle represents hybrid genetic group and crossed square represents purebred Baltic group.



A10: Annual temperature measurements in the Baltic Sea (ICEC Report on Ocean Climate)



A11: Annual temperature measurements in the Southern Norwegian Sea (ICEC Report on Ocean Climate)



A12: Annual temperature measurements in the Southern Norwegian Sea (ICEC Report on Ocean Climate)

A13: Statistical models and tests

A13a: Statistical models and tests for parental populations

```
AnoPalder <- lm(Alder_lest~Population, data=newdataPa_1.df)
anova(AnoPalder)

Analysis of Variance Table

Response: Alder_lest
           Df Sum Sq Mean Sq F value Pr(>F)
Population  1  1.059  1.0588    0.83 0.3691
Residuals  32 40.824  1.2757

summary(AnoPalder)

Call:
```

```
lm(formula = Alder_lest ~ Population, data = newdataPa_1.df)

Residuals:
    Min      1Q  Median      3Q     Max 
-1.1177 -0.7647 -0.1177  0.2353  2.2353 

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)    
(Intercept)        4.7647     0.2739  17.393   <2e-16 ***
PopulationBaltic   0.3529     0.3874   0.911    0.369    
Residual standard error: 1.129 on 32 degrees of freedom
Multiple R-squared:  0.02528,	Adjusted R-squared:  -0.005179 
F-statistic:  0.83 on 1 and 32 DF,  p-value: 0.3691
```

```
AnoPkjonn <- lm(Alder_lest~Kj.nn, data = newdataPa_1.df)
anova(AnoPkjonn)

Analysis of Variance Table

Response: Alder_lest
          Df Sum Sq Mean Sq F value Pr(>F)
Kj.nn      1  0.423  0.4227  0.3263 0.5719
Residuals 32 41.460  1.2956               

summary(AnoPkjonn)

Call:
lm(formula = Alder_lest ~ Kj.nn, data = newdataPa_1.df)

Residuals:
     Min       1Q   Median       3Q      Max 
-1.06667 -0.84211 -0.06667  0.15789  2.15789 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)    
(Intercept)   4.8421     0.2611  18.543   <2e-16 ***
Kj.nnM        0.2246     0.3931   0.571    0.572    
Residual standard error: 1.138 on 32 degrees of freedom
Multiple R-squared:  0.01009,	Adjusted R-squared:  -0.02084 
F-statistic: 0.3263 on 1 and 32 DF,  p-value: 0.5719
```

```
AnoPvekt<-lm(Vekt~Lengde_TL+Population, data=newdataPa_1.df)
anova(AnoPvekt)

Analysis of Variance Table

Response: Vekt
           Df Sum Sq Mean Sq   F value  Pr(>F)    
Lengde_TL   1 439365  439365 1444.2339 < 2e-16 ***
Population  1   1894    1894    6.2244 0.01814 *  
Residuals  31   9431     304                      

summary(AnoPvekt)

Call:
lm(formula = Vekt ~ Lengde_TL + Population, data = newdataPa_1.df)
```

```
Residuals:
    Min      1Q  Median      3Q     Max 
-35.232  -5.929  -1.934   4.772  51.669 

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)    
(Intercept)      -165.764     62.726  -2.643   0.0128 *  
Lengde_TL          13.934      1.979   7.040 6.63e-08 ***
PopulationBaltic  -59.984     24.043  -2.495   0.0181 *  

Residual standard error: 17.44 on 31 degrees of freedom
Multiple R-squared:  0.9791,	Adjusted R-squared:  0.9777
```

```
 Ano1.1<-lme(INCR~KUMRAD+Population, random=~+1|Indivcode, data=Padultss)
 anova(Ano1.1)
             numDF denDF  F-value p-value
 (Intercept)     1   757 803.7590  <.0001
 KUMRAD          1   757 512.2992  <.0001
 Population      1    32   7.1626  0.0116

 summary(Ano1.1)
 Linear mixed-effects model fit by REML
  Data: Padultss 
        AIC      BIC    logLik
   1506.479 1529.833 -748.2395

 Random effects:
  Formula: ~+1 | Indivcode
         (Intercept)  Residual
 StdDev:   0.5348407 0.5786454

 Fixed effects: INCR ~ KUMRAD + Population 
                      Value  Std.Error  DF   t-value p-value
 (Intercept)      1.0768082 0.14489362 757  7.431716  0.0000
