Respiration rates of herring larvae at different salinities and effects of previous environmental history

Running title: Salinity effects on respiration rates of herring

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Abstract

Metabolic rates of early life history stages of marine fishes show considerable inter-individual differences and are highly influenced by extrinsic factors like temperature or food availability. Measuring oxygen uptake rates is a proxy for estimating metabolic rates. Still, the relationship between respiration rates and ambient or previous salinity conditions as well as parental and developmental acclimation to changes in salinity is largely unexplored. In the present study, we conducted experiments to investigate salinity effects on the routine metabolic rates (RMR) of euryhaline Atlantic herring (*Clupea harengus*) larvae at three levels of salinity: low (6 psu), intermediate (16 psu) and high (35 psu) reflecting ecological relevant conditions for its populations in the Atlantic and Baltic Sea. The larvae originated from different genetic backgrounds and salinity adaptations to account for cross-generation effects on metabolic rates. Closed respirometry carried out over 24 h on individual fish larvae generally confirmed near isometric respiration rates at all salinity regimes, with rates being 15.4% higher at 6 psu and 7.5% higher at 35 psu compared to 16 psu conditions. However, transgenerational acclimation to different salinity regimes of parents had no effect on the salinity specific metabolic rates of their offspring. Our study demonstrates the ability of herring to cope with a wide range of salinity conditions, irrespective of parental environmental history and genetic origin. This phenotypic plasticity is considered to be one of the main contributing factors to the success of herring as a widely distributed fish species in the North Atlantic and adjacent waters.

Keywords: respiration, salinity, *Clupea harengus*, larval fish, osmoregulation

1. Introduction

The metabolic rate, which is proportional to the oxygen consumption (Gillooly et al. 2001), acts as a "pacemaker" for life and resulting energy can be used for other biological processes like survival, growth or reproduction (Brown et al. 2004). Metabolic rates of ectothermic organisms, like fish, are strongly dependent on the body mass as well as temperature (Clarke & Johnston 1999, Brown et al. 2004). However, even within the same environmental conditions, large variations among individuals occur which can partly be explained by the heritability of metabolic rates (Pettersen et al. 2018). This individual variation in metabolic rates is also affecting the behaviour of organisms (Biro & Stamps 2010), e.g. active individuals having higher metabolic rates will experience higher growth if sufficient food resources are available (Burton et al. 2011, Metcalfe et al. 2016). Other extrinsic and intrinsic factors (e.g. nutrition or temperature changes, genetic differences, and hormonal actions) are known to influence metabolic rates and can disentangle the metabolism and other biological processes like growth (Ishikawa & Namikawa 1987, Nijhout et al. 2006). Therefore, the general "pacemaker" theory is often challenged (Glazier 2015). For early life history stages of marine fishes, a decoupling between metabolism and development can occur when fish experience changes in salinity (Swanson 1998). Still, the relationship between metabolic rates and salinity as well as potential of adaptations to the environment is largely unexplored.

In general, salinity is an important factor influencing key physiological processes such as osmoregulation (Bœuf & Payan 2001) and ambient physical properties such as buoyancy of early life stages of fishes (Sundby & Kristiansen 2015). Due to the small size and high surface-to-volume ratio, larvae are relatively sensitive to changes in salinity, mainly related to osmoregulation (Holliday 1969). In some cases, osmoregulation can account for 20 to >50% of the total energy budget of euryhaline fishes (Rao 1968, Nordlie

1978), but in most cases the cost of osmoregulation is probably modest (Ern et al. 2014, Christensen et al. 2019). Usually, fishes in intermediate salinity conditions have better growth rates which are often correlated with lower metabolic rates (Bœuf & Payan 2001). Changes in salinity can impose physiological stress to euryhaline fishes (Kültz 2015, Kijewska et al. 2016). Nonetheless, physiological adaptations and mechanisms allowing euryhaline fishes to successfully cope with different salinities are not fully explored, yet (Eliason & Farrell 2016). The minimum energy an organism needs to survive in the absence of movements and digestion is defined as the standard metabolic rate (SMR) (Chabot et al. 2016), whereas the routine metabolic rate (RMR) is measured during routine activity. Claireaux and Lagardère (1999) demonstrated that the effect of salinity on SMR and RMR is reverse suggesting that also other mechanisms, apart from osmoregulation, are involved to compensate changes in salinity. An example for how salinity can affect energetic expenditures through mechanisms unrelated to metabolic rates is the potential loss of buoyancy (Saborido-Rey et al. 2003). A dramatic decrease in salinity can be observed from the Atlantic (35 psu) to the inner Baltic Sea (as low as 2 psu). The Baltic Sea is a relatively young habitat (~10,000 years old) and was successfully colonized by several marine fish species after successful adaptation.

One species that has successfully colonized and rapidly adapted to the Baltic Sea is Atlantic herring (*Clupea harengus*) known for its phenotypic plasticity and adaptability (Geffen 2009). Herring is a euryhaline species and has formed distinct populations throughout the transition zone of the Atlantic Ocean and the Baltic Sea that reflect environmental gradients, such as salinity (André et al. 2011, Teacher et al. 2013). Recently, herring populations from the Atlantic and Baltic Sea have further been differentiated by whole-genome sequencing (Lamichhaney et al. 2012, Martinez Barrio et al. 2016, Pettersson et al. 2019). The genetic differentiation between herring from the

Atlantic and Baltic Sea is assumed to include genetic changes in osmoregulation as an adaptation to the striking differences in salinity (Lamichhaney et al. 2012). Despite the ecological role of herring, studies measuring the oxygen uptake in herring larvae is rare (Peck & Moyano 2016). Recent studies demonstrated that physiological response of herring larvae are dependent on feeding conditions (Illing et al. 2018), body mass and temperature (Moyano et al. 2017) suggesting the existence of metabolic flexibility. Existing information on how salinity influences metabolic rate of herring larvae is derived from mainly two studies (Holliday et al. 1964, Almatar 1984). Furthermore, no studies have yet accounted for parental origin or developmental acclimation to new salinities, and there is still a lack of information about the ability of herring larvae to adapt to salinity changes in terms of metabolic rates.

Given this lack of knowledge, we conducted experiments to investigate salinity effects on the growth and RMR of Atlantic herring (*Clupea harengus*) larvae at three levels of salinity: low (6 psu), intermediate (16 psu) and high (35 psu). The larvae originated from parental fish with different genetic backgrounds and salinity adaptations to account for cross-generation effects on metabolic rates. In a first experiment, we used larvae from wild-caught Baltic herring that were reared at intermediate salinity to investigate the effect of acute salinity changes on the RMR. In a second experiment, we used laboratory-reared herring larvae from known parental salinity regimes to test if their developmental acclimation to different salinities will affect their RMR. We hypothesised that metabolic rates of larvae experiencing acute salinity changes (experiment 1) have higher RMR. For the second experiment, we hypothesised that larvae reared near iso-osmotic (intermediate) salinity have higher growth rates and lower RMR. Further, we hypothesised that the developmental acclimation of larvae is more effective in the salinity of parental origin resulting in lower RMR.

