RESEARCH ARTICLE

Sperm pre-fertilization thermal environment shapes offspring phenotype and performance

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ABSTRACT

The sperm pre-fertilization environment has recently been suggested to mediate remarkable transgenerational consequences for offspring phenotype (transgenerational plasticity, TGB), but the adaptive significance of the process has remained unclear. Here, we studied the transgenerational effects of sperm pre-fertilization thermal environment in a cold-adapted salmonid, the European whitefish (*Coregonus lavaretus*). We used a full-factorial breeding design where the eggs of five females were fertilized with the milt of 10 males that had been pre-incubated at two different temperatures (3.5°C and 6.5°C) for 15 h prior to fertilization. Thermal manipulation did not affect sperm motility, cell size, fertilization success or embryo mortality. However, offspring that were fertilized with 6.5°C-exposed milt were smaller and had poorer swimming performance than their full-siblings that had been fertilized with the 3.5°C-exposed milt. Furthermore, the effect of milt treatment on embryo mortality varied among different females (treatment×female interaction) and male–female combinations (treatment×female×male interaction). Together, these results indicate that sperm pre-fertilization thermal environment shapes offspring phenotype and post-hatching performance and modifies both the magnitude of female (dam) effects and the compatibility of the gametes. Generally, our results suggest that short-term changes in sperm thermal conditions may have negative impact for offspring fitness. Thus, sperm thermal environment may have an important role in determining the adaptation potential of organisms to climate change. Detailed mechanism(s) behind our findings require further attention.

KEY WORDS: Climate change, *Coregonus lavaretus*, Paternal effect, Sperm, Temperature, Transgenerational plasticity

INTRODUCTION

Environmental conditions often vary considerably, which poses a major challenge for organisms to maintain their performance and fitness across a broad range of environments (Beaman et al., 2016). However, most (if not all) organisms are typically capable of adjusting their phenotypes in response to environmental changes (phenotypic plasticity), although the adaptive significance of this plasticity is often unclear (Merilä and Hendry, 2014). Besides affecting individual phenotypes within a single generation, phenotypic plasticity can also operate across generations (transgenerational plasticity, TGP: Donelson et al., 2011; Shama et al., 2014; Forsman, 2015; Guillaume et al., 2016; Luquet and Tariel, 2016). TGP refers to processes in which the phenotype or environment experienced by the parents (and possibly their ancestors) modifies the phenotype of the offspring without changing the DNA sequence of the offspring (Luquet and Tariel, 2016). TGP can occur via both maternal (Mousseau and Fox, 1998) and paternal environments (Crean and Bonduriansky, 2014), although paternally-mediated TGB (paternal effects) has received much less attention.

In many species, the only provisioning of the males to the next generation is their semen. Thus, in such species, paternal effects are mediated predominantly via sperm or other ejaculate (non-sperm) factors (e.g. Puri et al., 2010; Garcia-Gonzalez and Dowling, 2015; Rodgers et al., 2015; Crean et al., 2016). Supporting this view, recent findings have demonstrated that both paternal (sperm pre-release) environment and sperm post-release environment can have remarkable transgenerational consequences (for fertilization success, offspring survival and developmental rate: Crean et al., 2012, 2013; Jensen et al., 2014; Ritchie and Marshall, 2013; Immler et al., 2014; Zajitschek et al., 2014; Marshall, 2015, see also Kekäläinen et al., 2015; Evans et al., 2017). However, although sperm environmental variation potentially has many important transgenerational ecological and evolutionary consequences, the mechanistic basis of these effects have remained unclear (Marshall, 2015). In any case, sperm environmental plasticity (and paternal effects in general) are often assumed to be adaptive if environmental conditions experienced by offspring are sufficiently predictable from the environment encountered by their fathers (or their gametes) (Burgess and Marshall, 2014). However, environmental conditions often vary unpredictably and in these situations sperm-mediated transgenerational effects may impede adaptation to changing conditions, for example since sperm environmental conditions may adjust offspring phenotypes for the 'wrong' environment. Accordingly, transgenerational effects may often act as conduit by which stressful conditions, such as rapidly changing temperature regimes, reduce the performance of offspring in the next generation (Guillaume et al., 2016).

Environmental temperature has a fundamental influence on various physiological processes, especially in ectothermic (i.e. cold-blooded) animals (e.g. Kortet and Vainikka, 2008; Muñoz et al., 2015; Sinclair et al., 2016). Many of these species, such as numerous salmonids, are capable of living only within a narrow temperature range (stenothermic species) and are thus particularly sensitive to relatively small increase (or fluctuations) in ambient water temperature (e.g. Cingi et al., 2010). Accordingly, relatively minor changes in the sperm thermal environment can have many fundamental effects on the sperm physiology and function in these species (Lahnsteiner and Mansour, 2012; Dadras et al., 2017a; Fenkes et al., 2017). Water temperature can, for example, affect the physiological state of lipids, properties of the plasma membrane and

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enzyme activity of sperm cells, as well as alter the composition of the seminal plasma (Dadras et al., 2017a).

Elevated temperatures also can trigger the generation of reactive oxygen species (ROS) in sperm that function as important signaling molecules in cells and are required during the fertilization process (Menezo et al., 2016). However, elevated concentrations of ROS can lead to oxidative stress that can damage sperm lipids, proteins and DNA and has been demonstrated to alter DNA methylation patterns of sperm (Lane et al., 2014; Menezo et al., 2016). For example, Lane et al. (2014) studied the effect of oxidative stress in mice and found that elevated ROS levels in sperm impaired embryo development, but did not affect sperm motility or fertilization ability. Together, these findings indicate that sperm pre-fertilization thermal environment and associated ROS fluctuations may mediate important transgenerational consequences for offspring. However, earlier studies on the transgenerational impacts of environmental stressors have mainly clarified the role of parents in altering the phenotype of their offspring (Donelson et al., 2011; Salinas and Munch, 2012; Shama et al., 2014), whereas the role of sperm thermal environment has remained unclear.

