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Egg size-dependent expression of growth hormone receptor accompanies compensatory growth in fish

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Large egg size usually boosts offspring survival, but mothers have to trade off egg size against egg number. Therefore, females often produce smaller eggs when environmental conditions for offspring are favourable, which is subsequently compensated for by accelerated juvenile growth. How this rapid growth is modulated on a molecular level is still unclear. As the somatotropic axis is a key regulator of early growth in vertebrates, we investigated the effect of egg size on three key genes belonging to this axis, at different ontogenetic stages in a mouthbrooding cichlid (*Simochromis pleurospilus*). The expression levels of one of them, the growth hormone receptor (*GHR*), were significantly higher in large than in small eggs, but remarkably, this pattern was reversed after hatching: young originating from small eggs had significantly higher *GHR* expression levels as yolk sac larvae and as juveniles. *GHR* expression in yolk sac larvae was positively correlated with juvenile growth rate and correspondingly fish originating from small eggs grew faster. This enabled them to catch up fully in size within eight weeks with conspecifics from larger eggs. This is the first evidence for a potential link between egg size, an important maternal effect, and offspring gene expression, which mediates an adaptive adjustment in a relevant hormonal axis.

Keywords: maternal effects; egg size; gene expression; compensatory growth; somatotropic axis; fish

1. INTRODUCTION

There is abundant evidence that females can contribute to the early offspring environment through maternal effects and can thereby influence the survival prospects of offspring [1–5]. Maternal effects are often passed on by the properties of eggs [6,7], and among these, egg size has received by far the greatest attention [8–13]. Egg size is not only easy to quantify, but also often reflects the amount of resources mothers invest in individual offspring [14,15]. Females must trade off an increased egg size against the number of eggs they can produce [16] and hence benefit from producing an egg size that maximizes their fitness in a given environment [9]. It is therefore not surprising that egg size often highly varies both between females of a species and between clutches of the same individual [8,12].

Most often, larger eggs result in larger offspring [17,18]. Size and growth is considered to be particularly important in early life-history stages as juveniles may benefit from reaching size refuges from competition [19], winter-mortality [20] and negative size-selective predation [21]. Therefore, we can expect that hatching from different egg sizes gives rise to different growth trajectories as a result of differentiation in the activity of genes involved in growth. For example, offspring hatched from smaller eggs often grow faster than offspring from larger eggs, and are thereby capable to catch up in size with the latter [22–27], or even overcompensate, so that they reach larger body sizes than offspring hatched from large eggs [28].

Although many studies stress that individual differences in growth are crucial for juvenile survival, so far the underlying molecular mechanisms that regulate growth compensation in juveniles hatched from small eggs have not been explored. A large variation in gene expression among eggs has been reported previously [29], but this study did not explore the possible link between maternal effects and gene expression. Moreover, no one has yet examined how egg-trait-induced differences in gene expression affect offspring phenotype. Hence, the ecological consequences of differential gene expression mediated by maternal effects are unknown.

The somatotropic axis is a key regulator of embryonic and postnatal growth in vertebrates, which makes it a candidate mediator of maternal regulation of offspring growth performance. In this axis, insulin-like growth factor 1 (IGF-1) mediates many growth hormone (GH) effects both as a circulating hormone and as a locally produced growth factor [30,31]. GH is postnatally produced by the pituitary and is the major regulator of growth in vertebrates. Cellular effects of GH are initiated by binding to GHR, a class I cytokine receptor, which triggers the JAK2-STAT signalling cascade leading to the transcriptional activation of mitogenic factors, such as IGF-1

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[30]. GHR, IGF-1 and IGF-2 are all widely produced during embryonic development in fishes and in other vertebrates [32–34].

In this study, we investigate the link between egg size and the expression of genes involved in the control of somatic growth. At the same time, we explore the possible ecological function of gene expression variation in the mouthbrooding cichlid *Simochromis pleurospilus*. We compared gene expression in clutches consisting of either large or small eggs at four stages during early development: within 1 day after fertilization, at an age of 2 days, at the yolk sac stage (age of 8 days) and as juvenile (age of 38 days). At each stage, we compared the transcription levels of growth hormone receptor (*GHR*) and *IGF-1* and *IGF-2* in siblings of representative clutches. Subsequently, we tested for an association between differently expressed genes and the growth of juveniles. To the best of our knowledge, this is the first study investigating if gene expression might be linked to maternal investment and if egg size-induced differences in gene expression may affect offspring phenotype.

2. METHODS

(a) *Study species and animal husbandry*

Simochromis pleurospilus is a maternally mouthbrooding cichlid endemic to Lake Tanganyika, one of the Great Lakes of East Africa. Females incubate their young for about two weeks in the mouth while the larvae consume their yolk sac, followed by a two week period in which the mother releases the young regularly for foraging [35]. Our laboratory stock originates from a population at the southern tip of the lake near Nkumbula Island, Zambia. The juveniles used for the experiment were third generation descendants from wild caught individuals. To obtain clutches, we placed groups of 4–15 females together with 1–5 males in 200 l and 400 l tanks. All fish are kept at a 13 : 11 h L : D regime and at water temperatures of 26.0–28.0°C. The adults were fed twice daily with TetraMin flakes and once a week with a mixture of small, frozen crustaceans. All tanks were equipped with a layer of fine grain sand, biological filters, and flower pot halves, which served as shelters.

(b) *Experimental design*

Twice daily we checked for females with eggs in their mouths to obtain the approximate time of spawning. As soon as possible after fertilization, the eggs were taken from the females by slightly pressing their cheeks. A female was fin-clipped before she was placed back in her group tank to prevent us from taking more than one clutch of the same female. All eggs were weighed individually on an electronic balance to the nearest 0.1 mg. Before weighing, the eggs were placed on a moistened cotton pad so that excess water could run off the egg. We selected clutches for this study with a mean egg weight smaller than 17.0 mg ('small eggs') and larger than 19.0 mg ('large eggs'). Egg size variance is larger between clutches than within clutches in *S. pleurospilus* (F. H. I. D. Segers & B. Taborsky 2011, unpublished data). As a result, all eggs from a clutch fell in the same size class in all cases. As it was impossible to manipulate egg size in these fish, we collected clutches with large and small eggs from different females, making it impossible to control for maternal genetic background (see §4). In most cases, multiple clutches were collected from the same breeding tank. Often, clutches from the same breeding tank fell in both egg size classes (see electronic supplementary material, table S1), indicating that egg size and the corresponding gene expression pattern were not simply caused by a tank effect. In addition, we corrected for breeding tank in our statistical models (see below) to exclude any confounding tank effects.