2. Material and Methods

2.1. Population samples and larval rearing

Adult herring from both wild and laboratory-reared populations were used to produce larvae for the two experiments. The wild population used in the first experiment (Exp 1) consisted of Baltic autumn spawning herring. Baltic herring were caught 13/09/2017 by gillnets at Hästskär approximately 80 km north of Uppsala, Sweden (60°38'52.0"N 17°48'44.2"E). These herring represent populations from a low saline environment (6 psu, Baltic Sea). After net retrieval, we euthanized herring and transported them to the wet lab at the University of Bergen for the fertilization experiment (12 h after capture). Herring gonads stay viable for up to 20 h after capture (Blaxter 1955, Blaxter & Hempel 1961). Fertilizations were conducted at a salinity of 16 psu to achieve high fertilization rates (Berg et al. 2019) and the ambient water temperature was approximately 10°C. Offspring from five parent pairs of autumn-spawning Baltic herring were incubated and co-reared together in the same tank at salinity of 16 psu and 10°C (Figure 1). The hatching date (defined as the day when 50% were hatched) of larvae was 25/09/2017.

For the second experiment (Exp 2), we used herring larvae generated by mating F1 laboratory-reared hybrids of wild spring-spawning populations caught in the Atlantic and Baltic Sea (Berg et al. 2018). The fertilization was conducted 07/06/2016. Spring-spawning Baltic herring were caught at the same location as the parents used in experiment 1 and the spring-spawning Atlantic herring were caught approximately 26 km west of Bergen, Norway (60°34'11.2"N 5°0'18.9"E). Hybrids were reared at 16 or 35 psu for three years until their first maturity. Further information about the fertilization and rearing of hybrids are described in Berg et al. (2018). From each salinity condition, 16 or 35 psu, one F1 intercross of laboratory-reared Atlantic/Baltic hybrids was used to produce offspring that had been fertilized, incubated and reared directly at 10°C and at a salinity

of 16 or 35 psu. By using only one pair per combination, non-environmental maternal effects were purposely and effectively minimized. Additionally, we included a larval group at 6 psu of hybrids originating from salinity of 16 psu (Figure 1). We did not include any fertilization at 6 psu of hybrids originating from salinity of 35 psu in the study design. This resulted in five experimental groups consisting of offspring with parents originating from 16 psu incubated and reared at salinities of 6, 16, and 35 psu, and offspring with parents reared at 35 psu incubated and reared at salinities of 16 and 35 psu. All larvae used in Exp 2 had the same grandparents. Hatching date of larvae was 20/06/2016.

The larval rearing and following experiments were reviewed and approved by Norwegian national animal ethics committee (Forsøksdyrutvalget-FOTS ID-8459). For both experiments, larvae were reared in round tanks (1 m diameter) including 300 l water and a 12 h day/night light regime. Larval stocking density was initially 1,500 larvae. Larvae were fed daily with natural filtered zooplankton in *ad libitum* (2,000 prey/liter). The natural zooplankton consist mainly of different copepods and nauplii stages and where stored in their original water (marine conditions) for a maximum of one day. Each day remaining plankton was counted within each tank, and plankton was added to reach the same level of prey per liter. Remaining plankton was found in all tanks each day. The high saline water (35 psu) was natural but filter seawater originating from approx. 90 m depth. For the intermediate (16 psu) and low (6 psu) salinity, the seawater was mixed with filtered freshwater. These are nominal values of the salinity because the actual values during the experiment fluctuated between 5−7, 15−17, and 34−35 psu, respectively.

2.2. Respiration measurements

The oxygen consumption of randomly selected larvae was measured for individual larvae for approximately 24 h to achieve adequate results (Chabot et al. 2016). We used closed respirometry with larvae kept in darkness and without anaesthetics, in line with common

practice of routine metabolic rate (RMR) measurements (Peck & Moyano 2016). Visual inspection of larvae indicated that some limited movement took place in the respiration vials at the end of the measurement period. The evening prior to respiration measurements, larvae were collected from the respective rearing tanks and gently transferred with ladles to 5 L buckets with appropriate temperature and salinity conditions. These were kept inside a thermo-controlled room overnight in darkness to enable complete digestion of any gut remains in the herring larvae. The following morning, individual larvae were placed in individual respirometry vials containing oxygen saturated water. This was obtained by vigorously shaking half-filled bottles with water of given salinity and temperature for a few minutes before filling the vials. Respiration vials with volumes of 4 and 20 mL (OXVIAL4, OXVIAL20 with integrated optical oxygen sensor, PyroScience ® GmbH, Aachen, Germany) were used. The net water volume of individual vials was determined and averaged 4.97 and 23.94 mL respectively. Younger aged larvae were placed in vials of 4 mL, while later measurements were conducted in 20 mL vials, with one larva per vial. Larvae from the acclimation buckets were inserted into the vials with a narrow pipette, transferring as little water from the buckets as possible. The vials were carefully closed with corresponding caps and inspected to confirm that no air bubbles were trapped inside. The respiration vials which were attached to separate optical cables were placed in a water bath inside a thermo-controlled room. No extra stirring devices were used to homogenize the water within the vials. All sensors attached to the respiration vials (up to 28 per series) had been individually calibrated to 100% saturation prior to insertion of larvae. Factory setting for the 0% calibration of each vial and sensor was provided. Measurements of oxygen concentration in four vials at a time were then repeatedly carried out and averaged over a period of 60-90 seconds by attaching 4 of the optical cables to a multichannel PyroScience

FireStingO2 (P/N: FSO2-x, PyroScience) measuring unit. The temperature was logged continuously with a sensor attached to the FireSting unit. After logging the corresponding oxygen concentrations [µmol/L] in the vials with the associated FireSting software (Pyro Oxygen Logger, version 3.0, PyroScience), four new cables were attached, and the procedure repeated until oxygen measurements had been made in all the vials. Typically, this procedure took about 15 min, and then repeated after 2-4 h during the day and the following morning. The respiration vials themselves were kept untouched in the water bath during the entire period of measurements. The first measurement was conducted 1 hour after placing larvae in the vials to ensure acclimation to the experimental salinity/temperature and setup. In addition to vials containing a larva, at least one blank vial without larva per salinity and temperature combination was used every day to quantify and account for the background respiration using the same water quality applied during calibration of the optodes.

Duration of the measurement procedure was typically 20-24 h, but occasionally some vials were terminated earlier if the oxygen levels fell below 50% saturation. On average, the saturation after 24 h was 74.2±12.6 and 82.1±9.8% for the different vial volumes 4 and 20 mL, respectively. Typically, six to seven separate averaged measurements were obtained for each larva over the entire period. Following the last measurement, larvae in vials were removed and placed in a petri dish, terminally sedated with tricaine mesylate (MS-222), and photographed under a stereomicroscope. These larvae were also transferred to Teflon plates and dried at 55°C for 24 hrs before being weighed on a Sartorius® microbalance (Type: M3P; Sartorius GmbH, Göttingen, Germany) to the nearest µg. Finally, standard lengths [mm] of larvae were measured from images using ImageJ software (Version 1.48, https://imagej.nih.gov/ij/). Vials containing dead larvae with noticeable shrinkage were excluded from further analysis.

2.3. Experimental setup

For Exp 1, larvae were reared in water at 16 psu and 10°C. Oxygen measurements were conducted in 6, 16 and 35 psu, and a constant temperature of 10°C (Table 1). Larvae were transferred to the new salinities for acclimation the evening prior to measuring. In total, six rounds of measurements were conducted with larvae of age 17-44 days (Table 1). Up to eight larvae were used for each salinity per round. In addition, one blank sample was included for salinity of 6 and 35 psu, and two blank samples for 16 psu.