In the present study, we investigated the transgenerational consequences of the sperm thermal environment in the European whitefish (*Coregonus lavaretus* Linnaeus 1758), a cold-adapted salmonid with many populations currently living at the upper end of its thermal tolerance range (Cingi et al., 2010). Given that, in salmonids (and many other poikilothermic animals), climate warming is particularly harmful for early life stages (e.g. Jensen et al., 2008), our primary aim was to study the effects of sperm thermal environment on the developing embryos and post-hatching larvae. Based on the above-mentioned findings on the close association between sperm ROS levels and offspring fitness, we hypothesized that increased sperm pre-fertilization temperature has negative impacts on embryo development and offspring performance post-hatching. In order to clarify these effects, we first pre-incubated the stripped milt (and non-activated sperm) from 10 males in two different temperature conditions (3.5°C and 6.5°C). We then fertilized the eggs of five standard (non-manipulated) females with the sperm of each of these 10 males from both temperature treatments in all possible male–female combinations. This full-factorial breeding design allowed us to partition the relative contribution (i.e. the proportion of variance explained) of dam effects, sire effects as well as paternally mediated genotype×environment interactions and transgenerational (milt temperature) effects on offspring phenotype (body and yolk size) and performance (aerobic swimming capacity). In this way, we were able to rule out potentially confounding differences in maternal investments in gametes and thus, fully disentangle sperm-mediated transgenerational effects from maternal effects (Guillaume et al., 2016). Furthermore, by splitting the ejaculates of the males into different treatments, we were able to compare the effect of pre-fertilization environmental manipulation within each sire, which is not possible at the whole-organism (individual) level.

MATERIALS AND METHODS
Experimental fish, gamete collection and milt treatments
Parental fish originated from the River Kokemäenjoki (Finland) anadromous whitefish (*C. lavaretus*) population and represented the second selectively bred and pedigreed hatchery generation (year class 2012) maintained at the Tervo Fish Farm by the Natural Resource Institute Finland (national Finnish breeding programme of the European whitefish; Kause et al., 2011). All the fish were reared together in an outdoor raceway over three summers. The sexes were

kept separated 10 days prior to gamete collection. Gametes from 10 males and five females were stripped on 12 and 13 November 2015, respectively. The surface water temperature at the Tervo station was 4.5°C at the time of milt collection and before that ranged from 4.1 to 5.3°C during November. For both sexes, the individuals were selected from different families, the average additive genetic relationship (a) being 0.046 and 0.019 for males and females, respectively. The inbreeding coefficient (F) of all stripped fish was zero. Milt (containing non-activated sperm) of all males was divided into two aliquots in plastic zipper bags (Minigrip, Georgia, USA) filled with air (n=20 bags in total). In order to imitate short-term changes in water temperature, one aliquot bag (within each male) was incubated in 3.5°C ('cold treatment') water and another in 6.5°C ('warm treatment') water for 15 h. Incubating the milt and non-activated sperm outside the male body cavity allowed us to discriminate the transgenerational effects mediated by sperm thermal environment from the paternal effects (i.e. transgenerational effects mediated by sperm pre-release environment). Given that average global air temperature has been predicted to rise Air temperature has been predicted to rise 4.8°C by 2100 (IPCC, 2014), both treatments can be expected to represent ecologically relevant temperatures.

Sperm motility measurements
Sperm motility both before (control measurements) and after sperm treatments (see above) was measured using computer-assisted sperm analysis (CASA) (integrated semen analysis system, ISAS v. 1: Proiser, Valencia, Spain) with a black and white CCD camera (capture rate 60 frames s^{-1}) and negative phase contrast microscope (100× magnification). Sperm motility analyses were performed after vortexing the milt samples for 5 s and then by adding 0.1 µl milt to two-chamber (chamber height, 20 µm; volume, 6 µl) microscope slides (Leja, Nieuw-Vennep, The Netherlands) and by activating sperm with 3 µl of 5.0°C natural (Tervo aquaculture station) water. In other words, sperm motility analyses for both thermal treatments were always conducted in identical water temperatures (representing the average temperature between two treatments). Sperm motility parameters (curvilinear velocity, VCL; percentage of motile sperm cells and linearity of sperm swimming tracks) and size of the sperm cells (head area) were measured 10 s after activation (at least two replicate measurements/male).

Artificial insemination and incubation of the eggs
After pre-incubation of milt batches (see above) for 15 h, artificial fertilizations were immediately conducted in all possible combinations (n=50 families) between 10 males and five females (North Carolina II design) (Fig. 1). Within each family, eggs were divided into two sub-batches: one batch was fertilized with cold-treated sperm and another with warm-treated sperm, resulting in 100 male–female combinations (split-clutch+split-ejaculate design). In order to minimize the potential time effects for measured sperm and offspring parameters, all the fertilizations (and CASA measurements, see above) were performed simultaneously (sequentially) for both sperm treatments within each male. The average a between males and females was 0.045 and the average F (calculated based on the knowledge of a full pedigree extending through all breeding generations) of the resulting families was 0.023. All the fertilizations were replicated twice (n=200 male–female combinations in total). In order to equalize sperm numbers across all fertilizations, we measured the spermatocrit (sperm volumes) for all the males by centrifuging the milt samples for 10 min (11,000 rpm) in a micro-hematocrit centrifuge. Then, by using the highest male-specific spermatocrit as a reference value, we equalized
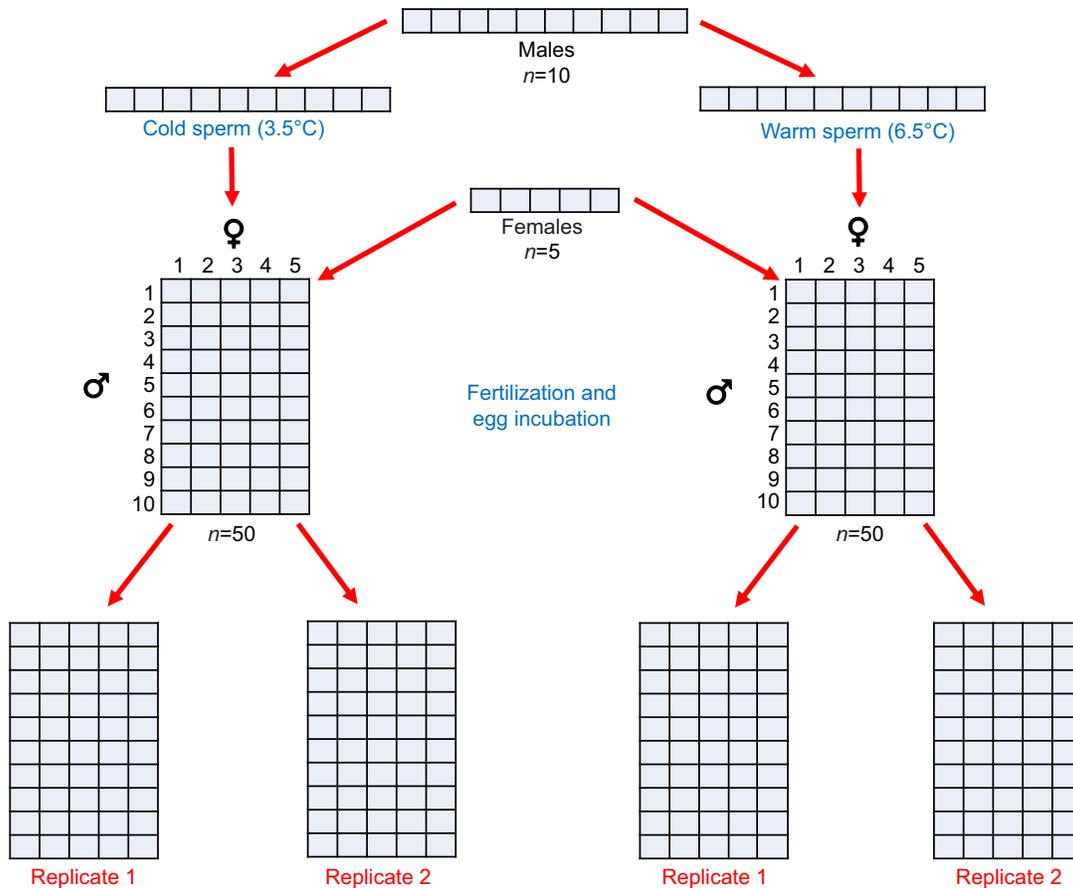
Fig. 1. Experimental design. Eggs of five standard (non-manipulated) females were fertilized with sperm of 10 males (n=50 families). Within each family, fertilizations were performed using both cold-treated and warm-treated sperm, resulting in 100 independent male–female combinations (split-clutch+split-ejaculate design). All the fertilizations were replicated twice (n=200 fertilizations in total). Fertilized eggs were incubated in these same combinations in respective containers until hatching.