We collected 17 clutches in the small egg size class with a range of 11.0–16.9 mg and 13 clutches in the large egg size class with a range of 19.9–25.8 mg mean egg weight. This represents the outer ends of the long-term egg-size distribution observed in our laboratory population of *S. pleurospilus* (i.e. 7.7–25.8 mg, with egg sizes below 11.0 mg being rare).

We randomly sampled one egg or hatchling per clutch at four stages during early ontogeny, whereas the remaining siblings were raised to obtain further growth measurements. Sample 1 was taken within 24 h of fertilization and always immediately after weighing the eggs. We placed one egg in RNA*later* (Ambion, USA) and left it overnight at −4°C. The next day, we transferred the sample to a freezer where it was stored at −20°C until analysis. The remaining eggs were hatched in a self-constructed cichlid egg tumbler designed to raise individual eggs (for methods see [18]). Sample 2 was taken approximately 48 h after fertilization. The egg was preserved as described for the first sample. We noted the time to hatch of the remaining eggs, which takes approximately 5 days in *S. pleurospilus*. On day 8 after fertilization, we sampled one yolk sac larva (sample 3). Before preservation, we measured the total length of the larvae under a binocular with a measuring eye piece. Larvae were anaesthetized in MS-222 (Sigma-Aldrich, Switzerland), sacrificed and immediately preserved as described above for the eggs. The next day the remaining hatchlings were placed in a net cage (16.5 × 12 × 13.5 cm) that was fixed at the surface of a 25 l tank. From then on, the fish were exposed to our general husbandry conditions (see above). The number of siblings that survived the egg tumbler varied between clutches (see electronic supplementary material, table S1), but did not differ between egg size classes (*t*-test, $t = 0.79$, $N_S = 13$, $Ncs_L = 10$, $p = 0.43$). No juveniles died after they were removed from the egg tumbler, apart from the individuals we sampled for the gene expression analysis. On day 14 after fertilization (corresponding to the age when mothers start releasing young periodically for foraging), we released the young into the 25 l tank and fed them 6 days a week ad libitum with TetraMin flakes. Food remains were removed regularly to prevent the deterioration of water quality. Sample 4 was taken on day 38 after fertilization. This sampling age was chosen because our previous experiments showed that compensatory growth occurs during this life stage [18].

On days 28, 42 and 56 after fertilization, we measured the standard length to the nearest 0.1 mm under a binocular microscope of the siblings that had not been sampled for gene expression analysis. We took the mean of these measurements for each clutch to calculate the specific growth rate (SGR) from day 28 to 56 as follows:

$$\mathrm{SGR}(\% \times \mathrm{d}^{-1}) = \frac{(\ln x_2 - \ln x_1)}{(\mathrm{age}_2 - \mathrm{age}_1)} \times 100,$$

where x_1, x_2, age_1 and age_2 are initial and final mean standard lengths and corresponding ages of the fish at two successive measurements.

(c) *RNA extraction and real-time PCR*

Gene expression was measured using the whole organism. Total RNA was extracted using TRIzol reagent (Invitrogen, Merelbeke, Belgium) according to the manufacturer's instructions. In short, the samples preserved in RNA*later* were homogenized in 100 µl TRIzol using TissueLyser II (Quiagen) homogenizer with glass beads. RNA was precipitated by 50 per cent isopropanol, washed with ethanol and air dryed. The pellet was resuspended in RNase-free water and quantified by photospectrometry. DNase digestion was performed with 1 U of RQ1 RNase-free DNase (Catalys AG, Switzerland). Single stranded cDNA was synthesized from 800 ng total RNA using TaqMan Reverse Transcription Reagents (Applied Biosystems, Rotkreuz, Switzerland) according to manufacturer's instructions in total amount of 80 µl. Two microlitres of this cDNA was subjected, in triplicates, to real-time PCR using FastStart Universal Probe Master (Rox) (Roche, Switzerland) with the addition of 300 nM of each primer and 150 nM of the fluorogenic probe. Amplification was performed in a reaction volume of a 10 µl in MicroAmp Fast Optical 96-Well Reaction Plate (Applied Biosystems, Rotkreuz, Switzerland). Real-time PCR runs were carried out using ABI 7500 Fast Real-Time PCR System (Applied Biosystems) with the following conditions: 95°C for 10 min of the enzyme activation followed by 40 cycles of 95°C for 15 s and 60°C for 1 min.

(d) *Design of primers and probes for quantitative real-time PCR*

Available sequences from related African cichlids were used to create TaqMan real-time PCR assays for the genes in consideration (see electronic supplementary material, table S2). TaqMan probes were labelled at the 5′ end with fluorescent reporter dye fluorescein (FAM) and at the 3′ end with the quencher TAMRA. We investigated the expression of three key genes of the GH–IGF axis, namely *GHR*, *IGF-1* and *IGF-2* (see electronic supplementary material, table S2), but we did not include *GH* as *GH* transcripts were not detected outside the pituitary neither in *S. pleurospilus* (this study) nor in another cichlid fish, *Oreochromis niloticus* [36]. Three housekeeping genes were tested as references for the quantification: ribosomal 18S (*R18S*, *Oreochromis mossambicus*), *β-Actin* (*O. niloticus*) and the elongation factor 1 alpha (*EF1α*, *O. niloticus*). A comparison of these three reference genes showed that *R18S* has the most stable expression pattern across the experimental groups. Therefore, *R18S* was chosen as a reference for qPCR data normalization. The target genes were: *GHR 1* (*O. niloticus*), *IGF-1* (*O. niloticus*), and *IGF-2* (*O. niloticus*).