For Exp 2, larvae were reared at a temperature of 10°C and at 6, 16, and 35 psu (Table 2). Oxygen consumption was measured at the same salinity as larvae were reared. However, as the parents of these larvae were Atlantic/Baltic hybrids that lived their entire life in either 16 or 35 psu, the setup allowed for comparisons of offspring adaptations to different parental salinity environments. In total, ten rounds of measurements, each time including three experimental groups, were conducted with larvae of age 11-39 days (Table 2). Up to seven larvae and one blank sample were used for each experimental group and sampling round.

2.4. Statistical analysis

All statistical analyses and plotting were conducted using the R software (R Core Team 2019). For all tests, we used $p < 0.05$ as the level of significance.

Growth rates [mm/day] for all larval groups (*ad libitum* feeding) were estimated using an ANCOVA using standard length as the response variable and full interaction between age and larval groups as predictor variables. The age of larvae used for respiration measurements was defined as the day of acclimatisation. The larval groups were separated by parental salinity (6 psu for Baltic autumn spawners, 16 psu and 35 psu for laboratory-reared Atlantic/Baltic hybrids) and actual rearing salinity of larvae. This resulted in six (one in Exp 1 and five in Exp 2) larval rearing groups. Only the five rearing

groups from Exp 2 were used for statistical analyses. The one group from Exp 1 was added for visualisation.

The oxygen consumption [µmol/h] for individual larvae was calculated from the slope of linear regressions of the oxygen concentration measurements over time (R^2 > 98%). In general, the oxygen consumption of larvae did not differ significantly between measurements before and after the night independent of oxygen saturation. Each oxygen consumption was corrected for the residual respiration of the blank samples (mean value if more than one) and multiplied by the actual volume of the used vial. There were no mass-specific differences in RMR of larvae when using the two different vial sizes (ANOVA: d.f. = 1;246, F = 0.4, p = 0.54).

Log-transformations were performed on the standard length, dry mass and oxygen consumption of each larva prior to the statistical analyses. For larvae with missing dry mass (n = 36), we used the length-dry mass relationship of live larvae (Figure 2, Table 3) to estimate the corresponding dry mass.

For statistical analyses, we followed the protocol of Zuur et al. (2010) for the data exploration. Mass-specific RMR measurements were tested for normality and homogeneity of variance and statistical outliers were removed (n = 10) when the values were outside 1.5 times the interquartile range above the upper quartile and below the lower quartile. Further, we used linear regression models to indicate how oxygen consumption was influenced by the dry mass of larvae. For the model selection, we started with full interaction models where higher order interactions were removed when not significant. The final models best explain the oxygen consumption *RMR* where:

$$RMR = \propto + \beta_1 \times DW + \beta_2 \times Sal$$

for Exp 1 and Exp 2 combined where *DW* is the log-transformed dry weight of each larvae and *Sal* the salinity during the measurement. For Exp 2 we additionally tested the influence of parental salinity with this model:

$$RMR = \propto + \beta_1 \times DW + \beta_2 \times Sal + \beta_3 \times ParSal$$

where *ParSal* represent the salinity origin of parents. Following, significant differences for variables with more than two levels, like salinity, were evaluated by a Tukey HSD post hoc test. For visualisation, we standardized the RMR for dry mass and presented the standardized RMR [nmol/µg/h] as boxplots.

3. Results

3.1. Length-dry mass relationship

A general observation during the respiration measurements was the higher mortality of larvae at 35 psu (Table 1&2). Herring larvae of wild caught Baltic herring (Exp 1) and laboratory reared Atlantic/Baltic herring hybrids (Exp 2) showed allometric length-dry mass relationships (ANOVA: d.f. = 1;226, F = 2964.7, $p < 0.001$; Figure 2) which differed between the two experiments (ANOVA: d.f. = 1;226, F = 5.3, $p < 0.05$). On average, larvae from wild Baltic autumn spawners (Exp 1) were 2.3% heavier at a given length than larvae from laboratory-reared Atlantic/Baltic hybrids (Exp 2).

The growth rates of herring larvae from Exp 2 were rather similar, except for larvae reared at 35 psu when parents originated from 16 psu and vice versa (ANCOVA: d.f. = 4;1916, F = 22.6, $p < 0.001$; Figure 3, Table A1). Herring reared at 16 psu when parents originated from 36 psu had the lowest growth rate of all groups, whereas larvae reared at 35 psu when parents originated from 16 psu had the highest growth rates. For the comparisons among groups with the same parental salinity, growth rates were higher at higher rearing salinity (Figure 3). The overall growth rate of all herring larvae in this study was 0.34±0.01 mm/day and there was no significant difference between larvae

sampled regularly or used for respiration measurements (ANCOVA: d.f. = 1;2110, F = 1.9, p = 0.17).

3.2. Salinity effects on oxygen consumption

The RMR of larvae was affected similarly with respect to dry mass and salinity between Exp 1 and 2 (ANOVA: d.f. = 1;244, F = 0.4, p = 0.54). There was a general isometric increase in oxygen consumption (RMR) with increasing body mass of larvae (ANOVA: d.f. = 1;244, F = 2703.4, $p < 0.001$), with a mean overall slope (± SE) of 1.01 (± 0.02; see Table 4 for group specific regression equations). The variation in Exp 1 based on multiple wild caught parent combinations was larger compared to Exp 2 using offspring from a single cross (overall residual standard error (RSE) Exp 1 = 0.14 versus RSE Exp 2= 0.08). The acute exposure to lower or higher salinities affected the RMR (ANOVA: d.f. = 2;244, F = 8.2, $p < 0.001$; Figure 4), and larvae at salinity of 6 psu had an 18.1% higher RMR than larvae at 16 psu (Tukey HSD test, $p < 0.001$). There was also a tendency that RMR was higher at 35 psu than at 16 psu (7.7%), but this effect was not significant (Tukey HSD test, p = 0.07; Figure 4). In Exp 2, the parental salinity did not influence the RMR of their offspring (ANOVA: d.f. = 1;98, F = 0.1, p = 0.79; Figure 5) independent of the salinity during respiration measurements.

4. Discussion

This is, to our knowledge, the first study to report the oxygen consumption of Atlantic herring larvae produced by wild and laboratory-reared herring over a wide range of salinities. Generally, the routine metabolic rates (RMR) of herring had an isometric increase with increasing body mass for all experimental groups. However, herring larvae had the lowest RMR at intermediate salinities and highest RMR in low saline waters. Offspring from herring that were reared their entire life at either 16 or 35 psu showed no significant differences in mass-specific RMR. This indicates that parental environmental

history has limited or no effect on the RMR of their progeny. Still, we cannot exclude that a longer acclimation time than just one night to different salinities might also have resulted in similar RMR. Growth rates of herring larvae were generally high and constant, consistent with previous studies (Folkvord et al. 2004, Folkvord et al. 2009). Larvae at 35 psu had the highest growth when parents originated from 16 psu and lowest growth for vice versa conditions. Further, there was a tendency that growth rates increased with increasing salinity within experimental groups.