sperm volumes across all fertilizations. The final sperm volume in all fertilizations was 1.2 µl of pure spermatozoa (equivalent to 10 µl of milt with 12% spermatocrit). Fertilizations were made on Petri dishes by injecting the sperm with a micropipette directly on the freshly stripped eggs. Immediately after this, 50 ml of 5.0°C natural water was poured on the Petri dish and each dish was gently shaken for 3 s. To allow eggs to be fertilized they were left undisturbed in the dishes for at least 5 min (ensuring that all the sperm had lost their motility). Selected fertilization temperature represented the average temperature between above-mentioned two milt temperature treatments, thus ensuring that the magnitude of the temperature change was identical for both milt treatments. Fertilization temperature also closely represented the natural water temperature at the Tervo station during the fertilizations (4.5°C). By conducting experimental fertilizations for both sperm treatments at the same (natural) temperature, we minimized the potentially confounding influence of fertilization environment on our results. Fertilized eggs were then randomly divided into individual incubating containers (two replicate containers per family within both sperm treatments) in four 600 liter temperature-controlled water tanks filled with 3.5°C non-chlorinated tap water, where they were incubated until all the eggs were hatched in April 2016. The mean (±s.e.m.) egg number was 176±2.7 eggs per container. In order to incubate the eggs in close to natural thermal conditions, 1 week after fertilization, water temperature was decreased to 3.0°C. Next (4–7 March 2016), water temperature was gradually raised to 6.0°C to imitate arrival of spring and to facilitate

hatching. Dead embryos were counted and removed weekly during the whole incubating period.

Offspring swimming performance and size

Offspring swimming performance at larval stage was determined on 10–17 March 2016 using a swimming tube system with gravity-driven flow and constant water velocity of 6.2 cm s^{-1} (Huuskonen et al., 2009; Kekäläinen et al., 2010). Offspring swimming performance of whitefish has been shown to predict predator avoidance ability of the offspring (Kekäläinen et al., 2010) and thus is strongly linked to fitness. In the experiments, individual larvae were forced to swim against a current at 6.0°C water temperature and their fatigue time was recorded (the time taken to drift against a net placed at the rear end of the tube). For each 200 male–female combinations, three haphazardly selected individuals were used in the experiments. All the larvae were 3–10 days old during the experiments (whitefish larvae are freely swimming immediately after hatching). Taking into account the length of the egg incubation period (~120 days), variation in the mean developmental time of tested larvae was less than ±3%. After the experiments, larvae were killed by overdose of tricaine methanesulfonate (MS-222, Sigma) and preserved in a solution of 70% ethanol and 1% neutralized formalin. The larvae were later measured for total length and mass. The length (l) and height (h) of yolks were also measured in order to calculate the yolk volume of the larvae according to the equation for a prolate spheroid: V=0.5236lh^2 (Blaxter and Hempel, 1963). All the experiments were conducted according to the license issued by the

Finnish Animal Experiment Board (ESAVI/3443/04.10.07/2015, modified in ESAVI/8062/04.10.07/2015).

Statistical analyses

The effect of sperm treatment (within-subjects factor) on sperm motility (VCL, linearity and percentage of motile sperm cells) and cell size were studied using repeated-measures ANOVA. Paired differences between three sperm treatments (before treatment, and after cold and warm treatment) were tested using Šidák post hoc tests. The effect of male, female, male–female interaction and sperm treatment on fertilization success (evaluated by determining the proportion of dead eggs 5 weeks after fertilization), total embryo mortality, offspring swimming performance and offspring size (length, fresh mass and yolk volume) were tested in linear mixed-effects models (LMMs). In the models, sperm treatment was used as a fixed factor, and male, female and all two- and three-level interactions between male, female and sperm treatment were used as random factors. Models were simplified by removing non-significant interaction terms based on a likelihood ratio test. In order to estimate the relative contribution of both parents (and their interaction) and sperm treatment on measured offspring traits, we calculated marginal R^2 (i.e. variance explained by the fixed factor) and conditional R^2 (i.e. variance explained by both fixed and random factors) for our models (Nakagawa and Schielzeth, 2013). Then, we partitioned variance of random factors further into dam, sire and dam×sire interaction variance (observational variance components) and finally calculated the relative proportions of variance explained by fixed factor (sperm treatment) and random factors (dam, sire and dam-sire interaction). Model assumptions were verified graphically using Q–Q plots and residual plots. All presented P-values are from two-tailed tests with α=0.05. LMM analyses were conducted using lmerTest (version 2.0-33) and R^2 calculations with MuMIn (version 1.15.6) package in R (version 3.3.2).