(e) *Relative quantification of treatment effects*

Differences in gene expression between small and large egg classes were evaluated using the relative gene expression model ($\Delta\Delta C_T$ method) [37]. Prior to analysis, amplification efficiencies of all qPCR assays were validated by plotting of C_T values of the dilution series of the DNA template against the logarithms of the dilution factors. Comparison of the slopes confirmed that the efficiencies of the compared assays were very similar [38]. Statistical analysis was performed on the ΔC_T values of each sample, and the data distribution is presented as a scatter plot of these values. As a higher ΔC_T value means a lower expression of a given gene, the axes of the plots were reversed for clarity in the presentations. Relative gene expression ratios (R) were calculated using the formula: $R = 2^{-\Delta\Delta C_T}$ with $\Delta C_T = C_T$ (target gene) $- C_T$ (reference gene), with $\Delta\Delta C_T = \Delta C_T$ (large egg class) $- \Delta C_T$ (small egg class). Thus, the reported R values represent n-fold ratios of gene expression levels between small and large egg classes (see electronic supplementary material, figure S1). In some samples, the gene expression analysis failed for unknown reasons, and we ended up with varying sample sizes for the analysis of the three target genes (see electronic supplementary material, table S1).

(f) *Statistical analysis*

All analyses were done in R v. 2.9.2 [39]. Exploratory data analysis revealed that the variance in gene expression among the samples collected within day 1 after fertilization (sample 1) was high compared to the other sampling days for all three genes (see §3). Therefore, we analysed sample 1 separately: gene expression levels of large and small eggs were compared with a general linear mixed-effects (LME) model, with breeding tank as a random effect to control for non-independence. To test for an effect of egg size and sampling day on gene expression among samples 2, 3 and 4, we used an LME, with clutch nested within breeding tank (see electronic supplementary material, table S1) as a random effect term. The interaction between egg size class and sampling day was tested by fitting two models, one with and one without the interaction, and subsequently comparing the two models using a likelihood ratio test [40]. Non-significant interactions were removed from the model. The effect of egg size on larva length was tested using an LME model with breeding tank as a random effect. The effect of egg size on juvenile growth was tested using LME models with clutch nested within breeding tank as random effect term and with experimental day as a continuous variable. We adjusted significance levels using the sequential Bonferroni correction when we did multiple pairwise comparisons. All significant p-values remained significant after correction. The relationships between the expression levels of the three considered genes and between gene expression and growth were explored by Spearman rank correlations.

3. RESULTS

(a) *Gene expression*

In the sample collected within one day after fertilization (sample 1), expression levels did not differ between the large and the small egg size classes for *GHR* (LME, $n = 13$, $t = -1.62$ $p = 0.17$), *IGF-1* (LME, $n = 15$, $t = -2.14$, $p = 0.07$) or *IGF-2* (LME, $n = 19$, $t = -1.32$, $p = 0.22$). It is noteworthy that mRNA quantities were highly variable in this sample for all three genes (figure 1*a–c*). This variance may largely result from the fact that the interval between spawning (i.e. fertilization) and sampling of eggs varied considerably (range: 2.0–26.5 h), and thus embryonic transcription might have started already in some eggs, but not in others.

There was a significant decrease in *GHR* expression with sampling day for the hatchlings from large eggs (LME, $N = 45$, egg size class × sampling day: $L = 33.13$, $p < 0.001$). This resulted in large eggs having a higher level of *GHR* gene transcription than small eggs on day 2, while on day 8 and day 38 (thus after hatching), juveniles from small eggs had a higher *GHR* gene transcription than juveniles from large eggs (pairwise comparisons,

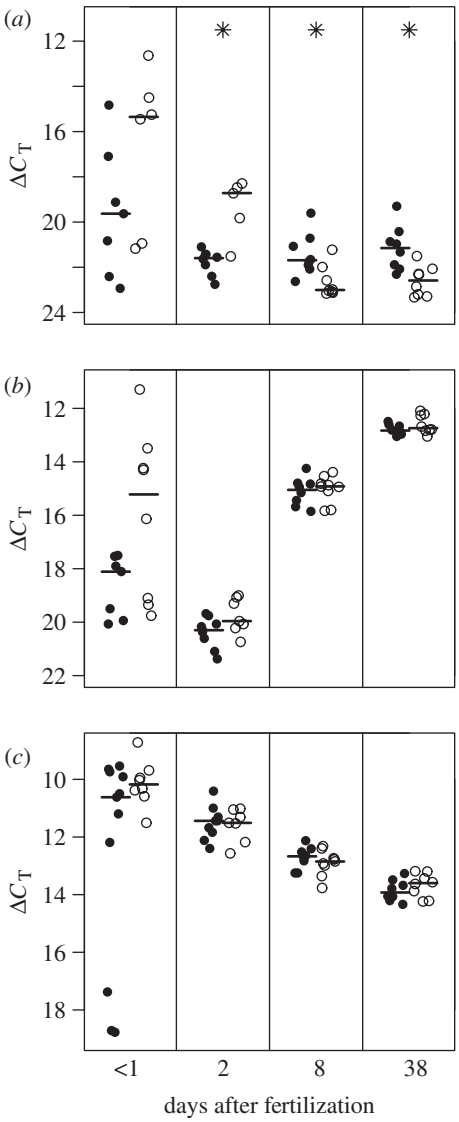

Figure 1. Results of the mRNA quantification of (*a*) *GHR*, (*b*) *IGF-1* and (*c*) *IGF-2*. The four panels of each graph represent samples taken at days 1, 2, 8 and 38 after fertilization, from left to right. Filled circles: small-egg size class; open circles: large-egg size class; horizontal lines show the medians. Significant differences in gene transcription levels between egg size classes are indicated by asterisks. Note that a higher ΔC_T value means a lower expression of a given gene.

$t = -4.92$, $p = 0.004$, $t = 2.84$, $p = 0.01$ and $t = 3.40$, $p = 0.005$, respectively). Sampling day as a main effect was not significant (pairwise comparisons, all $p > 0.10$).

Overall, *IGF-1* expression tended to be higher in large eggs than in small eggs (LME, $n = 49$, $t = -2.14$, $p = 0.052$; figure 1*b*). *IGF-1* expression increased with sampling day (pairwise comparisons, day 2 versus 8: $t = -30.77$, $p < 0.001$ and day 8 versus 38: $t = -14.68$, $p < 0.001$), but this increase did not differ between egg size classes (egg size class × sampling day: $L = 2.61$, $p = 0.27$).

There was no effect of egg size class on *IGF-2* expression (LME, $n = 49$, egg size class: $t = 0.09$, $p = 0.93$ and egg size class × sampling day: $L = 1.66$, $p = 0.44$; figure 1*c*). Overall *IGF-2* transcription decreased with sampling day (pairwise comparisons, day 2 versus 8: $t = 7.84$, $p < 0.001$ and day 8 versus 38: $t = 5.91$, $p < 0.001$).