Studies on the metabolic rates of Atlantic herring have a long history. Most of these studies focus on the effect of temperature (e.g. de Silva & Tytler 1973, Moyano et al. 2017) or food availability (e.g. Kiørboe et al. 1987, Illing et al. 2018). Almatar (1984) demonstrated that first feeding herring larvae consume less oxygen at intermediate salinities which is supported by the present study. Independent of parental environment or if larvae experienced abrupt changes in salinity, metabolic rates were lowest at 16 psu. It has previously been shown in Pacific herring that highest viability of hatched larvae occurred at intermediate salinities of 13.2-19 psu (Alderdice et al. 1979). Herring larvae used within this study were offspring from Baltic autumn spawners and laboratory-reared Atlantic/Baltic hybrid spring spawners. There are clear genetic differences between autumn and spring spawning herring (Martinez Barrio et al. 2016, Lamichhaney et al. 2017, Kerr et al. 2019), but the mass-specific respiration rates and the effect of salinity were similar between the offspring of these two groups.

Hyperosmotic (35 psu) and hypoosmotic (6 psu) conditions presumably increase metabolic expenditures due to higher osmoregulatory activities (Christensen et al. 2018). This is in line with our findings where acute exposures to hyper- and hypoosmotic conditions induce higher metabolic costs. RMR was highest at low salinities (hypoosmotic), even when the parents originated from the Baltic Sea representing an

environment with low salinity (Exp 1). Atlantic herring is one of a few marine fish species that successfully colonized the brackish Baltic Sea and genetic differentiation between Atlantic and Baltic herring is expected to involve efficient osmoregulation as an adaptation to the differences in salinity (Lamichhaney et al. 2012). Still, given the fact that the Baltic herring larvae after acute salinity changes had the highest metabolic rates at 6 psu suggest that adaptation in terms of osmoregulation might not be complete.

Within the present study, we tested if the parental environment has an effect on metabolic rates. Offspring from laboratory-reared Atlantic/Baltic hybrids living their entire life at salinity of either 16 or 35 psu were used to test potential adaptations on RMR. There was no effect of parental environmental history on RMR independent of the rearing salinity of the offspring. Offspring used in the present study were the second filial generation and the segregation of alleles from Atlantic and Baltic herring should permit detection of gene variants underlying adaptations to their environmental conditions, but a larger experiment and individual genotyping of larvae would be required to accomplish this. Such an experiment is well justified by the fact that whole genome sequencing has revealed hundreds of loci underlying ecological adaptation in the Baltic herring and some of these are expected to involve osmoregulation (Pettersson et al. 2019). The variation of RMR was relatively low (overall RSE for Exp 2 = 0.08). However, genetic analyses on e.g. larvae with highest deviation from the mean (positive and negative statistical residuals) are essential to entangle if individual adaptations are causing this deviation.

Herring larvae used in Exp 2 experienced the same salinity during incubation, hatching, and metabolic rate measurements, in contrast to larvae from Exp 1 which were exposed to abrupt salinity changes (from 16 psu to either 6 or 35 psu) 24 h prior the measurements. Fish larvae are very vulnerable to sudden changes in environmental conditions (Houde 1994). Acute salinity changes were shown to result in up to 80% higher

oxygen consumption in sea bass fingerlings (Dalla Via et al. 1998). Larvae might not have acclimatized within 24 h to the sudden change in salinity resulting in larger variations in metabolic rates (overall RSE for Exp 1 = 0.146). Changes in osmoregulation, which will influence the metabolic rates, occurred up to a week after abrupt salinity changes in Atlantic menhaden (Engel et al. 1987). This would also indicate that larvae can adapt to a constant environment, but this adaptation might not be heritable. Another factor that needs to be considered is the number of parents used per experiment. Larvae from Exp 1 are the offspring from five parent pairs, while only one full sibling hybrid cross per salinity was used in Exp 2. This design ensured segregation of Atlantic and Baltic alleles and thus a considerable genetic variability in the tested F2 generation. Therefore, the variation within an experiment could also partly be explained by individual/parental differences rather than the ability to adapt to current environmental conditions.

Osmoregulation is probably the most important driver affecting the metabolic rates in different salinities (Rao 1968, Nordlie 1978). Higher energy costs of larvae also occur with increased swimming costs due to negative buoyancy at low salinities (Sundby & Kristiansen 2015). This could explain why larvae at 6 psu have even higher metabolic rates compared with larvae at high salinities because they need to compensate for a potential negative buoyancy. To what extent this affects the RMR in relatively small respiration vials is unclear. Activity measurements would provide more insight because individuals with higher activity levels and consequently higher metabolic rates are also known to exhibit higher growth rates (Burton et al. 2011). However, we found a tendency that growth rates increased with salinity, which would contradict the notion that fish with highest growth rates are having higher metabolic rates. On the other hand, the growth

rates of herring can be negatively influenced when living at the extremes of their salinity tolerance range (Rajasilta et al. 2011).

Another observation of this study is that higher mortality occurred during respiration measurements at highest salinity (Table 1&2) for both experiments. Since the handling of larvae was identical for all salinities, there must be additional factors besides higher metabolic rates due to osmoregulation that can cause higher mortality. Also, saturation levels and actual oxygen content [µmol/L] for living and dead larvae did not differ after 24 h or for the last measurement before the night. Higher metabolic rates at 6 psu might also be the indirect result of higher activity levels caused by negative buoyancy (Burton et al. 2011, Sundby & Kristiansen 2015). A higher activity level might also increase the risk of injuries during the measurements were larvae are kept in relatively small volumes (4 or 20 ml). The trend of higher mortality is probably not linked to higher growth rates for larvae reared at 35 psu because this pattern was also observed in Exp 1 where larvae experienced abrupt salinity changes.

It is essential to know the mass-specific metabolic rates when developing bioenergetic models (Chabot et al. 2016). Here, we estimated mass-specific RMR over a wide range of body sizes (5-22 mm) and estimate the body size scaling of RMR (*b-value* in Table 3) at different salinities. The body scaling of RMR ranged from 0.92-1.13 which is in accordance with recent studies (Peck & Moyano 2016, Moyano et al. 2017). The development of equipment used for respiration measurements resulting in more precise and accurate results might explain why our results were slightly higher compared to traditional findings (de Silva & Tytler 1973, Kiørboe et al. 1987). Even though Moyano et al. (2017) used populations (North Sea autumn spawners and Western Baltic spring spawners) that are genetically different than populations from our study over a wide range of temperatures, the body size scaling of respiration rates from there study were

comparable with our findings. However, the growth rates of herring from that study were significantly lower than for larvae from the present study. This indicates that body size is much more important than genetic origin and growth rates in influencing RMR of herring larvae.

In general, it seems that Atlantic herring are well-adapted to their environmental conditions and can cope with a wide range of salinity in terms of metabolic rates. The salinity in the Baltic Sea is dependent on inflow of marine water from the North Sea through the Skagerrak and Kattegat, which is an important transition zone for herring. However, in times of climate change, several climate models project a decline in salinity in the Baltic Sea (Meier et al. 2006, Vuorinen et al. 2015). Our findings suggest that rapid changes in salinity entail higher metabolic costs in Atlantic herring larvae due to increased osmoregulatory activity. In addition, increasing temperatures will have additive effects on metabolic rates (Almatar 1984). Thus, early life stages of herring and potentially also other marine species occurring in the Baltic Sea will be negatively affected under future climate projections. Ultimately, this can lead to marine habitat loss (Illing et al. 2016, Dippner et al. 2019) and impact the recruitment of fish stocks (Heikinheimo 2008, Pécuchet et al. 2015). However, the demonstrated transgenerational adaptive potential of herring larvae will allow this species to live in even less saline environments in the future (Donelson et al. 2012). Consequently, the ability of rapid adaptation to the surrounding salinity environment might reduce the negative effect of climate change on herring in the Baltic Sea.