RESULTS

Sperm traits

Sperm swimming velocity (VCL) and percentage of static sperm cells did not differ between sperm treatments (repeated-measures ANOVA, VCL: $F_{2,18}$=1.42, P=0.267; static sperm cells: $F_{2,18}$=1.65, P=0.220, Fig. 2). Sperm treatment affected the linearity of the sperm swimming trajectory and the head area of the sperm cells (repeated-measures ANOVA, linearity: $F_{1.14,10.28}$=5.48, P=0.037; head area: $F_{2,18}$=6.41, P=0.008). For linearity, paired comparisons showed no differences between cold and warm treatments (before vs cold: P=0.164; before vs warm: P=0.081; cold vs warm: P=0.787). However, sperm cell size tended to be larger before the sperm treatments than after cold (P=0.033) and warm (P=0.050) treatments. Again, no difference was found between cold and warm treatments (P=0.779). In other words, the effect of sperm thermal manipulation on the above-mentioned four sperm traits was similar for cold- and warm-treated sperm (Fig. 2).

Fertilization success and embryo mortality

Fertilization success and embryo total mortality differed between females, but no differences were found between males, male×female combinations or sperm treatments (Table 1). However, both mortality variables were affected by sperm treatment×female interactions, as well as sperm treatment×male×female interactions. In other words, the effect of sperm treatment on embryo mortality varied among different females and male–female combinations (Fig. 3).

Offspring size and swimming performance

Offspring post-hatching length, fresh mass and swimming performance were all affected by females and sperm treatment and in all cases, mean values of the traits were larger in the cold-treated sperm group (Table 2, Fig. 4). Offspring yolk size was affected by both parents and tended to also be affected by sperm treatment (P=0.083). In all four models (offspring length, fresh mass, yolk size and swimming performance), the interaction terms between sperm treatment and males/females (or male–female combinations) were statistically non-significant and were thus removed from the final models. Offspring swimming performance was not affected by offspring length, fresh mass or yolk size (removed from the final model).
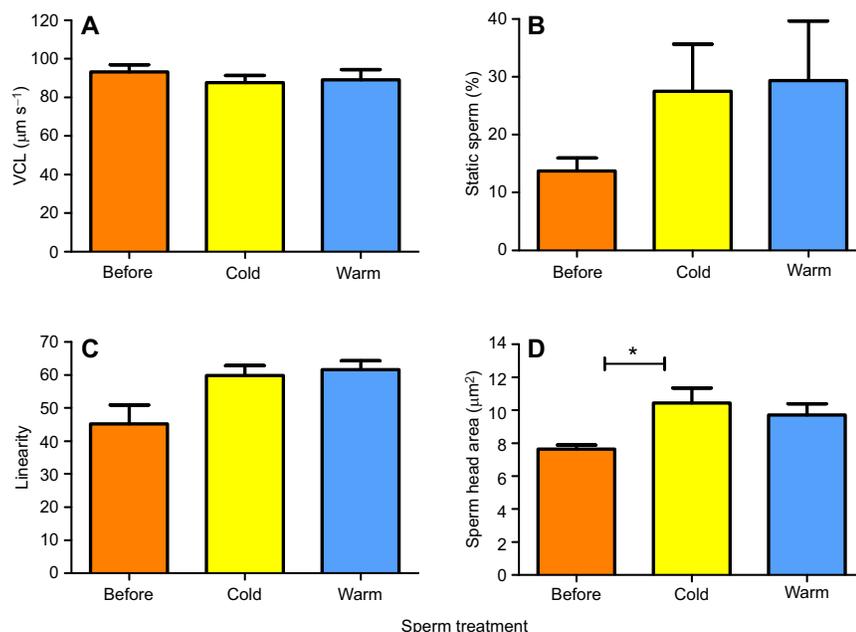
Fig. 2. Measured sperm traits of Coregonus lavaretus males before and after sperm incubation (15 h) at two different temperatures (3.5°C and 6.5°C). Mean±s.e.m. sperm curvilinear velocity (VCL, µm s^{-1}) (A), the proportion of static sperm cells (B), linearity of the sperm swimming trajectory (C) and head area (µm^2) of the sperm cells (D) before and after sperm treatments (n=10 males). An asterisk indicates statistically significant difference between treatments (repeated-measures ANOVA, P<0.05).

Table 1. Linear mixed model (LMM) statistics for fertilization success and embryo mortality

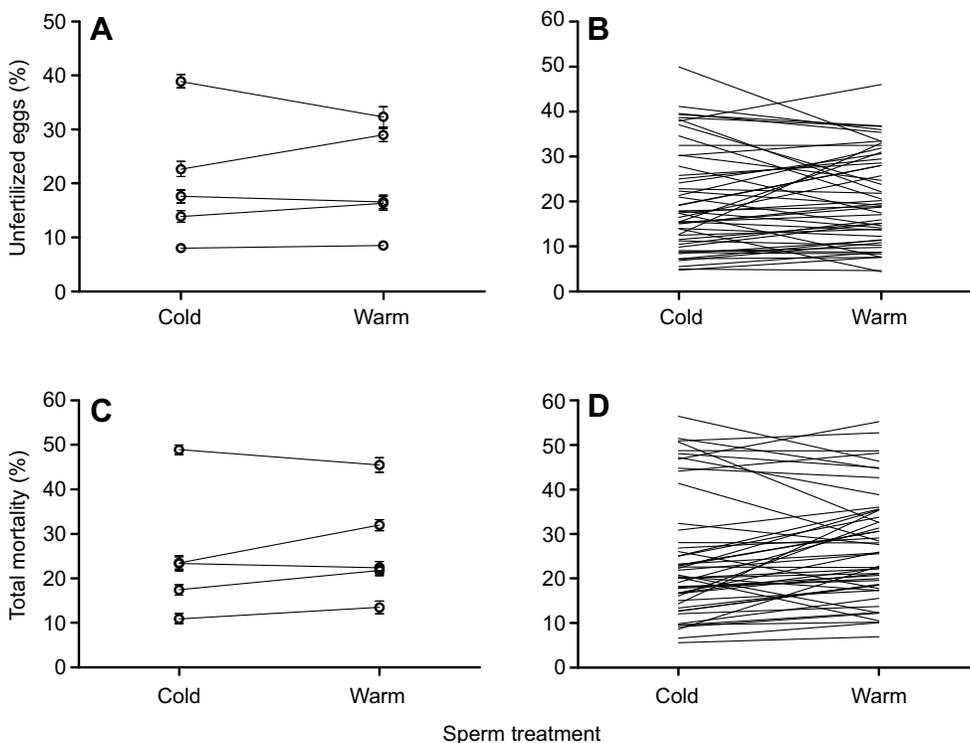|	Effect	Fertilization success			Total embryo mortality		
		χ^2	d.f.	P	χ^2	d.f.	P
Random effects	Male	0.419	1	0.517	0.524	1	0.469
	Female	6.790	1	**0.009**	8.570	1	**0.003**
	Male×female	1.173	1	0.279	1.578	1	0.209
	ST×male	0.000	1	1.000	0.000	1	1.000
	ST×female	8.849	1	**0.003**	7.565	1	**0.006**
	ST×male×female	24.724	1	**<0.001**	11.367	1	**<0.001**
		t-value	d.f.	P	t-value	d.f.	P
Fixed effects	Intercept	3.482	7.05	**0.010**	3.394	6.11	**0.014**
	ST	0.161	4.00	0.880	1.056	4.00	0.350