 KUMRAD           0.0277550 0.00122826 757 22.597031  0.0000
 PopulationBaltic 0.5038498 0.18826273  32  2.676312  0.0116
  Correlation: 
                  (Intr) KUMRAD
 KUMRAD           -0.401       
 PopulationBaltic -0.641 -0.013

 Standardized Within-Group Residuals:
         Min          Q1         Med          Q3         Max 
 -2.62744909 -0.64287597 -0.05910777  0.59087560  3.17880925 

 Number of Observations: 792
 Number of Groups: 34
```

```
Ano1.1<-lme(INCR~KUMRAD+Population, random=~+1|Indivcode, data=Padultss)
anova(Ano1.1)
            numDF denDF  F-value p-value
(Intercept)     1   250 737.8349  <.0001
KUMRAD          1   250   7.4490  0.0068
Population      1    32   0.2301  0.6347

summary(Ano1.1)
Linear mixed-effects model fit by REML
 Data: Padultss 
       AIC     BIC    logLik
  751.6245 769.834 -370.8122

Random effects:
```

```
 Formula: ~+1 | Indivcode
        (Intercept)  Residual
StdDev:   0.7466509 0.7695345

Fixed effects: INCR ~ KUMRAD + Population
                     Value Std.Error  DF  t-value p-value
(Intercept)      2.2440989 0.5448877 250 4.118462  0.0001
KUMRAD           0.0146497 0.0053740 250 2.726028  0.0069
PopulationBaltic 0.1307350 0.2725380  32 0.479694  0.6347
 Correlation:
                 (Intr) KUMRAD
KUMRAD           -0.936
PopulationBaltic -0.243 -0.007

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.24589091 -0.60035628 -0.08417202  0.54533560  2.70574770

Number of Observations: 285
Number of Groups: 34
```

A13b: Statistical models and tests for F1 larva life stages

```
 AnotidlegSL <- lm(LogSL~SalinityF, data = tidlegSL)
 anova(AnotidlegSL)
 Analysis of Variance Table

 Response: LogSL
            Df Sum Sq   Mean Sq F value Pr(>F)
 SalinityF   2  0.003 0.0014755  0.0644 0.9376
 Residuals 503 11.526 0.0229151
 > summary(AnotidlegSL)

 Call:
 lm(formula = LogSL ~ SalinityF, data = tidlegSL)

 Residuals:
      Min       1Q   Median       3Q      Max
 -0.42696 -0.10724  0.03212  0.12016  0.24867

 Coefficients:
             Estimate Std. Error t value Pr(>|t|)
 (Intercept) 1.261859   0.013328  94.677   <2e-16 ***
 SalinityF16 0.002688   0.016678   0.161    0.872
 SalinityF35 0.006450   0.018205   0.354    0.723

 Residual standard error: 0.1514 on 503 degrees of freedom
   (44 observations deleted due to missingness)
 Multiple R-squared:  0.000256, Adjusted R-squared:  -0.003719
 F-statistic: 0.06439 on 2 and 503 DF,  p-value: 0.9376


 test<-lsmeans(AnotidlegSL, pairwise~SalinityF, adjust="tukey")
 summary(test)
 $lsmeans
  SalinityF   lsmean         SE  df lower.CL upper.CL
  6         1.261859 0.01332802 503 1.235674 1.288045
  16        1.264547 0.01002520 503 1.244851 1.284244
  35        1.268310 0.01240130 503 1.243945 1.292674
```

```
Confidence level used: 0.95

$contrasts
 contrast     estimate         SE  df t.ratio p.value
 6 - 16   -0.002688268 0.01667755 503  -0.161  0.9858
 6 - 35   -0.006450497 0.01820518 503  -0.354  0.9332
 16 - 35  -0.003762229 0.01594669 503  -0.236  0.9698

P value adjustment: tukey method for comparing a family of 3 estimates


Ano4<-lm(LogOR~LogDW*Genetics+SalinityF,  data=ss_utan6_stat)
anova(Ano4)
Analysis of Variance Table

Response: LogOR
               Df  Sum Sq Mean Sq   F value    Pr(>F)
LogDW           1 1.31040 1.31040 1504.5567 < 2.2e-16 ***
Genetics        1 0.00095 0.00095    1.0883   0.29996
SalinityF       1 0.04007 0.04007   46.0117 1.762e-09 ***
LogDW:Genetics  1 0.00393 0.00393    4.5133   0.03668 *
Residuals      81 0.07055 0.00087

summary(Ano4)

Call:
lm(formula = LogOR ~ LogDW * Genetics + SalinityF, data = ss_utan6_stat)