In conclusion, our study demonstrates the ability of herring to cope with a wide range of salinity conditions, irrespective of parental environmental history and genetic origin. This phenotypic plasticity is considered to be one of the main contributing factors to the success of herring as a widely distributed fish species in the North Atlantic.

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Literature cited

Alderdice DF, Rao TR, Rosenthal H (1979) Osmotic responses of eggs and larvae of the Pacific herring to salinity and cadmium. Helgol Mar Res 32:508-538

Almatar SM (1984) Effects of acute changes in temperature and salinity on the oxygen uptake of larvae of herring (*Clupea harengus*) and plaice (*Pleuronectes platessa*). Mar Biol 80:117-124

André C, Larsson LC, Laikre L, Bekkevold D, Brigham J, Carvalho GR, Dahlgren TG, Hutchinson WF, Mariani S, Mudde K, Ruzzante DE, Ryman N (2011) Detecting population structure in a high gene-flow species, Atlantic herring (*Clupea harengus*): direct, simultaneous evaluation of neutral vs putatively selected loci. Heredity 106:270-280

Berg F, Almeland OW, Skadal J, Slotte A, Andersson L, Folkvord A (2018) Genetic factors have a major effect on growth, number of vertebrae and otolith shape in Atlantic herring (*Clupea harengus*). PLoS ONE 13:e0190995

Berg F, Slotte A, Andersson L, Folkvord A (2019) Genetic origin and salinity history influence reproductive success of Atlantic herring. Mar Ecol Prog Ser 617-618:81-94

Biro PA, Stamps JA (2010) Do consistent individual differences in metabolic rate promote consistent individual differences in behavior? Trends Ecol Evol 25:653-659

Blaxter JHS (1955) Herring rearing - I. The storage of herring gametes. Mar Res Scot 3:12

Blaxter JHS, Hempel G (1961) Biologische Beobachtungen bei der Aufzucht von Heringsbrut. Helgol Mar Res 7:260-283

Bœuf G, Payan P (2001) How should salinity influence fish growth? Comp Biochem Physiol 130:411-423

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771-1789

Burton T, Killen SS, Armstrong JD, Metcalfe NB (2011) What causes intraspecific variation in resting metabolic rate and what are its ecological consequences? Proc R Soc B 278:3465-3473

Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. Journal of Fish Biology 88:81-121

Christensen EAF, D. SJ, Grosell M, Steffensen JF (2019) Intra-specific difference in the effect of salinity on physiological performance in European perch (*Perca fluviatilis*) and its ecological importance for fish in estuaries. Biology 8:89

Christensen EAF, Illing B, Iversen NS, Johansen JL, Domenici P, Steffensen JF (2018) Effects of salinity on swimming performance and oxygen consumption rate of shiner perch *Cymatogaster aggregata*. J Exp Mar Biol Ecol 504:32-37

Claireaux G, Lagardère JP (1999) Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J Sea Res 42:157-168

Clarke A, Johnston NM (1999) Scaling of metabolic rate with body mass and temperature in teleost fish. J Anim Ecol 68:893-905

Dalla Via J, Villani P, Gasteiger E, Niederstätter H (1998) Oxygen consumption in sea bass fingerling *Dicentrarchus labrax* exposed to acute salinity and temperature changes: metabolic basis for maximum stocking density estimations. Aquaculture 169:303-313

de Silva CD, Tytler P (1973) The influence of reduced environmental oxygen on the metabolism and survival of herring and plaice larvae. Netherlands Journal of Sea Research 7:345-362

Dippner JW, Fründt B, Hammer C (2019) Lake or sea? The unknown future of central Baltic Sea herring. Frontiers in Ecology and Evolution 7

Donelson JM, Munday PL, McCormick MI, Pitcher CR (2012) Rapid transgenerational acclimation of a tropical reef fish to climate change. Nature Climate Change 2:30-32

Eliason EJ, Farrell AP (2016) Oxygen uptake in Pacific salmon *Oncorhynchus spp*.: when ecology and physiology meet. Journal of Fish Biology 88:359-388

Engel DW, Hettler WF, Coston-Clements L, Hoss DE (1987) The effect of abrupt salinity changes on the osmoregulatory abilities of the Atlantic menhaden *Brevoortia tyrannus*. Comp Biochem Physiol 86:723-727

Ern R, Huong DTT, Cong NV, Bayley M, Wang T (2014) Effect of salinity on oxygen consumption in fishes: a review. Journal of Fish Biology 84:1210-1220

Folkvord A, Høie H, Johannessen A, Solbakken T (2009) Effects of prey concentration, light regime, and parental origin on growth and survival of herring larvae under controlled experimental conditions. ICES J Mar Sci 66:1702-1709

Folkvord A, Johannessen A, Moksness E (2004) Temperature-dependent otolith growth in Norwegian spring-spawning herring (*Clupea harengus* L.) larvae. Sarsia 89:297-310

Geffen AJ (2009) Advances in herring biology: from simple to complex, coping with plasticity and adaptability. ICES J Mar Sci 66:1688-1695

Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and temperature on metabolic rate. Science 293:2248-2251

Glazier DS (2015) Is metabolic rate a universal ‘pacemaker’ for biological processes? Biol Rev 90:377-407

Heikinheimo O (2008) Average salinity as an index for environmental forcing on cod recruitment in the Baltic Sea. Boreal Environment Research 13:457-464

Holliday FGT (1969) The effects of salinity on the eggs and larvae of teleosts. In: Hoar WS, Randall DJ (eds) Fish Physiology, Book 1. Academic Press

Holliday FGT, Blaxter JHS, Lasker R (1964) Oxygen uptake of developing eggs and larvae of the herring (*Clupea harengus*). J Mar Biol Assoc UK 44:711-723

Houde ED (1994) Differences between marine and freshwater fish larvae: implications for recruitment. ICES J Mar Sci 51:91-97

Illing B, Moyano M, Berg J, Hufnagl M, Peck MA (2018) Behavioral and physiological responses to prey match-mismatch in larval herring. Estuar Coast Shelf Sci 201:82-94

Illing B, Moyano M, Hufnagl M, Peck MA (2016) Projected habitat loss for Atlantic herring in the Baltic Sea. Mar Environ Res 113:164-173

Ishikawa A, Namikawa T (1987) Postnatal growth and development in laboratory strains of large and small musk shrews (*Suncus murinus*). Journal of Mammalogy 68:766-774

Kerr Q, Fuentes-Pardo AP, Kho J, McDermid JL, Ruzzante DE (2019) Temporal stability and assignment power of adaptively divergent genomic regions between herring (*Clupea harengus*) seasonal spawning aggregations. Ecol Evol 9:500-510