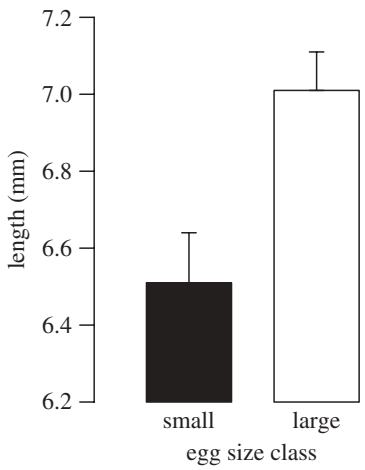

Figure 2. Mean total length of larvae + s.e. at day 8 after fertilization.

As GHR signalling influences *IGF-1* and *IGF-2* transcription in fish [41–43], we examined the correlations between the expression levels of *GHR* and *IGFs*. In both samples taken before hatching, the quantities of *GHR* and *IGF-1* mRNA were positively correlated (sample 1: $n = 13$, $r_s = 0.75$, $p = 0.005$; sample 2: $n = 13$, $r_s = 0.72$, $p = 0.005$). In contrast, after hatching the relationship between the expression levels of the two genes had vanished (sample 3: $n = 16$, $r_s = 0.06$, $p = 0.83$; sample 4: $n = 16$, $r_s = 0.25$, $p = 0.34$). The expression levels of *GHR* and *IGF-2* were not correlated on any of the sampling days (sample 1: $n = 12$, $r_s = -0.02$, $p = 0.96$; sample 2: $n = 13$, $r_s = -0.02$, $p = 0.95$; sample 3: $n = 16$, $r_s = 0.28$, $p = 0.30$; sample 4: $n = 16$, $r_s = 0.06$, $p = 0.83$).

(b) *Growth*

On day 8 after fertilization larvae hatched from large eggs were significantly larger than larvae hatched from small eggs (LME, $n = 24$, $t = 3.08$, $p = 0.008$; figure 2). Because of the considerable noise in the gene expression data of sample 1 (see above), we did not test for relationships between gene expression levels in this sample and later larval size or growth. Two days after spawning (sample 2), gene expression levels in the eggs were not related to body size of larvae from the same clutch on day 8 after fertilization for either *GHR* ($n = 10$, $r_s = -0.18$, $p = 0.62$), *IGF-1* ($n = 12$, $r_s = 0.37$, $p = 0.21$) or *IGF-2* ($n = 12$, $r_s = 0.01$, $p = 0.96$).

We monitored juvenile growth between day 28 and 56 after fertilization. At the beginning of this period, the juveniles from large-egg clutches were significantly larger than those from small-egg clutches (LME, $n = 207$, egg size class: $t = 2.87$, $p = 0.01$). Naturally, both groups increased in size during this period (LME, $n = 207$, experimental day: $t = 51.82$, $p < 0.001$). However, the small egg size class grew significantly faster (experimental day × egg size class: $L = 4.07$, $p = 0.04$; figure 3). Consequently, there was no significant difference in size between juveniles from large and small eggs at day 56 after fertilization (LME, $n = 55$, $t = 0.75$, $p = 0.47$).

As gene expression levels differed between egg sizes only for the *GHR* gene, we restricted our analysis to this gene when testing for a relationship between

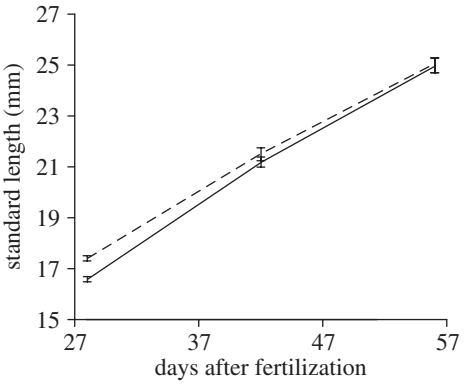

Figure 3. Growth trajectory for juvenile standard length. The mean ± s.e. of the small egg size class and the large egg size class are shown by the solid and the dashed line, respectively.

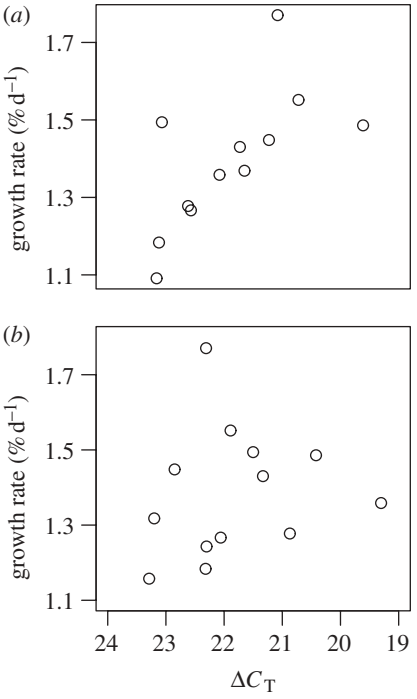

Figure 4. Relationship between *GHR* gene expression level of an individual on (*a*) day 8 and (*b*) day 38 and the mean growth rate of its siblings between day 28 and 56 after fertilization.

expression levels and growth. *GHR* expression on day 8 after fertilization (as measured in one sibling per clutch) was positively correlated with the growth rate of the remaining siblings between day 28 and day 56 ($n = 12$, $r_s = -0.66$, $p = 0.007$; figure 4*a*). In contrast, *GHR* expression levels on day 38 after fertilization were not related to sibling growth rates between 28 and 56 days of age ($n = 13$, $r_s = -0.35$, $p = 0.25$; figure 4*b*).

4. DISCUSSION

Fish hatched from small eggs expressed higher levels of *GHR* and grew faster, which enabled them to catch up in body size with conspecifics hatched from large eggs within 8 weeks after fertilization. These results suggest that differences in maternal egg provisioning can mediate differential gene expression programmes in offspring. This study is the first to investigate if differences in gene expression are associated with variance in egg size. Moreover, our growth results suggest an ecological function of the differences in gene expression [44], namely, that it allows young to compensate during early ontogeny for a lower maternal investment in egg size.