Residuals:
      Min        1Q    Median        3Q       Max
-0.101026 -0.013591  0.002368  0.014094  0.068103

Coefficients:
                            Estimate Std. Error t value Pr(>|t|)
(Intercept)                 0.114885   0.107630   1.067   0.2890
LogDW                       0.475357   0.027943  17.012  < 2e-16 ***
GeneticsHeterozygote        0.271542   0.122360   2.219   0.0293 *
SalinityF35                 0.041129   0.006557   6.272 1.64e-08 ***
LogDW:GeneticsHeterozygote -0.066355   0.031234  -2.124   0.0367 *

Residual standard error: 0.02951 on 81 degrees of freedom
Multiple R-squared:  0.9505,	Adjusted R-squared:  0.9481
F-statistic:   389 on 4 and 81 DF,  p-value: < 2.2e-16


Ano4<-lm(LogDW~LogSL*Genetics+SalinityF,  data=ss_utan6_stat)
anova(Ano4)
Analysis of Variance Table

Response: LogDW
               Df Sum Sq Mean Sq   F value    Pr(>F)
LogSL           1 7.1469  7.1469 4374.1188 < 2.2e-16 ***
Genetics        1 0.0009  0.0009    0.5429    0.4634
SalinityF       1 0.0348  0.0348   21.2881 1.462e-05 ***
LogSL:Genetics  1 0.0439  0.0439   26.8427 1.583e-06 ***
Residuals      81 0.1323  0.0016



summary(Ano4)
```

```
Call:
lm(formula = LogDW ~ LogSL * Genetics + SalinityF, data = ss_utan6_stat)

Residuals:
      Min        1Q    Median        3Q       Max 
-0.095692 -0.019792  0.000135  0.022754  0.132292 

Coefficients:
                            Estimate Std. Error t value Pr(>|t|)    
(Intercept)                -2.559255   0.251576 -10.173 3.89e-16 ***
LogSL                       4.466040   0.175776  25.408  < 2e-16 ***
GeneticsHeterozygote       -1.534905   0.295299  -5.198 1.48e-06 ***
SalinityF35                 0.048021   0.008997   5.337 8.40e-07 ***
LogSL:GeneticsHeterozygote  1.058183   0.204243   5.181 1.58e-06 ***


Residual standard error: 0.04042 on 81 degrees of freedom
Multiple R-squared:  0.982,	Adjusted R-squared:  0.9811 
F-statistic:  1106 on 4 and 81 DF,  p-value: < 2.2e-16
```

```
Ano1<-lme(INCR~KUMRAD*SalinityF, random=~+1|ID, data=larvae_ss)
anova(Ano1)
                 numDF denDF  F-value p-value
(Intercept)          1  3518 5938.031  <.0001
KUMRAD               1  3518 2673.540  <.0001
SalinityF            1   114    3.471   0.065
KUMRAD:SalinityF     1  3518   55.819  <.0001

summary(Ano1)
Linear mixed-effects model fit by REML
 Data: larvae_ss 
       AIC      BIC    logLik
  4750.694 4787.879 -2369.347

Random effects:
 Formula: ~+1 | ID
        (Intercept) Residual
StdDev:   0.3469464 0.440583

Fixed effects: INCR ~ KUMRAD * SalinityF 
                        Value  Std.Error   DF  t-value p-value
(Intercept)         1.5201296 0.05391792 3518 28.19340  0.0000
KUMRAD              0.0182056 0.00044366 3518 41.03499  0.0000
SalinityF35         0.1507073 0.07559761  114  1.99355  0.0486
KUMRAD:SalinityF35 -0.0045711 0.00061183 3518 -7.47122  0.0000
 Correlation: 
                   (Intr) KUMRAD SlnF35
KUMRAD             -0.484              
SalinityF35        -0.713  0.345       
KUMRAD:SalinityF35  0.351 -0.725 -0.485

Standardized Within-Group Residuals:
         Min           Q1          Med           Q3          Max 
-3.822644351 -0.637931474  0.006609914  0.638581099  4.918690894 

Number of Observations: 3636
Number of Groups: 116 


Ano1<-lme(INCR~KUMRAD*SalinityF*Genetics, random=~+1|ID, data=larvae_ss)
```

```
anova(Ano1)
                           numDF denDF  F-value p-value
(Intercept)                    1  3516 6887.427  <.0001
KUMRAD                         1  3516 2689.000  <.0001
SalinityF                      1   112    4.036  0.0469