Kijewska A, Kalamarz-Kubiak H, Arciszewski B, Guellard T, Petereit C, Wenne R (2016) Adaptation to salinity in Atlantic cod from different regions of the Baltic Sea. J Exp Mar Biol Ecol 478:62-67

Kiørboe T, Munk P, Richardson K (1987) Respiration and growth of larval herring *Clupea harengus*: relation between specific dynamic action and growth efficiency. Mar Ecol Prog Ser 40:1-10

Kültz D (2015) Physiological mechanisms used by fish to cope with salinity stress. Journal of Experimental Biology 218:1907-1914

Lamichhaney S, Fuentes-Pardo AP, Rafati N, Ryman N, McCracken GR, Bourne C, Singh R, Ruzzante DE, Andersson L (2017) Parallel adaptive evolution of geographically distant herring populations on both sides of the North Atlantic Ocean. Proc Natl Acad Sci 114:E3452-E3461

Lamichhaney S, Martinez Barrio A, Rafati N, Sundström G, Rubin C-J, Gilbert ER, Berglund J, Wetterbom A, Laikre L, Webster MT, Grabherr M, Ryman N, Andersson L (2012) Population-scale sequencing reveals genetic differentiation due to local adaptation in Atlantic herring. Proc Natl Acad Sci 109:19345-19350

Martinez Barrio A, Lamichhaney S, Fan G, Rafati N, Pettersson M, Zhang H, Dainat J, Ekman D, Höppner M, Jern P, Martin M, Nystedt B, Liu X, Chen W, Liang X, Shi C, Fu Y, Ma K, Zhan X, Feng C, Gustafson U, Rubin C-J, Sällman Almén M, Blass M, Casini M, Folkvord A, Laikre L, Ryman N, Ming-Yuen Lee S, Xu X, Andersson L (2016) The genetic basis for ecological adaptation of the Atlantic herring revealed by genome sequencing. eLife 5:e12081

Meier HEM, Kjellström E, Graham LP (2006) Estimating uncertainties of projected Baltic Sea salinity in the late 21st century. Geophysical Research Letters 33:L15705

Metcalfe NB, Van Leeuwen TE, Killen SS (2016) Does individual variation in metabolic phenotype predict fish behaviour and performance? Journal of Fish Biology 88:298-321

Moyano M, Illing B, Christiansen L, Peck MA (2017) Linking rates of metabolism and growth in marine fish larvae. Mar Biol 165:5

Nijhout HF, Davidowitz G, Roff DA (2006) A quantitative analysis of the mechanism that controls body size in *Manduca sexta*. Journal of Biology 5:16

Nordlie FG (1978) The influence of environmental salinity on respiratory oxygen demands in the euryhaline teleost, *Ambassis interrupta* bleeker. Comp Biochem Physiol 59:271-274

Peck MA, Moyano M (2016) Measuring respiration rates in marine fish larvae: challenges and advances. Journal of Fish Biology 88:173-205

Pécuchet L, Nielsen JR, Christensen A (2015) Impacts of the local environment on recruitment: a comparative study of North Sea and Baltic Sea fish stocks. ICES J Mar Sci 72:1323-1335

Pettersen AK, Marshall DJ, White CR (2018) Understanding variation in metabolic rate. J Exp Biol 221:jeb166876

Pettersson ME, Rochus CM, Han F, Chen J, Hill J, Wallerman O, Fan G, Hong X, Xu Q, Zhang H, Liu S, Liu X, Haggerty L, Hunt T, Martin FJ, Flicek P, Bunikis I, Folkvord A, Andersson L (2019) A chromosome-level assembly of the Atlantic herring genome—detection of a supergene and other signals of selection. Genome Research:doi: 10.1101/gr.253435.253119

R Core Team (2019) R: A language and environment for statistical computing (version 3.6.1). R Foundation for Statistical Computing, Vienna, Austria (http://wwwR-projectorg)

Rajasilta M, Laine P, Paranko J (2011) Current growth, fat reserves and somatic condition of juvenile Baltic herring (*Clupea harengus membras*) reared in different salinities. Helgol Mar Res 65:59-66

Rao GMM (1968) Oxygen consumption of rainbow trout (*Salmo gairdneri*) in relation to activity and salinity. Can J Zool 46:781-786

Saborido-Rey F, Kjesbu OS, Thorsen A (2003) Buoyancy of Atlantic cod larvae in relation to developmental stage and maternal influences. J Plankton Res 25:291-307

Sundby S, Kristiansen T (2015) The principles of buoyancy in marine fish eggs and their vertical distributions across the world oceans. PLoS ONE 10:e0138821

Swanson C (1998) Interactive effects of salinity on metabolic rate, activity, growth and osmoregulation in the euryhaline milkfish (*Chanos chanos*). J Exp Biol 201:3355-3366

Teacher AGF, André C, Jonsson PR, Merilä J (2013) Oceanographic connectivity and environmental correlates of genetic structuring in Atlantic herring in the Baltic Sea. Evol Appl 6:549-567

Vuorinen I, Hänninen J, Rajasilta M, Laine P, Eklund J, Montesino-Pouzols F, Corona F, Junker K, Meier HEM, Dippner JW (2015) Scenario simulations of future salinity and ecological consequences in the Baltic Sea and adjacent North Sea areas–implications for environmental monitoring. Ecological Indicators 50:196-205

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1:3-14

Table 1 Number of larvae included for the analyses of the respiration rates of experiment 1. Age (days post hatching = DPH) of larvae and mean temperatures ± standard deviation for each sampling day are shown. Dead or injured larvae (in brackets) were not included in the analyses.

Age (DPH)	Temp (°C)	Salinity		
		6 psu	16 psu	35 psu
Total	**10.04±0.17**	**42 (1)**	**39 (1)**	**29 (11)**
17	9.89±0.00	8 (0)	7 (0)	6 (0)
18	9.84±0.00	7 (0)	5 (1)	5 (1)
23	10.23±0.00	7 (0)	8 (0)	6 (2)
24	10.21±0.00	8 (0)	7 (0)	5 (3)
31	10.20±0.00	4 (1)	4 (0)	3 (1)
44	9.87±0.00	8 (0)	8 (0)	4 (4)

Table 2 Number of larvae included for the analyses of the respiration rates of experiment 2. Age (days post hatching = DPH) of larvae and mean temperatures ± standard deviation for each sampling day are shown. Dead or injured larvae (in brackets) were not included in the analyses.

Age (DPH)	Temp (°C)	Larval salinity – parental salinity				
		6 - 16	16 - 16	35 - 16	16 - 35	35 - 35
Total	**10.07±0.09**	**37 (4)**	**32 (2)**	**28 (9)**	**18 (6)**	**24 (12)**
11	9.86±0.02	4 (0)	7 (0)	5 (0)		
15	10.17±0.01			4 (1)	1 (0)	4 (1)
18	10.05±0.01	7 (0)	5 (0)	4 (0)		
20	10.04±0.00		5 (0)		3 (0)	4 (1)
24	10.10±0.00	2 (3)	4 (1)	0 (5)		
25	10.08±0.01	7 (0)			5 (2)	3 (3)
29	10.06±0.00		4 (1)		5 (1)	6 (1)
32	10.18±0.00	11 (0)		9 (2)		
36	10.17±0.00	6 (1)		6 (1)		3 (3)
39	10.02±0.00		7 (0)		4 (3)	4 (3)

Table 3 Model regressions for standard length (TL, mm)-dry mass (DM, µg) relationships of Atlantic herring larvae from experiment 1-2. There was no difference between larvae reared at different salinities within an experiment. RSE = residual standard error.