ST, Sperm treatment. Statistically significant P-values are indicated in bold.

DISCUSSION

The present results show that the offspring that were fertilized with 'cold' (+3.5°C-treated) sperm attained larger body size and had better swimming performance than offspring of the same parents that were fertilized with 'warm' (+6.5°C-treated) sperm. However, sperm motility, cell size of the sperm, fertilization success and embryo mortality were not affected by the sperm thermal environment, indicating that these findings most likely cannot be explained by treatment-specific differences in sperm fertilization ability or viability. Together, these results suggest that the sperm thermal environment has transgenerational consequences for offspring phenotype, and especially for post-hatching performance. This is remarkable given that, in whitefish, larvae swimming performance has been demonstrated to predict predator avoidance ability of the offspring and thus offspring post-hatching fitness in nature (Kekäläinen et al., 2010). Furthermore, since the material contribution of the eggs to the zygote is generally much higher than that of sperm, maternal effects typically dominate early life-history traits (see Table 2; Crean and Bonduriansky, 2014). Thus, it is possible that the relative importance

of paternal effects is even greater later in ontogeny. Observed strong dam effects may also partly explain why measured traits were not affected by male or male×female interaction effects. However, it is possible that the strength of these effects would be higher if the statistical power of the breeding design could be increased (e.g. by increasing the number of sires).

Males of many species are capable of adjusting sperm phenotype and motility in response to local conditions (Crean et al., 2013; Zajitschek et al., 2014; Marshall, 2015; Reinhardt et al., 2015). Similarly, several environmental factors can directly alter the phenotype of the sperm when sperm cells have been released into the fertilization environment (Alavi and Cosson, 2005, 2006; Mehlis and Bakker, 2014). Accumulating evidence suggests that both modifications in sperm phenotype can shape the phenotype of the offspring in the next generation (or possibly the next few generations) (Marshall, 2015). In the present study, sperm thermal manipulation was performed directly to the sperm (i.e. stripped milt) and prior to sperm activation. Thus, the observed results cannot be explained by a male phenotypic manipulation of the sperm.

Fig. 3. Fertilization success and embryo mortality when *C. lavaretus* sperm were pre-incubated at two different temperatures (3.5°C and 6.5°C) for 15 h prior to fertilization. Reaction norms of fertilization success (proportion of unfertilized eggs) and embryo total mortality (means±s.e.m.) for five females (A,C) and for male–female combinations (n=50 families) (B,D) in different sperm treatments.

Table 2. Linear mixed model (LMM) statistics for offspring size (length, fresh mass and yolk volume) and swimming performance

|	Effects	Length				Mass				Yolk volume				Swimming performance			
		χ^2	d.f.	P	% Var	χ^2	d.f.	P	% Var	χ^2	d.f.	P	% Var	χ^2	d.f.	P	% Var
Random effects	Male	0.274	1	0.60	0.9	0.228	1	0.60	0.1	5.52	1	**0.020**	4.6	2.164	1	0.10	3.5
	Female	45.362	1	**<0.001**	43.3	142.89	1	**<0.001**	88.3	47.05	1	**<0.001**	39.7	29.95	1	**<0.001**	30.2
	Male×female	0.086	1	0.80	1.1	0.000	1	1.0	0.0	0.00	1	1.00	0.00	0.176	1	0.70	1.7
	Residual				53.3				11.3				54.9				59.5
		t	d.f.	P	% Var	t	d.f.	P	% Var	t	d.f.	P	% Var	t	d.f.	P	% Var
Fixed effects	Intercept	47.523	6.52	**<0.001**		13.785	4.24	**<0.001**		0.682	7.32	0.52		17.94	8.63	**<0.001**	
	ST	−2.333	149	**0.021**	1.5	−2.289	185	**0.023**	0.3	−1.744	185	0.083	0.8	−4.096	149	**<0.001**	5.0

Statistically non-significant interactions (ST×male, ST×female and ST×male×female) were removed from the final models (*P*>0.76). The percentages of variance explained by the random and fixed factors are also given (calculated according to Nakagawa and Schielzetz, 2013, see Materials and Methods for detailed explanation). ST, Sperm treatment. Statistically significant *P*-values are indicated in bold.

Furthermore, since all the fertilizations and sperm motility measurements were conducted in identical conditions (in average temperature between two thermal treatments), our results are independent of the sperm fertilization environment. However, given that fertilization temperature in our experiment was different from either of the sperm incubation temperatures, our results may also partly be explained by rapid temperature change at fertilization. Future studies are needed to determine if the observed group-specific differences in offspring swimming performance remain the same in different temperature conditions (sperm temperature× offspring rearing temperature interaction).

We can find several potential, mutually non-exclusive alternative mechanisms for our findings. First, although milt temperature treatments did not affect sperm fertilization success or embryo mortality, it is possible that especially warmer (6.5°C) treatment increased ROS production in sperm (Dadras et al., 2017b). Increased ROS levels have been demonstrated to induce changes in sperm cellular structures and physiology (e.g. lipids, proteins, plasma membrane structure or enzyme activity; Menezo et al., 2016; Dadras et al., 2017a) and such changes occur also in quiescent salmonid sperm (Lahnsteiner et al., 1997). Thus, observed transgenerational changes in offspring phenotype and performance may be a consequence of sperm thermal environment-induced changes in sperm physiology or structure.