Genetics                       1   112   14.874  0.0002
KUMRAD:SalinityF               1  3516   55.158  <.0001
KUMRAD:Genetics                1  3516    2.854  0.0912
SalinityF:Genetics             1   112    6.984  0.0094
KUMRAD:SalinityF:Genetics      1  3516   10.389  0.0013

summary(Ano1)
Linear mixed-effects model fit by REML
 Data: larvae_ss
      AIC      BIC    logLik
  4755.032 4816.997 -2367.516

Random effects:
 Formula: ~+1 | ID
        (Intercept)  Residual
StdDev:   0.3208032 0.4398373

Fixed effects: INCR ~ KUMRAD * SalinityF * Genetics
                                            Value  Std.Error   DF   t-value p-value
(Intercept)                             1.3460193 0.11194900 3516 12.023505  0.0000
KUMRAD                                  0.0143007 0.00114035 3516 12.540632  0.0000
SalinityF35                             0.2288703 0.14457723  112  1.583032  0.1162
GeneticsHeterozygote                    0.2351891 0.12577529  112  1.869915  0.0641
KUMRAD:SalinityF35                     -0.0003069 0.00139168 3516 -0.220533  0.8255
KUMRAD:GeneticsHeterozygote             0.0045254 0.00123762 3516  3.656538  0.0003
SalinityF35:GeneticsHeterozygote       -0.0994974 0.16666002  112 -0.597008  0.5517
KUMRAD:SalinityF35:GeneticsHeterozygote -0.0050068 0.00155338 3516 -3.223164  0.0013
 Correlation:
                                        (Intr) KUMRAD SlnF35 GntcsH KUMRAD:SlF35 KUMRAD:G SF35:G
KUMRAD                                  -0.519
SalinityF35                             -0.774  0.402
GeneticsHeterozygote                    -0.890  0.462  0.689
KUMRAD:SalinityF35                       0.425 -0.819 -0.520 -0.379
KUMRAD:GeneticsHeterozygote              0.478 -0.921 -0.370 -0.516  0.755
SalinityF35:GeneticsHeterozygote         0.672 -0.349 -0.867 -0.755  0.451        0.390
KUMRAD:SalinityF35:GeneticsHeterozygote -0.381  0.734  0.466  0.411 -0.896       -0.797   -0.517

Standardized Within-Group Residuals:
          Min            Q1           Med            Q3           Max
```

```
-3.8847393910 -0.6403811103 -0.0001613325  0.6433340009  4.9216715974

Number of Observations: 3636
Number of Groups: 116
```

A13c: Statistical models and tests for F1 adult life stages

```
Ano1<-lm(Lengde_TL~SalinityF+Genetikk,  data=Distance_IND_1)
anova(Ano1)
Analysis of Variance Table

Response: Lengde_TL
          Df Sum Sq Mean Sq F value    Pr(>F)    
SalinityF  1  0.132   0.132  0.1287    0.7208    
Genetikk   1 20.152  20.151 19.6602 3.204e-05 ***
Residuals 73 74.824   1.025                      

summary(Ano1)

Call:
lm(formula = Lengde_TL ~ SalinityF + Genetikk, data = Distance_IND_1)

Residuals:
     Min       1Q   Median       3Q      Max 
-3.11031 -0.58907 -0.07862  0.64969  2.58969 

Coefficients:
                 Estimate Std. Error t value Pr(>|t|)    
(Intercept)      23.55031    0.19782 119.052  < 2e-16 ***
SalinityF35       0.02832    0.23268   0.122    0.903    
GenetikkPurebred  1.03168    0.23268   4.434  3.2e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.012 on 73 degrees of freedom
Multiple R-squared:  0.2133,   Adjusted R-squared:  0.1917 
F-statistic: 9.894 on 2 and 73 DF,  p-value: 0.0001576

test<-lsmeans(Ano1, pairwise~SalinityF+Genetikk, adjust="tukey")
summary(test)
$lsmeans
 SalinityF Genetikk   lsmean        SE df lower.CL upper.CL
 16        Hybrid   23.55031 0.1978161 73 23.15606 23.94455
 35        Hybrid   23.57862 0.2012943 73 23.17744 23.97980
 16        Purebred 24.58199 0.2086164 73 24.16622 24.99776
 35        Purebred 24.61031 0.1978161 73 24.21606 25.00455

Confidence level used: 0.95 

$contrasts