Exp	Equation	Adj R^2	n	RSE
1	$DM = 0.0653 * TL^{3.271}$	0.91	107	0.10
2	$DM = 0.0414 * TL^{3.418}$	0.94	122	0.08

Table 4 Model regressions for body size scaling of routine metabolic rate (RMR, nmol/ind/h) of Atlantic herring larvae from one wild population (Baltic Autumn Spawners = A Baltic) as well as two laboratory-reared groups (Atlantic/Baltic Hybrid 16 and Hybrid 35). Larvae were reared at different salinities (Sal) and ambient water temperature of 10°C. RSE = residual standard error.

Group	Sal	Equation	Adj R^2	n	RSE
A Baltic	6	$RMR = 0.0721 * DM^{1.044}$	0.92	42	0.13
	16	$RMR = 0.0343 * DM^{1.132}$	0.87	39	0.15
	35	$RMR = 0.0834 * DM^{0.992}$	0.78	29	0.16
Hybrid 16	6	$RMR = 0.0792 * DM^{1.000}$	0.96	37	0.07
	16	$RMR = 0.0732 * DM^{1.006}$	0.95	32	0.10
	35	$RMR = 0.1121 * DM^{0.942}$	0.98	28	0.07
Hybrid 35	16	$RMR = 0.0761 * DM^{0.997}$	0.87	18	0.10
	35	$RMR = 0.1307 * DM^{0.920}$	0.92	24	0.08

Figure 1 Illustration of the experimental design used for the factorial crossing experiments and respiration measurements. Parental Baltic herring used in experiment 1 were wild caught, A = autumn. Herring used in experiment 2 were offspring of full sibling F1 Atlantic/Baltic hybrids that had been reared three years under common garden conditions in a salinity of either 16 or 35 psu.

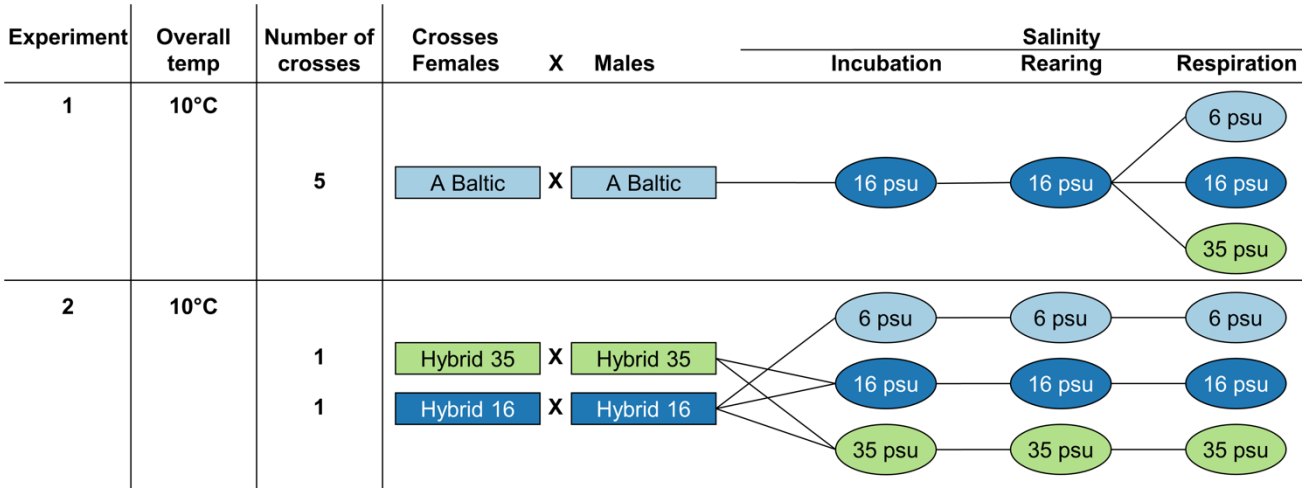


Figure 2 Length-dry mass relationship for larvae of experiment 1-2. Regression lines for the statistical model are shown. Individual regression equations are given in the Appendix (Table A1).

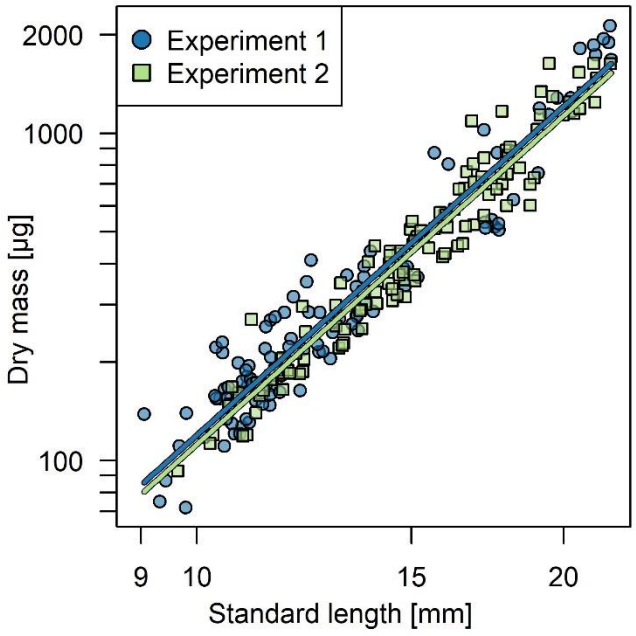


Figure 3 Growth rates ± 95% confidence intervals [mm/day] of 8-56 days old herring larvae originating from one wild population of Baltic autumn spawners (6 psu/parental salinity) as well as two laboratory-reared groups (Atlantic/Baltic Hybrid, 16 and 35 psu/parental salinity) reared at three different salinities (6, 16, and 35 psu). Groups that do not share any letters are significantly different ($p < 0.05$). Larvae from Exp 1 (left of dashed line) were added for visual comparison.

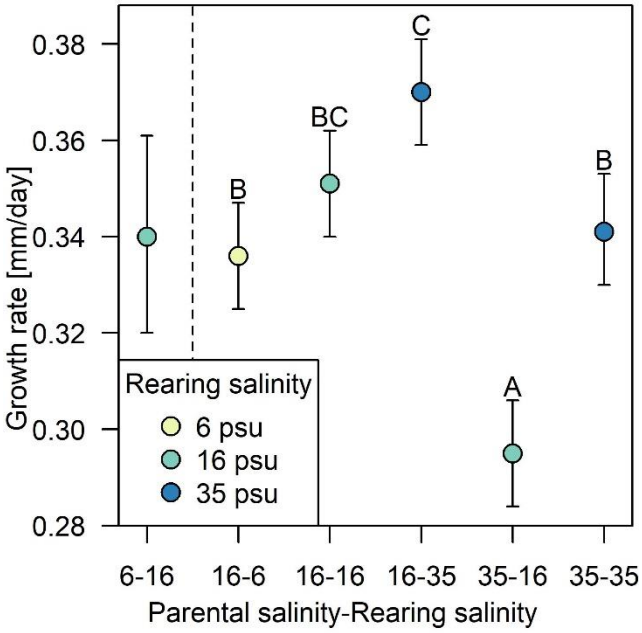


Figure 4 Standardized routine metabolic rate (nmol/µg/h) of larvae separated by experiment and salinity. The salinity exposure time differed between experiments; acute vs. long-term for experiment 1 and 2, respectively. The median (black) and mean (red) are indicated in the boxes, which represent the interquartile range. Whiskers represent the lowest and highest observations within 1.5× the interquartile range. Observations outside the whiskers are outliers indicated as individual points. Individual regression equations are given in Table 4.