Interestingly, of the three genes of the somatotropic axis analysed in this study, only *GHR* mRNA levels differed with egg size, suggesting an IGF-independent action of GH. The binding of GH to its receptor leads to an increase in GHR density and subsequent increase in cellular sensitivity for GH, which can then promote enhanced growth [45,46]. Previously a strong association between the transcription of *GHR* and *IGF-1* was detected, suggesting that a higher GHR density may mediate a higher *IGF-1* transcription [46]. In our samples, the expression of *GHR* and *IGF-1* was only significantly correlated at the two sampling stages taken before the hatching of the larvae. Although IGF-1 is considered to be the main mediator of somatotropic actions of GH [31], IGF-1-independent GH function has also been characterized [47]. One of the effects of GH is the ability to promote lipid metabolism [48]. Interestingly, this function of GH is independent as well as antagonistic to the lipogenic properties of IGF-1 [31]. This suggests that an elevation of GHR signalling might enhance the utilization of yolk in faster growing larvae. Moreover, in the post-hatching stages, a higher sensitivity to GH through a higher receptor density might serve to increase appetite and foraging activity [49].

Growth compensation during early life-history stages is commonly observed in fishes [20,50,51]. A large size is thought to be highly beneficial during early life history as it reduces negative size-dependent mortality [21,52] and increases larval competitive abilities [53]. If being larger has clear benefits, then why do juveniles hatched from large eggs not grow faster? There is abundant evidence showing that substantial costs arise from fast growth, for example, various physiological costs and an enhanced predation risk because of increased activity [54]. Thus, the abovementioned benefits of a large size have to be traded off against the costs of fast growth, and it is conceivable that the benefits of fast growth only outweigh the costs for individuals that hatched from small eggs.

Expression levels of *GHR* were higher in the small-egg clutches already at an age when the larvae still have yolk reserves (day 8) and do not yet take up external food. It is hence unlikely that the larvae were able to predict future food abundance. It has been shown, however, that female *S. pleurospilus* produce smaller eggs when the expected food availability for the young is high [55]. Thus, it is tempting to speculate that the upregulated *GHR* gene in larvae from smaller eggs might enable individuals hatched from small eggs to use the expected high food abundance more efficiently. Maternal diet appears to serve as a cue to predict the offspring rearing environment in a broad range of taxa, which usually triggers the production of smaller eggs [25,27,55,56], and/or of faster post-natal growth [57] when the offspring environment is predicted to be favourable. Possibly, mothers anticipating

favourable conditions for their offspring can only afford to reduce egg size if a hormonal mechanism exists to result in an enhanced growth of young hatching from small eggs. We propose that a higher *GHR* expression is a promising candidate for such a mechanism. As we did not control for early maternal nutrition, we cannot be entirely sure if the egg sizes of our sampled clutches were environmentally induced or genetically determined. A long-term experiment tracking gene expression and growth for several generations under different environmental conditions would clarify whether maternal nutrition can indirectly influence offspring growth through differences in egg size.

GHR expression at 8 days after fertilization correlated positively with the average growth rates during the second month of life. Surprisingly, however, gene expression at 38 days after fertilization was not correlated with growth during this period, although an effect of egg size on *GHR* expression was still present. Furthermore, gene expression at day 8 and day 38 did not correlate (Spearman rank correlation, $n = 10$, $r_s = 0.12$, $p = 0.76$). In general, maternal effects are thought to be most important in the earliest life stages, whereas with advancing age, other factors, such as the offspring's genotype and its current environment, are assumed to become more important [1,27,58,59]. Thus, the expression of *GHR* may start to be influenced by these other factors at day 38. More gene expression samples and growth measurements during the later life of the juveniles would be needed to see how long the effect of egg size on gene expression persists and if *GHR* expression is still able to affect growth during later life.

We propose three possible mechanisms for the enhanced *GHR* expression in young hatched from small eggs that are not mutually exclusive: (i) a maternal signal might have altered the activity state of embryonic genes. For example, maternal transcripts of *GH*, *GHR*, *IGF-1* and *IGF-2* have been detected in the unfertilized eggs and early embryos of various fish species [60–64], but their role is not well understood. It is unlikely, however, that a maternal signal consisting of *GHR* mRNA is responsible for faster growth in small-egg young because before hatching *GHR* transcripts were more abundant in large eggs. Possibly, a maternal signal might have been produced by genes not quantified in this study. For example, insulin and thyroid hormones are known to modulate *GHR* expression and function [65], and thyroid hormones are found in the unfertilized eggs of numerous fish species [66]. To test whether maternal signals can explain the differential *GHR* expression observed in our study, future studies should compare the quantities of maternally derived hormones and transcripts (e.g. by transcriptome analysis) that are present in eggs of different sizes *before* the onset of embryonic transcription. This requires the analysis of freshly spawned eggs within a few hours after spawning because, for example, in the cichlid fish *O. niloticus*, embryonic transcription has been estimated to start within 8 to 12 hours after fertilization [67]). These analyses could be initial steps in exploring which maternal mRNAs and hormones in the egg are potential candidates for transgenerational cues, which upregulate *GHR* expression in larvae. (ii) Alternatively, yolk quantity itself might have acted as a signal influencing juvenile growth. For example, sea urchins develop more slowly when hatched from experimentally yolk-reduced eggs [68]. To distinguish this possibility from the aforementioned mechanism, an experiment in which yolk is extracted from eggs [69] should be performed. A mechanism by which juveniles can control their growth gene expression independently of maternal cues might allow them to respond more flexibly to environmental conditions which require further adjustment of juvenile growth trajectories. For example, juveniles have been observed to grow slower when food is limited [35] and to grow faster when predation risk is higher [70]. However, a maternal cue might be preferable if mothers have more reliable information about the environment in which their young will grow [55,71]. (iii) Finally, the regulation of *GHR* gene expression might be an epigenetic effect, where chromosomal regions of offspring are structurally adapted to propagate an altered activity state of the gene, for example through DNA methylation or histone modification [72,73]. One should note that this mechanism could be a consequence of the first two mechanisms: changes in methylation marks might be induced by a maternal signal or by larval sampling of yolk quantity. Thus experiments in which the maternal signals have been identified (e.g. by transcriptome analysis) or in which the yolk amount was manipulated could be followed up by a study which quantifies the methylation of the promoter regions upstream of the *GHR* gene.