 contrast                      estimate        SE df t.ratio p.value
 16,Hybrid - 35,Hybrid      -0.02831625 0.2326766 73  -0.122  0.9993
 16,Hybrid - 16,Purebred    -1.03168375 0.2326766 73  -4.434  0.0002
 16,Hybrid - 35,Purebred    -1.06000000 0.3201547 73  -3.311  0.0077
 35,Hybrid - 16,Purebred    -1.00336750 0.3377196 73  -2.971  0.0205
 35,Hybrid - 35,Purebred    -1.03168375 0.2326766 73  -4.434  0.0002
 16,Purebred - 35,Purebred -0.02831625 0.2326766 73  -0.122  0.9993

P value adjustment: tukey method for comparing a family of 4 estimates
```

```
Ano1<-lm(LogW~LogTL*SalinityF*Genetikk,  data=Distance_IND_1)
anova(Ano1)
Analysis of Variance Table

Response: LogW
                         Df  Sum Sq Mean Sq  F value    Pr(>F)    
LogTL                     1 0.43526 0.43526 312.6168 < 2.2e-16 ***
```

```
SalinityF                  1 0.00216 0.00216   1.5524   0.21755
Genetikk                   1 0.03315 0.03315  23.8066 7.991e-06 ***
LogTL:SalinityF            1 0.00816 0.00816   5.8598   0.01848 *
LogTL:Genetikk             1 0.00027 0.00027   0.1962   0.65935
SalinityF:Genetikk         1 0.00588 0.00588   4.2239   0.04415 *
LogTL:SalinityF:Genetikk  1 0.00698 0.00698   5.0136   0.02881 *
Residuals                 61 0.08493 0.00139

summary(Ano1)

Call:
lm(formula = LogW ~ LogTL * SalinityF * Genetikk, data = Distance_IND_1)

Residuals:
      Min        1Q    Median        3Q       Max
-0.109281 -0.017659  0.002356  0.019588  0.068143

Coefficients:
                                   Estimate Std. Error t value Pr(>|t|)
(Intercept)                         -2.2260     0.7617  -2.922  0.00487 **
LogTL                                3.0506     0.5544   5.502 7.87e-07 ***
SalinityF35                          1.5859     1.2433   1.276  0.20694
GenetikkPurebred                     0.9971     1.0692   0.933  0.35473
LogTL:SalinityF35                   -1.1744     0.9081  -1.293  0.20081
LogTL:GenetikkPurebred              -0.6981     0.7744  -0.901  0.37090
SalinityF35:GenetikkPurebred        -3.3992     1.5379  -2.210  0.03085 *
LogTL:SalinityF35:GenetikkPurebred   2.5024     1.1176   2.239  0.02881 *


Residual standard error: 0.03731 on 61 degrees of freedom
  (7 observations deleted due to missingness)
Multiple R-squared:  0.8528,   Adjusted R-squared:  0.8359
F-statistic: 50.47 on 7 and 61 DF,  p-value: < 2.2e-16


Ano1<-lme(INCR~KUMRAD..Radius.*SalinityF, random=~+1|Indivcode, data=Adult_
ss)
 anova(Ano1)
                            numDF denDF  F-value p-value
 (Intercept)                    1  2635 6979.164  <.0001
 KUMRAD..Radius.                1  2635 2286.965  <.0001
 SalinityF                      1    75   30.470  <.0001
 KUMRAD..Radius.:SalinityF      1  2635   64.754  <.0001

 summary(Ano1)
 Linear mixed-effects model fit by REML
  Data: Adult_ss
       AIC      BIC   logLik
   4188.32 4223.748 -2088.16

 Random effects:
  Formula: ~+1 | Indivcode
         (Intercept)  Residual
 StdDev:   0.2564379 0.5025067

 Fixed effects: INCR ~ KUMRAD..Radius. * SalinityF
                                Value  Std.Error   DF  t-value p-value
 (Intercept)                1.4721888 0.05569717 2635 26.43202  0.0000
 KUMRAD..Radius.            0.0209560 0.00055140 2635 38.00486  0.0000
 SalinityF35                0.0239912 0.07649341   75  0.31364  0.7547
 KUMRAD..Radius.:SalinityF35 -0.0059716 0.00074209 2635 -8.04700  0.0000
  Correlation:
                            (Intr) KUMRAD..Rd. SlnF35
 KUMRAD..Radius.            -0.600
```

```
SalinityF35                   -0.728  0.437
KUMRAD..Radius.:SalinityF35  0.446 -0.743      -0.592