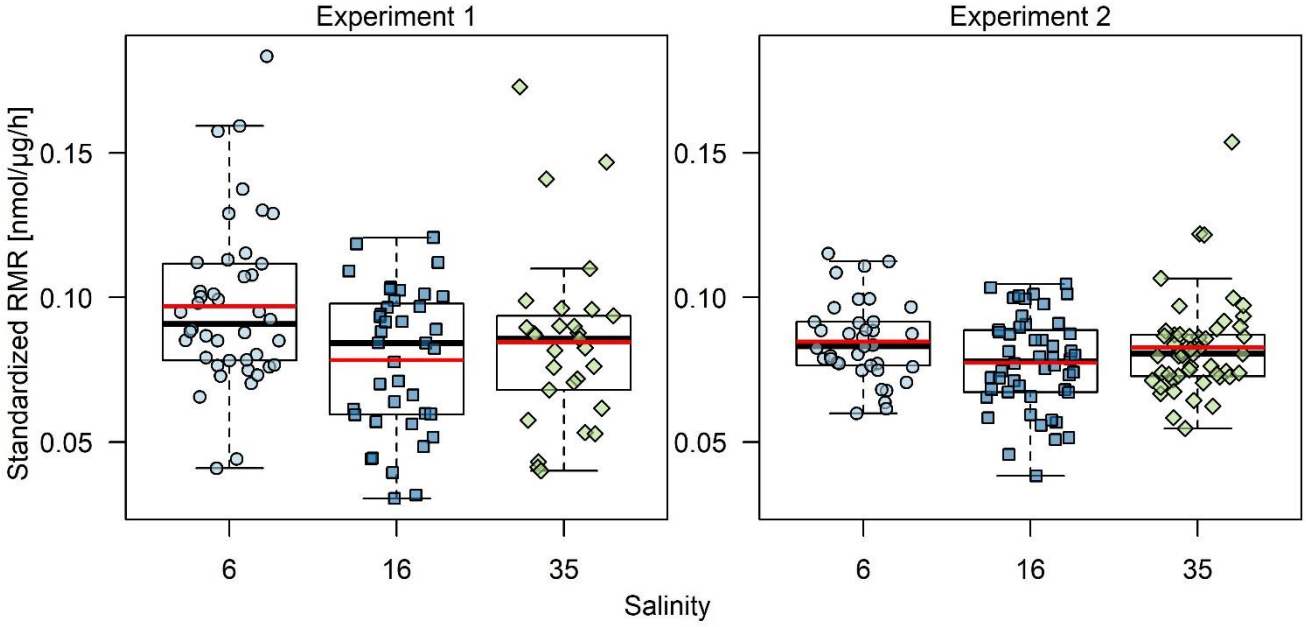
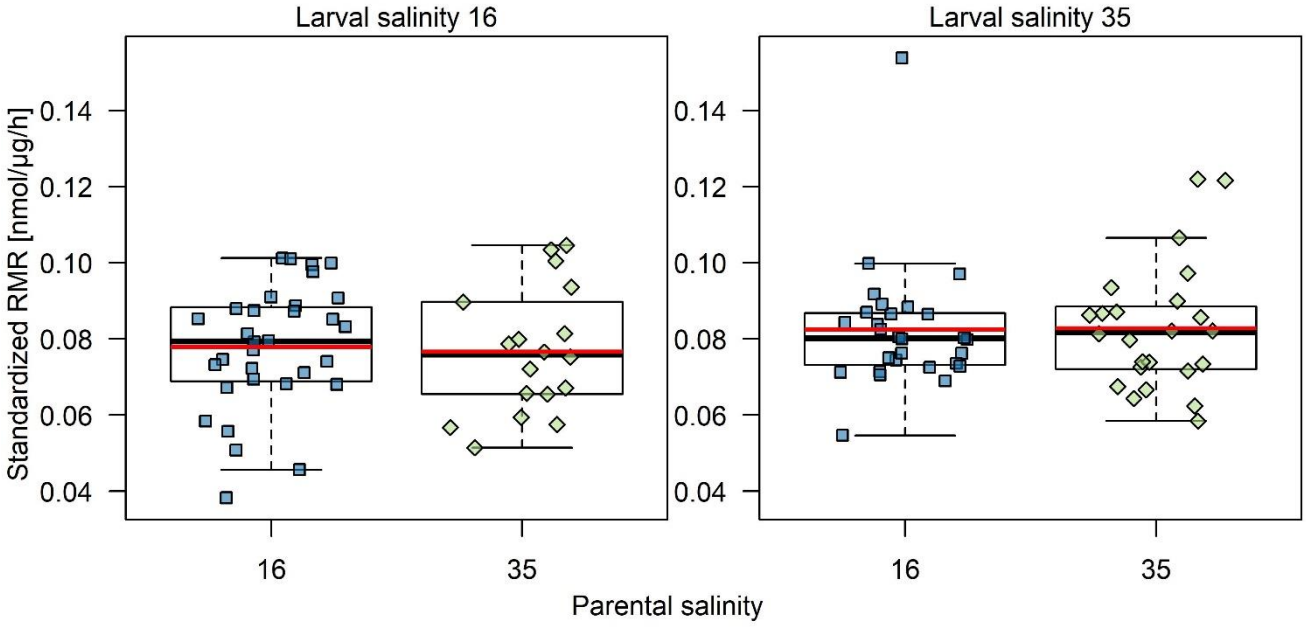


Figure 5 Standardized routine metabolic rate (nmol/µg/h) of larvae from experiment 2 reared and measured at salinity of 16 and 35 psu, separated by parental salinity of 16 or 35. The median (black) and mean (red) are indicated in the boxes, which represent the interquartile range. Whiskers represent the lowest and highest observations within 1.5× the interquartile range. Observations outside the whiskers are outliers indicated as individual points. Individual regression equations are given in Table 4.



Appendix

Table A1 Individual model regressions for growth rate (SL, standard length (mm)-at-age (Day)) of herring larvae from one wild population of Baltic autumn spawners as well as two laboratory-reared groups (Atlantic/Baltic Hybrid 16 and Hybrid 35). Larvae were reared at different salinities (Sal) and ambient water temperature of 10°C. RSE = residual standard error.

Group	Sal	Equation	Adj R^2	n	RSE
Autumn Baltic	16	SL = 5.07 + 0.34 Day	0.89	197	1.43
Hybrid 16	6	SL = 6.17 + 0.34 Day	0.93	388	1.42
	16	SL = 5.12 + 0.35 Day	0.89	391	1.92
	35	SL = 4.71 + 0.37 Day	0.92	379	1.71
Hybrid 35	16	SL = 6.87 + 0.30 Day	0.84	382	1.97
	35	SL = 6.21 + 0.34 Day	0.92	386	1.53