Second, sperm thermal environment may have induced changes in offspring gene expression via certain epigenetic factor(s) (Danchin et al., 2011; Bonduriansky et al., 2012; Jenkins and Carrell, 2013). Epigenetic inheritance is currently argued to be mediated mainly via epigenetic marks, such as DNA methylation or acetylation, histone modifications, cytoplasmic RNA molecules, prions and sperm nuclear proteins (Danchin, 2013; Castillo et al., 2014). Interestingly, elevated sperm ROS levels have been demonstrated to shape the methylation patterns of the sperm DNA, lipids and proteins, which can, in turn, shape embryonic development and metabolism (Lane et al., 2014; Menezo et al., 2016). Thus, it is possible that our sperm treatments induced such methylation changes in sperm, leading to observed changes in offspring phenotype and performance. Third, it is possible that other (non-sperm) components of the ejaculate mediated the observed phenotypic changes in the offspring (García-González and Simmons, 2007). Seminal fluid contains a wide array of proteins, peptides, lipids, sugars, enzymes, hormones and RNA molecules, all of which can potentially act as mediators of paternal effects and thus influence offspring development (Crean and Bonduriansky, 2014; Chen et al., 2016; Crean et al., 2016).
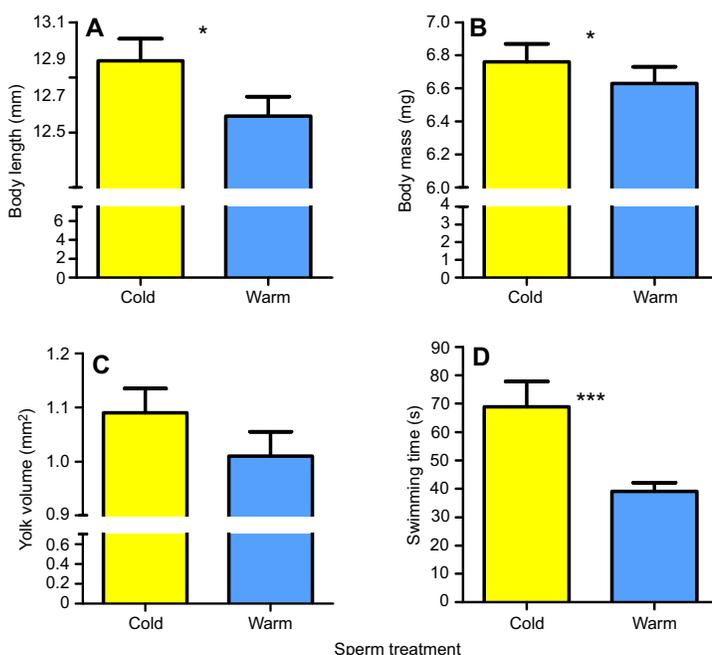
Fig. 4. Measured offspring traits in *C. lavaretus* embryos produced from sperm pre-incubated at two different temperatures (3.5°C and 6.5°C) for 15 h prior to fertilization. The effect (means±s.e.m.) of sperm temperature treatments of 10 males on offspring body length (A), body mass (B), yolk volume (C) and swimming performance (D). Asterisks indicate statistically significant differences between treatments (linear mixed model, **P*<0.05; ***P<0.001).

Finally, even if we found no evidence for differential phenotypic selection of sperm across temperature treatments, we cannot entirely rule out the possibility that offspring phenotypic differences reflect differential selection among sperm genotypes. We also found a significant female×treatment as well as male×female×treatment interaction for fertilization success and embryo mortality. Thus, fertilization success and embryo mortality was not completely random in different sperm treatments, which could potentially contribute to generating differences in offspring phenotype (Burgess and Marshall, 2014). The above-mentioned interactions also indicate that both the effect of female and male–female combinations on fertilization success and embryo mortality was dependent on sperm thermal environment. In other words, females and male–female combinations that produced the most viable offspring when eggs were fertilized with cold-treated sperm did not necessarily have the highest fitness when eggs were fertilized with warm-treated sperm. This indicates that sperm pre-fertilization temperature conditions may shape both the magnitude of dam effects as well as the reproductive (or genetic) compatibility of the gametes (see also Lymbery and Evans, 2013; Sherman et al., 2015). Thus, evolutionary responses of the individuals on water temperature fluctuations may be influenced, and perhaps constrained, by the sperm pre-fertilization environment. The mechanistic basis of this finding needs to be elucidated in future studies.

Irrespective of the underlying mechanism, present results clearly indicate that sperm pre-fertilization thermal environment mediates transgenerational effects for offspring phenotype and performance. This is important as transgenerationally mediated effects of sperm environmental variation can have many important ecological implications, but currently represent a largely unexplored source of variation in offspring phenotypes (Marshall, 2015). Importantly, sperm-mediated transgenerational effects may constitute particularly important mechanisms in determining the adaptation potential of species to climate change (Guillaume et al., 2016). However, the adaptive value of such effects (and TGP in general) has remained unclear, since the impact of these effects for offspring fitness are not always positive (Day and Bonduriansky, 2011; Uller et al., 2013). Sperm environmental plasticity may represent adaptive response, especially in situations where parents (or males) can anticipate the environmental conditions of their offspring, but it may be less common when environmental conditions vary unpredictably (Burgess and Marshall, 2014). Accordingly, sperm environmental plasticity may not be adaptive when environmental conditions show rapid fluctuations (Guillaume et al., 2016, see also Salinas and Munch, 2012, for similar results). In the present study, the duration of sperm thermal treatments was only 15 h, which, along with the above-mentioned findings, may at least partly explain why warmer sperm pre-incubation conditions decreased offspring post-hatching size (i.e. embryonic development rate) and swimming performance. Therefore, our findings indicate that short-term temperature spikes just prior to spawning may have negative impact on offspring fitness in whitefish and, potentially, in many other cold-adapted species. This is important as in the future, sudden temperature alterations are expected to be more common (Thompson et al., 2013).

In conclusion, our results suggest that the sperm pre-fertilization thermal environment modifies embryonic development and swimming performance of the offspring, thus representing an important source of variation in offspring phenotype and fitness. Observed results cannot be explained by male phenotypic manipulation of the sperm or differential maternal investments and are independent of the fertilization environment. Interestingly, we also found no major phenotypic (or viability) differences of sperm between the thermal treatments. Together, our results can have many potentially important ecological implications and can help to understand factors that determine adaptation of organisms to climate change and associated environmental fluctuations. However, further studies are required to determine potential mechanism(s) behind our findings.