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-3.67474495 -0.68108836 -0.01239545  0.65610329  3.57047579

Number of Observations: 2714
Number of Groups: 77
```

A13d: Statistical models and tests for comparing otolith increment widths between larva and adult life stages

```
Ano2<-lme(INCR~KUMRAD*SalinityF*Genetics+KUMRAD*SalinityF*Generation_F1+KUM
RAD
*Genetics*Generation_F1, random=~+1|ID, data=ssall)
anova(Ano2)
```

```
                             numDF denDF   F-value p-value
(Intercept)                      1  6150 13521.600  <.0001
KUMRAD                           1  6150  5035.132  <.0001
SalinityF                        1   186    23.022  <.0001
Genetics                         1   186     8.525  0.0039
Generation_F1                    1   186     0.934  0.3350
KUMRAD:SalinityF                 1  6150   120.185  <.0001
KUMRAD:Genetics                  1  6150     9.594  0.0020
SalinityF:Genetics               1   186     6.026  0.0150
KUMRAD:Generation_F1             1  6150    11.994  0.0005
SalinityF:Generation_F1          1   186     9.539  0.0023
Genetics:Generation_F1           1   186     7.217  0.0079
KUMRAD:SalinityF:Genetics        1  6150    10.496  0.0012
KUMRAD:SalinityF:Generation_F1   1  6150     6.215  0.0127
KUMRAD:Genetics:Generation_F1    1  6150    16.590  <.0001

summary(Ano2)
Linear mixed-effects model fit by REML
 Data: ssall 
      AIC      BIC    logLik
  8992.51 9100.574 -4480.255

Random effects:
 Formula: ~+1 | ID
        (Intercept)  Residual
StdDev:   0.2942348 0.4669005

Fixed effects: INCR ~ KUMRAD * SalinityF * Genetics + KUMRAD * SalinityF * Generation_F1 +      KUMRAD * Genetics * Generat
ion_F1 
                                               Value  Std.Error   DF   t-value p-value
(Intercept)                                1.5684201 0.07526088 6150 20.839779  0.0000
KUMRAD                                     0.0208605 0.00066037 6150 31.589111  0.0000
SalinityF35                               -0.0063514 0.09787865  186 -0.064891  0.9483
GeneticsPurebred                          -0.2075595 0.10122356  186 -2.050505  0.0417
Generation_F1Larvae                        0.0075631 0.08822220  186  0.085728  0.9318
KUMRAD:SalinityF35                        -0.0080954 0.00085156 6150 -9.506520  0.0000
KUMRAD:GeneticsPurebred                    0.0002118 0.00090261 6150  0.234641  0.8145
SalinityF35:GeneticsPurebred               0.0715545 0.11389744  186  0.628236  0.5306
KUMRAD:Generation_F1Larvae                -0.0020793 0.00079175 6150 -2.626276  0.0087
SalinityF35:Generation_F1Larvae            0.1433196 0.10995668  186  1.303418  0.1940
GeneticsPurebred:Generation_F1Larvae      -0.0149495 0.11374203  186 -0.131434  0.8956
KUMRAD:SalinityF35:GeneticsPurebred        0.0043715 0.00105803 6150  4.131704  0.0000
KUMRAD:SalinityF35:Generation_F1Larvae     0.0029097 0.00099482 6150  2.924840  0.0035
KUMRAD:GeneticsPurebred:Generation_F1Larvae -0.0042860 0.00105228 6150 -4.073087  0.0000
```

```
 Correlation:
                                            (Intr) KUMRAD SlnF35 GntcsP Gn_F1L KUMRAD:SlF35 KUMRAD:GnP SF35:GP
KUMRAD                                      -0.529
SalinityF35                                 -0.664  0.362
GeneticsPurebred                            -0.620  0.336  0.308
Generation_F1Larvae                         -0.796  0.421  0.481  0.438
KUMRAD:SalinityF35                           0.365 -0.698 -0.524 -0.188 -0.268
KUMRAD:GeneticsPurebred                      0.330 -0.631 -0.183 -0.531 -0.234  0.366
SalinityF35:GeneticsPurebred                 0.370 -0.220 -0.557 -0.597 -0.162  0.318        0.348