(a) *Conclusions*

The significance of egg properties and particularly egg size for life-history evolution has been studied extensively. However, the potential influence of egg size on offspring gene expression has been ignored so far. Here, we investigate gene expression patterns in relation to egg size. We detected an enhanced expression of *GHR* in *S. pleuropilus* juveniles originating from smaller eggs, and higher *GHR* mRNA levels were correlated to faster growth, such that juveniles hatching from small eggs fully compensated for their initial size disadvantage. Hitherto mechanisms of maternal effects have been studied mainly by quantifying deposited and circulating hormone levels in eggs and young. Our results demonstrate the importance of scrutinizing an additional level, the transcription of hormones and particularly their receptors, which ultimately determine tissue sensitivity to secreted hormones. Moreover, our study highlights the ecological and evolutionary significance of differential gene expression in relation to maternal effects.

The experiments carried out for this study conformed to the legal requirements of Switzerland (license number 57/07 and 21/08).

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REFERENCES

1 Bernardo, J. 1996 The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. *Am. Zool.* **36**, 216–236. (doi:10.1093/icb/36.2.216)

2 Mousseau, T. A. & Fox, C. W. 1998 *Maternal effects as adaptations*. Oxford, UK: Oxford University Press.
3 Fox, C. W. & Czesak, M. E. 2000 Evolutionary ecology of progeny size in arthropods. *Annu. Rev. Entomol.* **45**, 341–369. (doi:10.1146/annurev.ento.45.1.341)
4 Marshall, D. J. & Uller, T. 2007 When is a maternal effect adaptive? *Oikos* **116**, 1957–1963. (doi:10.1111/j.2007.0030-1299.16203.x)
5 Marshall, D. J., Allen, R. M. & Crean, A. J. 2008 The ecological and evolutionary importance of maternal effects in the sea. *Oceanogr. Mar. Biol.* **46**, 203–250. (doi:10.1201/9781420065756.ch5)
6 Eising, C. M., Eikenaar, C., Schwabl, H. & Groothuis, T. G. G. 2001 Maternal androgens in black-headed gull (*Larus ridibundus*) eggs: consequences for chick development. *Proc. R. Soc. Lond. B* **268**, 839–846. (doi:10.1098/rspb.2001.1594)
7 Uller, T., Astheimer, L. & Olsson, M. 2007 Consequences of maternal yolk testosterone for offspring development and survival: experimental test in a lizard. *Funct. Ecol.* **21**, 544–551. (doi:10.1111/j.1365-2435.2007.01264.x)
8 Fox, C. W., Thakar, M. S. & Mousseau, T. A. 1997 Egg size plasticity in a seed beetle: an adaptive maternal effect. *Am. Nat.* **149**, 149–163. (doi:10.1086/285983)
9 Einum, S. & Fleming, I. A. 1999 Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proc. R. Soc. Lond. B* **266**, 2095–2100. (doi:10.1098/rspb.1999.0893)
10 Cunningham, E. J. A. & Russell, A. F. 2000 Egg investment is influenced by male attractiveness in the mallard. *Nature* **404**, 74–77. (doi:10.1038/35003565)
11 Svensson, E. & Sinervo, B. 2000 Experimental excursions on adaptive landscapes: density-dependent selection on egg size. *Evolution* **54**, 1396–1403. (doi:10.1111/j.0014-3820.2000.tb00571.x)
12 Kudo, S. 2001 Intraclutch egg-size variation in acanthosomatid bugs: adaptive allocation of maternal investment? *Oikos* **92**, 208–214. (doi:10.1034/j.1600-0706.2001.920202.x)
13 McCormick, M. I. 2003 Consumption of coral propagules after mass spawning enhances larval quality of damselfish through maternal effects. *Oecologia* **136**, 37–45. (doi:10.1007/s00442-003-1247-y)
14 Brown, R. W. & Taylor, W. W. 1992 Effects of egg composition and prey density on the larval growth and survival of lake whitefish (*Coregonus clupeaformis* Mitchill). *J. Fish Biol.* **40**, 381–394. (doi:10.1111/j.1095-8649.1992.tb02585.x)
15 Kamler, E. 2005 Parent–egg–progeny relationships in teleost fishes: an energetic perspective. *Rev. Fish Biol. Fish.* **15**, 399–421. (doi:10.1007/s11160-006-0002-y)
16 Smith, C. C. & Fretwell, S. D. 1974 The optimal balance between size and number of offspring. *Am. Nat.* **108**, 499–506. (doi:10.1086/282929)
17 Hutchings, J. A. 1991 Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution* **45**, 1162–1168. (doi:10.2307/2409723)
18 Segers, F. H. I. D. & Taborsky, B. 2011 Egg size and food abundance interactively affect juvenile growth and behaviour. *Funct. Ecol.* **25**, 166–167. (doi:10.1111/j.1365-2435.2010.01790.x)
19 Arendt, J. D. & Wilson, D. S. 1997 Optimistic growth: competition and an ontogenetic niche-shift select for rapid growth in pumpkinseed sunfish (*Lepomis gibbosus*). *Evolution* **51**, 1946–1954. (doi:10.2307/2411015)
20 Bertram, D. F., Chambers, R. C. & Leggett, W. C. 1993 Negative correlations between larval and juvenile growth rates in winter flounder: implications of compensatory growth for variation in size-at-age. *Mar. Ecol-Prog. Ser.* **96**, 209–215. (doi:10.3354/meps096209)
21 Sogard, S. M. 1997 Size-selective mortality in the juvenile stage of teleost fishes: a review. *Biol. Mar. Sci.* **60**, 1129–1157.
22 Eldridge, M. B., Whipple, J. A. & Bowers, M. J. 1982 Bioenergetics and growth of striped bass, *Morone saxatilis*, embryos and larvae. *Fish B-NOAA* **80**, 461–474.
23 Semlitsch, R. D. & Gibbons, J. W. 1990 Effects of egg size on success of larval salamanders in complex aquatic environments. *Ecology* **71**, 1789–1795. (doi:10.2307/1937586)
24 Parichy, D. M. & Kaplan, R. H. 1992 Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. *Oecologia* **91**, 579–586. (doi:10.1007/BF00650334)
25 Rotem, K., Agrawal, A. A. & Kott, L. 2003 Parental effects in *Pieris rapae* in response to variation in food quality: adaptive plasticity across generations? *Ecol. Entomol.* **28**, 211–218. (doi:10.1046/j.1365-2311.2003.00507.x)
26 Wagner, E. C. & Williams, T. D. 2007 Experimental (antiestrogen mediated) reduction in egg size negatively affects offspring growth and survival. *Physiol. Biochem. Zool.* **80**, 293–305. (doi:10.1086/512586)
27 Donelson, J. M., Munday, P. L. & McCormick, M. I. 2009 Parental effects on offspring life histories: when are they important? *Biol. Lett.* **5**, 262–265. (doi:10.1098/rsbl.2008.0642)
28 Heath, D. D., Fox, C. W. & Heath, J. W. 1999 Maternal effects on offspring size: variation through early development in Chinook salmon. *Evolution* **53**, 1605–1611. (doi:10.2307/2640906)
29 Diz, A. P., Dudley, E., MacDonald, B. W., Piña, B., Kenchington, E. L. R., Zouros, E. & Skibinski, D. O. F. 2009 Genetic variation underlying protein expression in eggs of the marine mussel (*Mytilus edulis*). *Mol. Cell Proteom.* **8**, 132–144. (doi:10.1074/mcp.M800237-MCP200)
30 Le Roith, D., Bondy, C., Yakar, S., Liu, J. L. & Butler, A. 2001 The somatomedin hypothesis: 2001. *Endocr. Rev.* **22**, 53–74. (doi:10.1210/er.22.1.53)
31 Reinecke, M., Björnsson, B. T., Dickhoff, W. W., McCormick, S. D., Navarro, I., Power, D. M. & Gutiérrez, J. 2005 Growth hormone and insulin-like growth factors in fish: where we are and where to go. *Gen. Comp. Endocr.* **142**, 20–24. (doi:10.1016/j.ygcen.2005.01.016)
32 Sanders, E. J. & Harvey, S. 2004 Growth hormone as an early embryonic growth and differentiation factor. *Anat. Embryol.* **209**, 1–9. (doi:10.1007/s00429-004-0422-1)
33 Gicquel, C. & Le Bouc, Y. 2006 Hormonal regulation of fetal growth. *Horm. Res.* **65**, 28–33. (doi:10.1159/000091503)
34 Wood, A. W., Duan, C. & Bern, H. A. 2005 Insulin-like growth factor signalling in fish. *Int. Rev. Cytol.* **243**, 215–285. (doi:10.1016/S0074-7696(05)43004-1)
35 Taborsky, B. 2006 The influence of juvenile and adult environments on life-history trajectories. *Proc. R. Soc. B* **273**, 741–750. (doi:10.1098/rspb.2005.3347)
36 Caelers, A., Maclean, N., Hwang, G., Eppler, E. & Reinecke, M. 2005 Expression of endogenous and exogenous growth hormone (GH) messenger (m) RNA in a GH-transgenic tilapia (*Oreochromis niloticus*). *Transgenic Res.* **14**, 95–104. (doi:10.1007/s11248-004-5791-y)
37 Livak, K. J. & Schmittgen, T. D. 2001 Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods* **25**, 402–408. (doi:10.1006/meth.2001.1262)

