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

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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 remains largely unexplored. In the present study, we conducted experiments to investigate the effects of salinity on the routine metabolic rates (RMR) of euryhaline Atlantic herring *Clupea harengus* larvae at 3 levels of salinity: low (6 psu), intermediate (16 psu) and high (35 psu), reflecting ecologically relevant conditions for herring populations in the Atlantic Ocean 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 the 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.

KEY WORDS: Respiration · Salinity · *Clupea harengus* · Larval fish · Osmoregulation

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1. INTRODUCTION

Metabolic rate, which is proportional to oxygen consumption (Gillooly et al. 2001), acts as a 'pacemaker' for life, and the resulting energy can be used for biological processes like survival, growth or reproduction (Brown et al. 2004). Metabolic rates of ectothermic organisms, like fish, are strongly dependent on body mass and temperature (Clarke & Johnston 1999, Brown et al. 2004). However, even within the same environmental conditions, large variations among individuals can occur, which can partly be explained by the heritability of metabolic rates (Pettersen et al. 2018). This individual variation in metabolic rates also affects 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 metabolism and other biological processes such as growth (Ishikawa & Namikawa 1987, Nijhout et al. 2006). Therefore, the general 'pacemaker' theory is often challenged (Glazier 2015). For early life history

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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 the potential for adaptations to the environment has been 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 their small size and high surface-to-volume ratio, larvae are relatively sensitive to changes in salinity, mainly in relation 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 on euryhaline fishes (Kültz 2015, Kijewska et al. 2016). Nonetheless, physiological adaptations and mechanisms allowing euryhaline fishes to successfully cope with different salinities have not yet been fully explored (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 & Lagardère (1999) demonstrated that salinity has opposite effects on SMR and RMR, suggesting that other mechanisms, apart from osmoregulation, are also involved in compensating for changes in salinity. An example of 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 occurs from the Atlantic Ocean (35 psu) to the inner Baltic Sea (as low as 2 psu). The Baltic Sea is a relatively young habitat (~10 000 yr 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 Baltic Sea that reflect environmental gradients such as salinity (André et al. 2011, Teacher et al. 2013). Recently, genetic differentiation of herring populations from the Atlantic and Baltic Sea has further been documented by whole-genome sequencing (Lamichhaney et al. 2012, Martinez Barrio et al. 2016, Pettersson et al. 2019). The genetic differentiation between herring from these two areas 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 oxygen uptake in herring larvae are rare (Peck & Moyano 2016). Recent studies have demonstrated that the physiological response of herring larvae is dependent on feeding conditions (Illing et al. 2018), body mass and temperature (Moyano et al. 2018), suggesting the existence of metabolic flexibility. Existing information on how salinity influences metabolic rate of herring larvae is derived mainly from 2 studies: Holliday et al. (1964) and Almatar (1984). Furthermore, no studies have yet accounted for parental origin or developmental acclimation to new salinities, and there is 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 *C. harengus* larvae at 3 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 (Expt 1) will have higher RMR. For the second experiment, we hypothesised that larvae reared near iso-osmotic (intermediate) salinity will 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. MATERIALS 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 2 experiments. The wild population used in the first experiment (Expt 1) consisted of Baltic autumn-spawning herring. Baltic herring were caught on 13 September 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); ambient water temperature was approximately 10°C. Offspring from 5 parent pairs of autumn-spawning Baltic herring were incubated and co-reared together in the same tank at 16 psu and 10°C (Fig. 1). The hatching date of larvae (defined as the day when 50% had hatched) was 25 September 2017.

For the second experiment (Expt 2), we used herring larvae generated by mating F1 laboratory-reared hybrids of wild spring-spawning populations caught in the Atlantic Ocean and Baltic Sea (Berg et al. 2018). Fertilization was conducted on 7 June 2016. Spring-spawning Baltic herring were caught at the same location as the parents used in Expt 1; 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 3 yr 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 were 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 of hybrids at 6 psu originating from salinity of 16 psu (Fig. 1). We did not include any fertilization of hybrids at 6 psu originating from a salinity of 35 psu in the study design because survival in previous experiments was low at 6 psu when herring originated from 35 psu (authors' unpubl. data). This resulted in 5 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 Expt 2 had the same grandparents. Larvae hatched on 20 June 2016.