Author contributions
Conceptualization: J.K.; Methodology: J.K., P.O., H.H.; Formal analysis: J.K.; Investigation: J.K., P.O., M.J., H.K., R.K., H.H.; Resources: J.K., M.J., H.K., H.H.; Data curation: J.K.; Writing - original draft: J.K.; Writing - review & editing: J.K., P.O., M.J., H.K., R.K., H.H.; Visualization: J.K.; Supervision: J.K., R.K., H.H.; Project administration: J.K., R.K., H.H.; Funding acquisition: J.K.

Data availability
Data from the study have been deposited in Dryad (Kekäläinen et al., 2018) available at: doi:10.5061/dryad.25rg2h3.

References
Alavi, S. M. H. and Cosson, J. (2005). Sperm motility in fishes. I. Effects of temperature and pH: a review. *Cell Biol. Int.* **29**, 101-110.

Alavi, S. M. H. and Cosson, J. (2006). Sperm motility in fishes. (II) Effects of ions and osmolality: a review. *Cell Biol. Int.* **30**, 1-14.

Beaman, J. E., White, C. R. and Seebacher, F. (2016). Evolution of plasticity: mechanistic link between development and reversible acclimation. *Trends Ecol. Evol.* **31**, 237-249.

Blaxter, J. H. S. and Hempel, G. (1963). The influence of egg size on herring larvae (*Clupea harengus* L.). *ICES J. Mar. Sci.* **28**, 211-240.

Bonduriansky, R., Crean, A. J. and Day, T. (2012). The implications of nongenetic inheritance for evolution in changing environments. *Evol. Appl.* **5**, 192-201.

Burgess, S. C. and Marshall, D. J. (2014). Adaptive parental effects: the importance of estimating environmental predictability and offspring fitness appropriately. *Oikos* **123**, 769-776.

Castillo, J., Amaral, A. and Oliva, R. (2014). Sperm nuclear proteome and its epigenetic potential. *Andrology* **2**, 326-338.

Chen, Q., Yan, W. and Duan, E. (2016). Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nat. Rev. Genet.* **17**, 733-743.

Cingi, S., Keinänen, M. and Vuorinen, P. J. (2010). Elevated water temperature impairs fertilization and embryonic development of whitefish *Coregonus lavaretus*. *J. Fish Biol.* **76**, 502-521.

Crean, A. J. and Bonduriansky, R. (2014). What is a paternal effect? *Trends Ecol. Evol.* **29**, 554-559.

Crean, A. J., Dwyer, J. M. and Marshall, D. J. (2012). Fertilization is not a new beginning: the relationship between sperm longevity and offspring performance. *PLoS ONE* **7**, e49167.

Crean, A. J., Dwyer, J. M. and Marshall, D. J. (2013). Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. *Ecology* **94**, 2575-2582.

Crean, A. J., Adler, M. I. and Bonduriansky, R. (2016). Seminal fluid and mate choice: new predictions. *Trends Ecol. Evol.* **31**, 253-255.

Dadras, H., Dzyuba, B., Cosson, J., Golpour, A., Siddique, M. A. M. and Linhart, O. (2017a). Effect of water temperature on the physiology of fish spermatozoon function: a brief review. *Aquacult. Res.* **48**, 729-740.

Dadras, H., Sampels, S., Golpour, A., Dzyuba, V., Cosson, J. and Dzyuba, B. (2017b). Analysis of common carp *Cyprinus carpio* sperm motility and lipid composition using different in vitro temperatures. *Anim. Reprod. Sci.* **180**, 37-43.

Danchin, E. (2013). Avatars of information: towards an inclusive evolutionary synthesis. *Trends Ecol. Evol.* **28**, 351-358.

Danchin, É., Charmantier, A., Champagne, F. A., Mesoudi, A., Pujol, B. and Blanchet, S. (2011). Beyond DNA: integrating inclusive inheritance into an extended theory of evolution. *Nat. Rev. Genet.* **12**, 475-486.

Day, T. and Bonduriansky, R. (2011). A unified approach to the evolutionary consequences of genetic and nongenetic inheritance. *Am. Nat.* **178**, E18-E36.

Donelson, J. M., Munday, P. L., McCormick, M. I. and Pitcher, C. R. (2011). Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nat. Clim. Change* **2**, 30-32.

Evans, J. P., Lymbery, R. A., Wiid, K. S., Rahman, M. M. and Gasparini, C. (2017). Sperm as moderators of environmentally induced paternal effects in a livebearing fish. *Biol. Lett.* **13**, 20170087.

Fenkes, M., Fitzpatrick, J. L., Ozolina, K., Shiels, H. A. and Nudds, R. L. (2017). Sperm in hot water: direct and indirect thermal challenges interact to impact on brown trout sperm quality. *J. Exp. Biol.* **220**, 2513-2520.

Forsman, A. (2015). Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity* **115**, 276-284.

Garcia-Gonzalez, F. and Dowling, D. K. (2015). Transgenerational effects of sexual interactions and sexual conflict: non-sires boost the fecundity of females in the following generation. *Biol. Lett.* **11**, 20150067.

García-González, F. and Simmons, L. W. (2007). Paternal indirect genetic effects on offspring viability and the benefits of polyandry. *Curr. Biol.* **17**, 32-36.

Guillaume, A. S., Monro, K. and Marshall, D. J. (2016). Transgenerational plasticity and environmental stress: do paternal effects act as a conduit or a buffer? *Funct. Ecol.* **30**, 1175-1184.

Huuskonen, H., Haakana, H. and Kekäläinen, J. (2009). Offspring performance is linked to parental identity and male breeding ornamentation in whitefish. *Biol. J. Linn. Soc.* **98**, 532-539.

Immler, S., Hotzy, C., Alavioon, G., Petersson, E. and Arnqvist, G. (2014). Sperm variation within a single ejaculate affects offspring development in Atlantic salmon. *Biol. Lett.* **10**, 20131040.

IPCC (2014). Climate change 2014: synthesis report. In *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. **Core Writing Team**, R. K. Pachauri and L. A. Meyer). Geneva, Switzerland: IPCC.

Jenkins, T. G. and Carrell, D. T. (2013). The sperm epigenome and potential implications for the developing embryo. *Reproduction* **143**, 727-734.

Jensen, L. F., Hansen, M. M., Pertoldi, C., Holdensgaard, G., Mensberg, K.-L. D. and Loeschcke, V. (2008). Local adaptation in brown trout early life-history traits: implications for climate change adaptability. *Proc. R. Soc. B* **275**, 2859-2868.