38 Caelers, A., Berishvili, G., Meli, M. L., Eppler, E. & Reinecke, M. 2004 Establishment of a real-time RT-PCR for the determination of absolute amounts of IGF-I and IGF-II gene expression in liver and extrahepatic sites of the tilapia. *Gen. Comp. Endocr.* **137**, 196–204. (doi:10.1016/j.ygcen.2004.03.006)

39 R Development Core Team. 2009 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

40 Faraway, J. J. 2006 *Extending the linear model with R* 1st edn. Boca Raton, FL: Chapman & Hall.

41 Tse, M. C., Vong, Q. P., Cheng, C. H. & Chan, K. M. 2002 PCR-cloning and gene expression studies in common carp (*Cyprinus carpio*) insulin-like growth factor-II. *Biochim. Biophys. Acta* **1575**, 63–74. (doi:10.1016/S0167-4781(02)00244-0)

42 Peterson, B. C., Waldbieser, G. C. & Bilodeau, L. 2005 Effects of recombinant bovine somatotropin on growth and abundance of mRNA for IGF-I and IGF-II in channel catfish (*Ictalurus punctatus*). *J. Anim. Sci.* **83**, 816–824.

43 Ponce, M., Infante, C., Funes, V. & Manchado, M. 2008 Molecular characterization and gene expression analysis of insulin-like growth factors I and II in the red-banded seabream, *Pagrus auriga*: transcriptional regulation by growth hormone. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **150**, 418–426. (doi:10.1016/j.cbpb.2008.04.013)

44 Aubin-Horth, N. & Renn, S. C. P. 2009 Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Mol. Ecol.* **18**, 3763–3780. (doi:10.1111/j.1365-294X.2009.04313.x)

45 Gabillard, J., Yao, K., Vandeputte, M., Gutierrez, J. & Le Bail, P. 2006 Differential expression of two GH receptor mRNAs following temperature change in rainbow trout (*Oncorhynchus mykiss*). *J. Endocrinol.* **190**, 29–37. (doi:10.1677/joe.1.06695)

46 Picha, M. E., Turano, M. J., Tipsmark, C. K. & Borski, R. J. 2008 Regulation of endocrine and paracrine sources of IGFs and GH receptor during compensatory growth in hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). *J. Endocrinol.* **199**, 81–94. (doi:10.1677/JOE-07-0649)

47 Kaplan, S. A. & Cohen, P. 2007 The somatomedin hypothesis 2007: 50 years later. *J. Clin. Endocrinol. Metab.* **92**, 4529–4535. (doi:10.1210/jc.2007-0526)

48 Vijayakumar, A., Novosyadlyy, R., Wu, Y., Yakar, S. & LeRoith, D. 2009 Biological effects of growth hormone on carbohydrate and lipid metabolism. *Growth Horm. IGF Res.* **20**, 1–7. (doi:10.1016/j.ghir.2009.09.002)

49 Björnsson, B. T. 2002 Growth hormone endocrinology of salmonids: regulatory mechanism and mode of action. *Fish Physiol. Biochem.* **27**, 227–242. (doi:10.1023/B:FISH.0000032728.91152.10)

50 Chambers, R. C., Leggett, W. C. & Brown, J. A. 1988 Variation in and among early life-history traits of laboratory-reared winter flounder *Pseudopleuronectes americanus*. *Mar. Ecol-Prog. Ser.* **47**, 1–15. (doi:10.3354/meps047001)