The larval rearing and the following experiments were reviewed and approved by the Norwegian National Animal Ethics Committee (Forsøksdyrutvalget-FOTS ID-8459). For both experiments, larvae were reared in 300 l round tanks (1 m diameter) on a 12 h day:12 h night light regime. Larval stocking density was initially 1500 larvae. Larvae were fed daily, ad libitum, with natural filtered zooplankton (2000 prey l^{-1}). The natural zooplankton consisted mainly of different copepods and nauplii stages and were stored in their original water (marine conditions) for a maximum of 1 d. Each day, any remaining plankton was counted within each tank, and additional plankton was added to reach the same level of prey per liter. Remaining plankton was found in all

Experiment	Overall temp	Number of crosses	Crosses Females	X	Males	Salinity Incubation	Salinity Rearing	Salinity Respiration
1	10°C	5	A Baltic	X	A Baltic	16 psu	16 psu	6 psu
								16 psu
								35 psu
2	10°C	1	Hybrid 35	X	Hybrid 35	6 psu	6 psu	6 psu
		1	Hybrid 16	X	Hybrid 16	16 psu	16 psu	16 psu
						35 psu	35 psu	35 psu

Fig. 1. Experimental design used for the factorial crossing experiments and respiration measurements. Parental Baltic herring used in Expt 1 were wild-caught (A: autumn). Herring used in Expt 2 were offspring of full sibling F1 Atlantic/Baltic hybrids that had been reared for 3 yr under common garden conditions in a salinity of either 16 or 35 psu. Colours of the rectangles and ellipses represent salinity of parental herring origin and rearing tanks, respectively (light blue = 6 psu, dark blue = 16 psu, green = 35 psu)

tanks each day. The high saline water (35 psu) was natural, filtered seawater originating from approx. 90 m depth. For the intermediate (16 psu) and low (6 psu) salinity, the seawater was mixed with filtered fresh water. The salinity values are nominal; 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 individual larvae was measured 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 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. The buckets 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®) were used. The net water volume of individual vials was determined and averaged 4.97 and 23.94 ml, respectively. Younger larvae were placed in 4 ml vials, while later measurements were conducted in 20 ml vials, with 1 larva vial^{-1}. 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 homogenise the water within the vials. All sensors attached to the respiration vials (up to 28 series^{-1}) had been individually calibrated to 100% saturation prior to insertion of larvae. The factory setting for 0% calibration of each vial and sensor was provided. Measurements of oxygen concentration in 4 vials at a time were then repeatedly carried out and averaged over a period of 60–90 s by attaching 4 of the optical cables to a multichannel PyroScience FireStingO2 (P/N: FSO2-x; PyroScience) measuring unit. Temperature was logged continuously with a sensor attached to the FireSting unit. After logging the corresponding oxygen concentrations (µmol l^{-1}) in the vials with the associated FireSting software (Pyro Oxygen Logger, version 3.0; PyroScience), 4 new cables were attached and the procedure was repeated until oxygen measurements had been made in all the vials. Typically, this procedure took about 15 min, and was repeated after 2–4 h during the day and again 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 h 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 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, saturation after 24 h was 74.2 ± 12.6 and 82.1 ± 9.8% for the 4 and 20 ml vial volumes, respectively. Typically, 6–7 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 then transferred to Teflon plates and dried at 55°C for 24 h before being weighed on a Sartorius® microbalance (Type M3P; Sartorius) 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 Expt 1, larvae were reared in water at 16 psu and 10°C. Oxygen measurements were conducted on larvae in 6, 16 and 35 psu and at a constant temperature of 10°C (Table 1). Larvae were transferred to the new

Table 1. Number of Baltic herring larvae included in analysis of respiration rates in Expt 1. Age (days post-hatching, DPH) of larvae and mean (±SD) temperature for each sampling day are shown. Dead or injured larvae (parentheses) 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)

salinities for acclimation the evening prior to measuring. In total, 6 rounds of measurements were conducted with larvae 17–44 d old (Table 1). Up to 8 larvae were used for each salinity per round. In addition, one blank sample was included for salinity trials of 6 and 35 psu; 2 blank samples were included for 16 psu.

For Expt 2, larvae were reared at a temperature of 10°C and at salinities of 6, 16 and 35 psu (Table 2). Oxygen consumption was measured at the same salinity in which the 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, this setup allowed for comparisons of the adaptations of the offspring to the different parental salinity environments. In total, 10 rounds of measurements, each including 3 experimental groups, were conducted with larvae 11–39 d old (Table 2). Up to 7 larvae and one blank sample were used for each experimental group and sampling round.