Jensen, N., Allen, R. M. and Marshall, D. J. (2014). Adaptive maternal and paternal effects: gamete plasticity in response to parental stress. *Funct. Ecol.* **28**, 724-733.

Kause, A., Quinton, C., Airaksinen, S., Ruohonen, K. and Koskela, J. (2011). Quality and production trait genetics of farmed European whitefish, *Coregonus lavaretus*. *J. Anim. Sci.* **89**, 959-971.

Kekäläinen, J., Huuskonen, H., Tuomaala, M. and Kortet, R. (2010). Both male and female sexual ornaments reflect offspring performance in a fish. *Evolution* **64**, 3149-3157.

Kekäläinen, J., Soler, C., Veentaus, S. and Huuskonen, H. (2015). Male investments in high quality sperm improve fertilization success, but may have negative impact on offspring fitness in whitefish. *PLoS ONE* **10**, e0137005.

Kekäläinen J, Oskoei P, Janhunen M, Koskinen H, Kortet R, Huuskonen H (2018) Data from: Sperm pre-fertilization thermal environment shapes offspring phenotype and performance. Dryad Digital Repository. https://doi.org/10.5061/dryad.25rg2h3.

Kortet, R. and Vainikka, A. (2008). Seasonality of innate immunity; evolutionary aspects and latest updates. In *New Research on Innate Immunity* (ed. M. Durand and C. V. Morel), pp. 13-45. New York: Nova Science Publishers, Inc.

Lahnsteiner, F. and Mansour, N. (2012). The effect of temperature on sperm motility and enzymatic activity in brown trout *Salmo trutta*, burbot *Lota lota* and grayling *Thymallus thymallus*. *J. Fish Biol.* **81**, 197-209.

Lahnsteiner, F., Weismann, T. and Patzner, R. A. (1997). Aging processes of rainbow trout semen during storage. *Prog. Fish Cult.* **59**, 272-279.

Lane, M., McPherson, N. O., Fullston, T., Spillane, M., Sandeman, L., Kang, W. X. and Zander-Fox, D. L. (2014). Oxidative stress in mouse sperm impairs embryo development, fetal growth and alters adiposity and glucose regulation in female offspring. *PLoS ONE* **9**, e100832.

Luquet, E. and Tariel, J. (2016). Offspring reaction norms shaped by parental environment: interaction between within- and trans-generational plasticity of inducible defenses. *BMC Evol. Biol.* **16**, 209.

Lymbery, R. A. and Evans, J. P. (2013). Genetic variation underlies temperature tolerance of embryos in the sea urchin *Heliocidaris erythrogramma armigera*. *J. Evol. Biol.* **26**, 2271-2282.

Marshall, D. J. (2015). Environmentally induced (co)variance in sperm and offspring phenotypes as a source of epigenetic effects. *J. Exp. Biol.* **218**, 107-113.

Mehlis, M. and Bakker, T. C. M. (2014). The influence of ambient water temperature on sperm performance and fertilization success in three-spined sticklebacks (*Gasterosteus aculeatus*). *Evol. Ecol.* **28**, 655-667.

Menezo, Y. J. R., Silvestris, E., Dale, B. and Elder, K. (2016). Oxidative stress and alterations in DNA methylation: two sides of the same coin in reproduction. *Reprod. Biomed. Online* **33**, 668-683.

Merilä, J. and Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol Appl.* **7**, 1-14.

Mousseau, T. A. and Fox, C. W. (1998). The adaptive significance of maternal effects. *Trends Ecol. Evol.* **13**, 403-407.

Muñoz, N. J., Farrell, A. P., Heath, J. W. and Neff, B. D. (2015). Adaptive potential of a Pacific salmon challenged by climate change. *Nat. Clim. Change* **5**, 163-166.

Nakagawa, S. and Schielzeth, H. (2013). A general and simple method for obtaining R^2 from generalized linear mixed-effects models. *Methods Ecol. Evol.* **4**, 133-142.

Puri, D., Dhawan, J. and Mishra, R. K. (2010). The paternal hidden agenda: epigenetic inheritance through sperm chromatin. *Epigenetics* **5**, 386-391.

Reinhardt, K., Dobler, R. and Abbott, J. (2015). An ecology of sperm: sperm diversification by natural selection. *Annu. Rev. Ecol. Evol. Syst.* **46**, 435-459.

Ritchie, H. and Marshall, D. J. (2013). Fertilisation is not a new beginning: sperm environment affects offspring developmental success. *J. Exp. Biol.* **216**, 3104-3109.

Rodgers, A. B., Morgan, C. P., Leu, N. A. and Bale, T. L. (2015). Transgenerational epigenetic programming via sperm microRNA recapitulates effects of paternal stress. *Proc. Natl. Acad. Sci. USA* **112**, 13699-13704.

Salinas, S. and Munch, S. B. (2012). Thermal legacies: transgenerational effects of temperature on growth in a vertebrate. *Ecol. Lett.* **15**, 159-163.

Shama, L. N. S., Strobel, A., Mark, F. C. and Wegner, K. M. (2014). Transgenerational plasticity in marine sticklebacks: maternal effects mediate impacts of a warming ocean. *Funct. Ecol.* **28**, 1482-1493.

Sherman, C. D. H., Ab Rahim, E. S., Olsson, M. and Careau, V. (2015). The more pieces, the better the puzzle: sperm concentration increases gametic compatibility. *Ecol. Evol.* **5**, 4354-4364.

Sinclair, B. J., Marshall, K. E., Sewell, M. A., Levesque, D. L., Willett, C. S., Slotsbo, S., Dong, Y., Harley, C. D. G., Marshall, D. J., Helmuth, B. S. et al. (2016). Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? *Ecol. Lett.* **19**, 1372-1385.

Thompson, R. M., Beardall, J., Beringer, J., Grace, M. and Sardina, P. (2013). Means and extremes: building variability into community-level climate change experiments. *Ecol. Lett.* **16**, 799-806.

Uller, T., Nakagawa, S. and English, S. (2013). Weak evidence for anticipatory parental effects in plants and animals. *J. Evol. Biol.* **26**, 2161-2170.

Zajitschek, S., Hotzy, C., Zajitschek, F. and Immler, S. (2014). Short-term variation in sperm competition causes sperm-mediated epigenetic effects on early offspring performance in a zebrafish. *Proc. R. Soc. B* **281**, 20140422.