51 Gagliano, M. & McCormick, M. I. 2007 Compensating in the wild: is flexible growth the key to early juvenile survival? *Oikos* **116**, 111–120. (doi:10.1111/j.2006.0030-1299.15418.x)

52 Gislason, H., Daan, N., Rice, J. C. & Pope, J. G. 2010 Size, growth, temperature and the natural mortality of marine fish. *Fish Fish.* **11**, 149–158. (doi:10.1111/j.1467-2979.2009.00350.x)

53 Marshall, D. J., Cook, C. N. & Emlet, R. B. 2006 Offspring size effects mediate competitive interactions in a colonial marine invertebrate. *Ecology* **87**, 214–225. (doi:10.1890/05-0350)

54 Metcalfe, N. B. & Monaghan, P. 2001 Compensation for a bad start: grow now, pay later? *Trends Ecol. Evol.* **16**, 254–260. (doi:10.1016/S0169-5347(01)02124-3)

55 Taborsky, B. 2006 Mothers determine offspring size in response to own juvenile growth conditions. *Biol. Lett.* **2**, 225–228. (doi:10.1098/rsbl.2005.0422)

56 Vijendravarma, R. K., Narasimha, S. & Kawecki, T. J. 2010 Effects of parental diet on egg size and offspring traits in *Drosophila*. *Biol. Lett.* **6**, 238–241. (doi:10.1098/rsbl.2009.0754)

57 van der Sman, J., Phillips, N. & Pfister, C. A. 2009 Relative effects of maternal and juvenile food availability for a marine snail. *Ecology* **90**, 3119–3125. (doi:10.1890/08-2009.1)

58 Einum, S. & Fleming, I. A. 2000 Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature* **405**, 565–567. (doi:10.1038/35014600)

59 Lindholm, A. K., Hunt, J. & Brooks, R. 2006 Where do all the maternal effects go? Variation in offspring body size through ontogeny in the live-bearing fish *Poecilia parae*. *Biol. Lett.* **2**, 586–589. (doi:10.1098/rsbl.2006.0546)

60 Yang, B., Greene, M. & Chen, T. T. 1999 Early embryonic expression of the growth hormone family protein genes in the developing rainbow trout (*Oncorhynchus mykiss*). *Mol. Reprod. Dev.* **53**, 127–134. (doi:10.1002/(SICI)1098-2795(199906)53:2<127::AID-MRD1>3.0.CO;2-H)

61 Ayson, F. G., de Jesus, E. G., Moriyama, S., Hyodo, S., Funkenstein, B., Gertlerm, A. & Kawauchi, H. 2002 Differential expression of insulin-like growth factor I and II mRNAs during embryogenesis and early larval development in rabbitfish, *Siganus guttatus*. *Gen. Comp. Endocr.* **126**, 165–174. (doi:10.1006/gcen.2002.7788)

62 Maures, T., Chan, S. J., Xu, B., Sun, H., Ding, J. & Duan, C. 2002 Structural, biochemical and expression analysis of two distinct insulin-like growth factor I receptors and their ligands in zebrafish. *Endocrinology* **143**, 1858–1871. (doi:10.1210/en.143.5.1858)

63 Aegerter, S., Jalabert, B. & Bobe, J. 2004 Messenger RNA stockpile of cyclin B, insulin-like growth factor I, insulin-like growth factor II, insulin-like growth factor receptor Ib, and p53 in the rainbow trout oocyte in relation with developmental competence. *Mol. Reprod. Dev.* **67**, 127–135. (doi:10.1002/mrd.10384)

64 Ozaki, Y., Fukada, H., Tanaka, H., Kagawa, H., Ohta, H., Adachi, S., Hara, A. & Yamauchi, K. 2006 Expression of growth hormone family and growth hormone receptor in the Japanese eel (*Anguilla japonica*). *Comp. Biochem. Phys. B* **145**, 27–34. (doi:10.1016/j.cbpb.2006.05.009)

65 Birzniece, V., Sata, A. & Ho, K. K. Y. 2009 Growth hormone receptor modulators. *Rev. Endocr. Metab. Disord.* **10**, 145–156. (doi:10.1007/s11154-008-9089-x)

66 Tagawa, M., Tanaka, M., Matsumoto, S. & Hirano, T. 1990 Thyroid hormones in eggs of various freshwater, marine and diadromous teleosts and their changes during egg development. *Fish Physiol. Biochem.* **8**, 515–520. (doi:10.1007/BF00003409)

67 Morrison, C. M., Miyake, T. & Wright, J. R. 2001 Histological study of the development of the embryo and early larva of *Oreochromis niloticus* (Pisces: Cichlidae). *J. Morphol.* **247**, 172–195. (doi:10.1002/1097-4687(200102)247:2<172::AID-JMOR1011>3.0.CO;2-H)

68 Sinervo, B. & McEdward, L. R. 1988 Developmental consequences of an evolutionary change in egg size: an experimental test. *Evolution* **42**, 885–899. (doi:10.2307/2408906)

69 Morley, S. A., Batty, R. S., Geffen, A. J. & Tytler, P. 1999 Egg size manipulation: a technique for investigating maternal effects on the hatching characteristics of herring. *J. Fish Biol.* **55**, 233–238. (doi:10.1111/j.1095-8649.1999.tb01059.x)

70 Bell, A. M., Dingemanse, N. J., Hankison, S. J., Langenhof, M. B. W. & Rollins, K. 2011 Early exposure to nonlethal predation risk increases somatic growth and decreases size at adulthood in threespined sticklebacks. *J. Evol. Biol.* **24**, 943–953. (doi:10.1111/j.1420-9101.2011.02247.x)

71 Kotrschal, A., Heckel, G., Bonfils, D. & Taborsky, B. 2011 Life-stage specific environments in a cichlid fish: implications for inducible maternal effects. *Evol. Ecol.* (doi:10.1007/s10682-011-9495-5)

72 Bossdorf, O., Richards, C. L. & Pigliucci, M. 2008 Epigenetics for ecologists. *Ecol. Lett.* **11**, 106–115. (doi:10.1111/j.1461-0248.2007.01130.x)

73 Ho, D. H. & Burggren, W. W. 2009 Epigenetics and transgenerational transfer: a physiological perspective. *J. Exp. Biol.* **213**, 3–16. (doi:10.1242/jeb.019752)