Table 2. Number of Atlantic/Baltic hybrid herring larvae included in analysis of respiration rates in Expt 2. Age (days post-hatching, DPH) of larvae and mean (±SD) temperature for each sampling day are shown. Dead or injured larvae (parentheses) 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)

2.4. Statistical analysis

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

Growth rates (mm d^{-1}) for all larval groups (ad libitum feeding) were estimated with ANCOVA using standard length as the response variable and the full interaction between age and larval groups as predictor variables. The age of larvae used for respiration measurements was defined as the day of acclimation. 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 the actual rearing salinity of larvae. This resulted in 6 (1 in Expt 1; 5 in Expt 2) larval rearing groups. Only the 5 rearing groups from Expt 2 were used for statistical analyses. The one group from Expt 1 was added for visualisation.

The oxygen consumption (µmol h^{-1}) 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 value 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 2 different vial sizes (ANOVA: $F = 0.4$, df = 1, 246, p = 0.54).

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

For statistical analyses, we followed the protocol of Zuur et al. (2010) for 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 the larvae. For model selection, we started with full

Table 3. Model regressions for length–mass relationships of Atlantic herring larvae from Expts 1 and 2. SL: standard length (mm); DM: dry mass (µg). There was no difference between larvae reared at different salinities within an experiment. RSE: residual standard error

Expt	Equation	Adj R^2	n	RSE
1	$DM = 0.0653 \times SL^{3.271}$	0.91	107	0.10
2	$DM = 0.0414 \times SL^{3.418}$	0.94	122	0.08

interaction models where higher order interactions were removed when not significant. The final model that best explained oxygen consumption as RMR was:

$$RMR = \alpha + \beta_1 \times DW + \beta_2 \times Sal \quad (1)$$

for Expts 1 and 2 combined, where β represents the estimated regression parameters for the log-transformed dry weight (DW) of each larvae and for the salinity (SAL) during the measurement. For Expt 2, we additionally tested the influence of parental salinity with this model:

$$RMR = \alpha + \beta_1 \times DW + \beta_2 \times Sal + \beta_3 \times ParSal \quad (2)$$

where ParSal represents the salinity origin of the parents. Following model selection, significant differences for variables with more than 2 levels, like salinity, were evaluated by Tukey's HSD post hoc test. For visualisation, we standardized the RMR for dry mass and present the standardised RMR (nmol µg^{-1} h^{-1}) as boxplots.

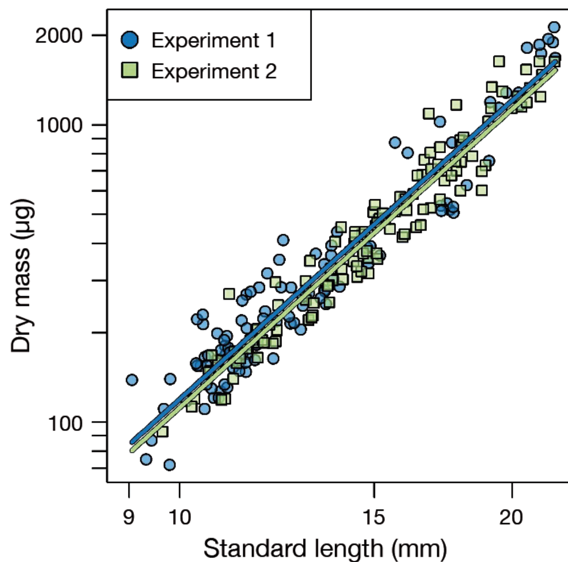

Fig. 2. Length–dry mass relationship for herring larvae used in Expts 1 and 2. Regression lines for the statistical model are shown. Individual regression equations are given in the Appendix (Table A1)

3. RESULTS

3.1. Somatic relationships

Larvae of wild-caught Baltic herring (Expt 1) and laboratory reared Atlantic/Baltic herring hybrids (Expt 2) showed allometric length–dry mass relationships (ANOVA: $F = 2964.7$, df = 1,226, $p < 0.001$; Fig. 2) which differed between the 2 experiments ($F = 5.3$, df = 1,226, $p < 0.05$). On average, larvae from wild Baltic autumn spawners (Expt 1) were 2.3% heavier at a given length than larvae from laboratory-reared Atlantic/Baltic hybrids (Expt 2).