KUMRAD:Generation_F1Larvae                   0.411 -0.781 -0.259 -0.232 -0.539  0.507        0.443      0.106
SalinityF35:Generation_F1Larvae              0.496 -0.272 -0.747 -0.120 -0.623  0.394        0.084      0.238
GeneticsPurebred:Generation_F1Larvae         0.342 -0.167  0.043 -0.551 -0.428 -0.023        0.264     -0.037
KUMRAD:SalinityF35:GeneticsPurebred         -0.208  0.409  0.298  0.334  0.103 -0.586       -0.649     -0.540
KUMRAD:SalinityF35:Generation_F1Larvae      -0.263  0.511  0.378  0.082  0.346 -0.732       -0.177     -0.144
KUMRAD:GeneticsPurebred:Generation_F1Larvae -0.159  0.280 -0.020  0.256  0.208  0.060       -0.444      0.023
                                            KUMRAD:G_ SF35:G_ GP:G_F KUMRAD:SF35:GP KUMRAD:SF35:G_
KUMRAD
SalinityF35
GeneticsPurebred
Generation_F1Larvae
KUMRAD:SalinityF35
KUMRAD:GeneticsPurebred
SalinityF35:GeneticsPurebred
KUMRAD:Generation_F1Larvae
SalinityF35:Generation_F1Larvae              0.348
GeneticsPurebred:Generation_F1Larvae         0.214    -0.074
KUMRAD:SalinityF35:GeneticsPurebred         -0.213    -0.142   0.026
KUMRAD:SalinityF35:Generation_F1Larvae      -0.654    -0.537   0.047  0.291
KUMRAD:GeneticsPurebred:Generation_F1Larvae -0.359     0.045  -0.542 -0.081         -0.110

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-3.94681960 -0.65381229 -0.00280175  0.64414401  4.66332854

Number of Observations: 6350
Number of Groups: 193
```

```
Ano3<-lme(INCR~KUMRAD+Generation_F1+Genetics+SalinityF, random=~+1|ID, data=ssall)
anova(Ano3)
              numDF denDF   F-value p-value
(Intercept)       1  5249 11152.726  <.0001
KUMRAD            1  5249  4027.495  <.0001
Generation_F1     1   160     1.072  0.3020
Genetics          1   160     7.786  0.0059
SalinityF         1   160    25.828  <.0001

summary(Ano3)
Linear mixed-effects model fit by REML
 Data: ssall
       AIC      BIC    logLik
  7730.026 7776.196 -3858.013

Random effects:
 Formula: ~+1 | ID
        (Intercept)  Residual
StdDev:   0.3019056 0.4727541

Fixed effects: INCR ~ KUMRAD + Generation_F1 + Genetics + SalinityF
                        Value  Std.Error   DF  t-value p-value
(Intercept)          1.7509547 0.05506260 5249 31.79934  0.0000
KUMRAD               0.0162692 0.00025630 5249 63.47629  0.0000
Generation_F1Larvae  0.0208366 0.05159206  160  0.40387  0.6868
GeneticsPurebred    -0.0194845 0.06542346  160 -0.29782  0.7662
SalinityF35         -0.2838342 0.05584990  160 -5.08209  0.0000
 Correlation:
                    (Intr) KUMRAD Gn_F1L GntcsP
KUMRAD              -0.284
Generation_F1Larvae -0.648  0.002
GeneticsPurebred    -0.103  0.005  0.156
SalinityF35         -0.504 -0.004  0.020 -0.436

Standardized Within-Group Residuals:
         Min           Q1          Med           Q3          Max
-3.463107148 -0.647609740 -0.002561665  0.641724257  4.838848244

Number of Observations: 5414
```

```
Number of Groups: 164

test<-lsmeans(Ano3, pairwise~SalinityF+Genetics+Generation_F1, adjustSigma=FALSE, adjust="tukey")
summary(test)
$lsmeans
 SalinityF Genetics Generation_F1   lsmean         SE  df lower.CL upper.CL
 16        Hybrid   Adult         2.745232 0.05279473 163 2.640982 2.849481
 35        Hybrid   Adult         2.461397 0.05288753 160 2.356950 2.565845
 16        Purebred Adult         2.725747 0.07960700 160 2.568531 2.882963
 35        Purebred Adult         2.441913 0.05624575 160 2.330833 2.552993
 16        Hybrid   Larvae        2.766068 0.04205012 160 2.683023 2.849113
 35        Hybrid   Larvae        2.482234 0.04351415 160 2.396298 2.568170