The growth rates of herring larvae from Expt 2 were similar, except for larvae reared at 35 psu when parents originated from 16 psu and vice versa (ANCOVA: $F = 22.6$, df = 4,1916, $p < 0.001$; Fig. 3, Table A1 in the Appendix). Herring reared at 16 psu when parents originated from 35 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 comparisons among groups with the same parental salinity, growth rates were higher at higher rearing salinity (Fig. 3). The overall growth rate of all herring larvae in this study was 0.34 ± 0.01 mm d^{-1}, and there was no significant difference between larvae sampled regularly or used

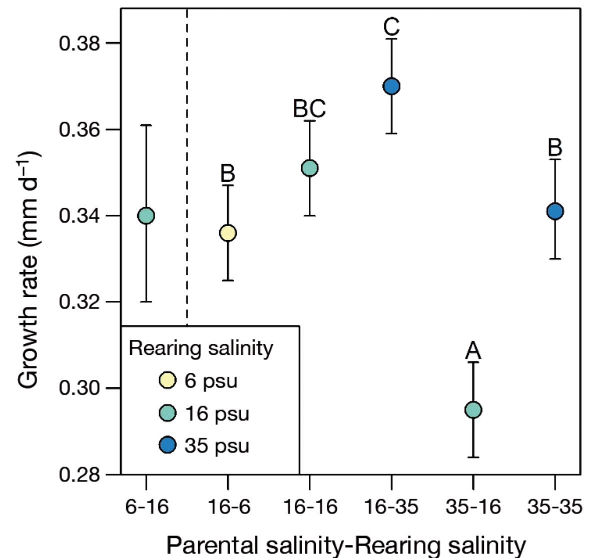

Fig. 3. Growth rates ± 95% confidence intervals of 8–56 d old herring larvae originating from one wild population of Baltic autumn spawners (6 psu parental salinity) as well as 2 laboratory-reared groups (Atlantic/Baltic hybrid, 16 and 35 psu parental salinity) reared at 3 different salinities (6, 16 and 35 psu). Groups that do not share common letters are significantly different ($p < 0.05$). Larvae from Expt 1 (left of dashed line) were added for visual comparison

for respiration measurements (ANCOVA: $F = 1.9$, df = 1, 2110, p = 0.17).

3.2. Salinity effects on oxygen consumption

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

Table 4. Model regressions for body size scaling of routine metabolic rate (RMR; nmol ind.$^{-1}$ h^{-1}) of Atlantic herring larvae from one wild population (A Baltic: Baltic autumn spawners) and 2 laboratory-reared groups (Atlantic/Baltic hybrid 16 and hybrid 35). Larvae were reared at different salinities (Sal) and ambient water temperature of 10°C. DM: dry mass; RSE: residual standard error

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

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 RMR of herring displayed 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 salinity waters. Offspring from herring that were reared their entire life at either 16 or 35 psu showed no significant differences in

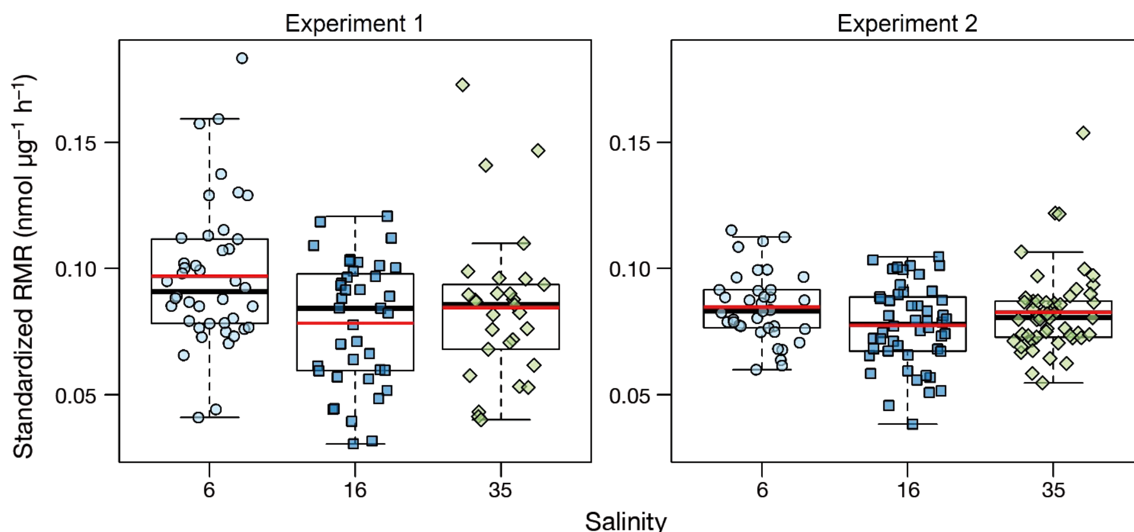

Fig. 4. Standardized routine metabolic rate (RMR) of herring larvae separated by experiment and salinity. The salinity exposure time differed between experiments; acute vs. long-term for Expts 1 and 2, respectively. The median (black line) and mean (red line) are indicated in the boxes, which represent 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

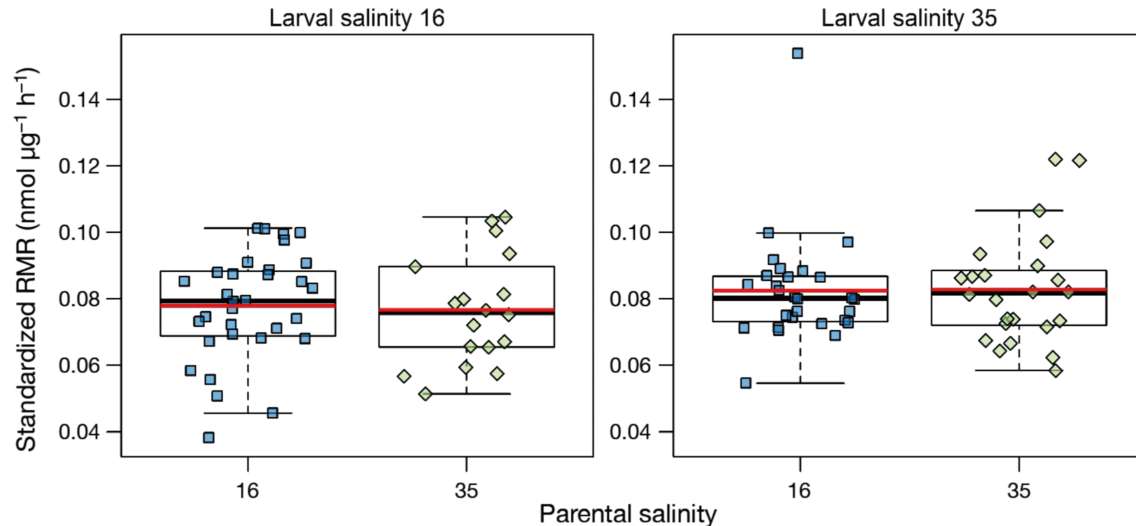

Fig. 5. Standardized routine metabolic rate (RMR) of herring larvae from Expt 2 reared and measured at salinities of 16 and 35 psu, separated by parental salinity of 16 or 35 psu. Boxplot parameters as in Fig. 4; individual regression equations are given in Table 4

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 an acclimation time to different salinities longer than just one night 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, 2009). Larvae at 35 psu had the highest growth when parents originated from 16 psu and lowest growth under the reverse 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 focused on the effect of temperature (e.g. de Silva & Tytler 1973, Moyano et al. 2018) 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 the 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 the offspring of 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 among the offspring of these 2 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 induced 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 (Expt 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.

In 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 a salinity of either 16 or 35 psu were used to test potential adaptations in 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; however, to determine if this is the case, a larger experiment and individual genotyping of larvae would be required. Such an experiment is well justified by the fact that

whole-genome sequencing has revealed hundreds of loci underlying ecological adaptations in 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 Expt 2 = 0.08). However, genetic analysis on e.g. larvae with the highest deviation from the mean (positive and negative statistical residuals) is essential to understand if individual adaptations are causing this deviation.

Herring larvae used in Expt 2 experienced the same salinity during incubation, hatching and metabolic rate measurements, in contrast to larvae from Expt 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 acclimated within 24 h to the sudden change in salinity, resulting in larger variations in metabolic rates (overall RSE for Expt 1 = 0.146). Changes in osmoregulation, which will influence metabolic rates, occurred up to 1 wk 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 Expt 1 were the offspring from 5 parent pairs, while only one full sibling hybrid cross per salinity was used in Expt 2. This design ensured segregation of Atlantic and Baltic alleles and thus 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 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 had even higher metabolic rates than larvae at high salinities — because they needed 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 that growth rates tended to increase with salinity, which would contradict the notion that fish with highest growth rates have 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 the highest salinity (Tables 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 caused the higher mortality. Also, saturation levels and actual oxygen content (µmol l^{-1}) 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 injury during measurement, as larvae were 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 Expt 1, where larvae experienced abrupt salinity changes.

It is essential to know 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 estimated the body size scaling of RMR (b-value, i.e. the exponent of the equation, 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. 2018). The development of equipment used for respiration measurements leading to 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. (2018) used populations (North Sea autumn spawners and Western Baltic spring spawners) that are genetically different to populations from our study over a wide range of temperatures, the body size scaling of respiration rates from their study were similar to our findings. However, the growth rates of herring from that study were significantly lower than 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 terms 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 other marine species 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 is likely to 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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Appendix.

Table A1. Individual model regressions for herring growth rate (standard length [SL, mm]-at-age [d]) of larvae from one wild population of Baltic autumn spawners as well as 2 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

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