 16        Purebred Larvae        2.746584 0.07983982 160 2.588908 2.904259
 35        Purebred Larvae        2.462750 0.05758618 160 2.349023 2.576477

Confidence level used: 0.95

$contrasts
 contrast                                   estimate         SE  df t.ratio p.value
 16,Hybrid,Adult - 35,Hybrid,Adult        0.28383416 0.05584990 160   5.082  <.0001
 16,Hybrid,Adult - 16,Purebred,Adult      0.01948447 0.06542346 160   0.298  1.0000
 16,Hybrid,Adult - 35,Purebred,Adult      0.30331863 0.06493030 160   4.671  0.0002
 16,Hybrid,Adult - 16,Hybrid,Larvae      -0.02083658 0.05159206 160  -0.404  0.9999
 16,Hybrid,Adult - 35,Hybrid,Larvae       0.26299758 0.07678811 160   3.425  0.0174
 16,Hybrid,Adult - 16,Purebred,Larvae    -0.00135211 0.08943245 160  -0.015  1.0000
 16,Hybrid,Adult - 35,Purebred,Larvae     0.28248205 0.08971811 160   3.149  0.0402
 35,Hybrid,Adult - 16,Purebred,Adult     -0.26434969 0.10287341 160  -2.570  0.1750
 35,Hybrid,Adult - 35,Purebred,Adult      0.01948447 0.06542346 160   0.298  1.0000
 35,Hybrid,Adult - 16,Hybrid,Larvae      -0.30467074 0.07526945 160  -4.048  0.0020
 35,Hybrid,Adult - 35,Hybrid,Larvae      -0.02083658 0.05159206 160  -0.404  0.9999
 35,Hybrid,Adult - 16,Purebred,Larvae    -0.28518627 0.11910252 160  -2.394  0.2513
 35,Hybrid,Adult - 35,Purebred,Larvae    -0.00135211 0.08943245 160  -0.015  1.0000
 16,Purebred,Adult - 35,Purebred,Adult    0.28383416 0.05584990 160   5.082  <.0001
 16,Purebred,Adult - 16,Hybrid,Larvae    -0.04032105 0.07671882 160  -0.526  0.9995
 16,Purebred,Adult - 35,Hybrid,Larvae     0.24351311 0.11092316 160   2.195  0.3602
 16,Purebred,Adult - 16,Purebred,Larvae  -0.02083658 0.05159206 160  -0.404  0.9999
 16,Purebred,Adult - 35,Purebred,Larvae   0.26299758 0.07678811 160   3.425  0.0174
 35,Purebred,Adult - 16,Hybrid,Larvae    -0.32415521 0.07553827 160  -4.291  0.0008
 35,Purebred,Adult - 35,Hybrid,Larvae    -0.04032105 0.07671882 160  -0.526  0.9995
```

```
 35,Purebred,Adult - 16,Purebred,Larvae  -0.30467074 0.07526945 160  -4.048  0.0020
 35,Purebred,Adult - 35,Purebred,Larvae  -0.02083658 0.05159206 160  -0.404  0.9999
 16,Hybrid,Larvae - 35,Hybrid,Larvae      0.28383416 0.05584990 160   5.082  <.0001
 16,Hybrid,Larvae - 16,Purebred,Larvae    0.01948447 0.06542346 160   0.298  1.0000
 16,Hybrid,Larvae - 35,Purebred,Larvae    0.30331863 0.06493030 160   4.671  0.0002
 35,Hybrid,Larvae - 16,Purebred,Larvae   -0.26434969 0.10287341 160  -2.570  0.1750
 35,Hybrid,Larvae - 35,Purebred,Larvae    0.01948447 0.06542346 160   0.298  1.0000
 16,Purebred,Larvae - 35,Purebred,Larvae  0.28383416 0.05584990 160   5.082  <.0001

P value adjustment: tukey method for comparing a family of 8 estimates

> cld(test)
 SalinityF Genetics Generation_F1   lsmean         SE  df lower.CL upper.CL .group
 35        Purebred Adult         2.441913 0.05624575 160 2.330833 2.552993  1
 35        Hybrid   Adult         2.461397 0.05288753 160 2.356950 2.565845  12
 35        Purebred Larvae        2.462750 0.05758618 160 2.349023 2.576477  1
 35        Hybrid   Larvae        2.482234 0.04351415 160 2.396298 2.568170  12
 16        Purebred Adult         2.725747 0.07960700 160 2.568531 2.882963   23
 16        Hybrid   Adult         2.745232 0.05279473 163 2.640982 2.849481    3
 16        Purebred Larvae        2.746584 0.07983982 160 2.588908 2.904259   23
 16        Hybrid   Larvae        2.766068 0.04205012 160 2.683023 2.849113    3

Confidence level used: 0.95
P value adjustment: tukey method for comparing a family of 8 estimates
significance level used: alpha = 0.05
```