

# Highly plastic resource allocation to growth and reproduction in females of an African annual fish

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**Abstract** – Phenotypic plasticity is an important mechanism to maximise fitness in unpredictable environments by fine-tuning phenotypes to a specific environmental setting. We used *Nothobranchius furzeri*, an African annual fish from temporary pools with erratic changes in habitat condition, to study changes in the allocation to growth and reproduction and to test the key trade-off between egg size and number. In an experimental setting, we quantitatively varied ration at two levels and over two time periods, including temporal switches in ration level. As predicted, female *N. furzeri* possessed the capacity for compensatory growth, which surprisingly came with no longer-term cost to fecundity. Females responded strongly to ration manipulation, with a pronounced decrease in fecundity associated with a low ration, even after accounting for body mass. Due to the unpredictability of offspring environment, we expected no adaptive change in oocyte size. However, females responded to the quality of their environment in accordance with an adaptive maternal effect, with females receiving a low ration producing larger eggs. Further, a switch in ration size in either direction was associated with a decrease in egg size. There was a trade-off between egg size and number in half of the treatments, but high variability in egg size among females made the relationship complex. Overall, *N. furzeri* females demonstrated high plasticity in both growth rate and fecundity parameters. Females appear able to track and respond adaptively to unpredictable changes in food availability in their environment.

**Key words:** *Nothobranchius*; compensatory growth; egg size; life history; diet restriction

## Introduction

Life-history theory assumes trade-offs in resource allocation among growth, reproduction and self-maintenance (Stearns 1992; Roff 2002). Given that self-maintenance typically takes priority, most life-history-relevant differences in energy allocation are predicted to arise from the trade-off between growth and reproduction (Heino & Kaitala 1999). Resource allocation among the three channels is assumed to have a genetic basis, but can be modified in response to environmental variation through phenotypic plasticity (West-Eberhard 1989).

Growth rate is a notably plastic trait (Arendt 1997). Deceleration or cessation of growth when conditions are suboptimal can be compensated by growth acceleration after an improvement in feeding

conditions, sometimes leading to full growth compensation (reviewed by Hector & Nakagawa 2012). While in some taxa growth ceases upon sexual maturation, in others (including fish, reptiles and molluscs) individuals continue growing throughout their entire lifespan (Stearns 1992). Indeterminate growth generates the opportunity for reversible changes in the pattern of growth over the lifetime of an individual that matches current environmental conditions (Arendt 1997; Dmitriew 2011). Individuals may invest in reproduction directly, in terms of allocation to gonads. However, in species with indeterminate growth, allocation to growth can also be viewed as an investment into future reproduction because fecundity is positively associated with body size (Stearns 1992; Wootton 1998). This creates an additional conflict between current and future

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reproduction, with its resolution depending on survival prospects (Stearns 1992).

Within species, the relative allocation to growth and reproduction generally appears to remain in stable proportion across wide levels of resource availability (Wootton 1977; McCoy et al. 2007; Inness & Metcalfe 2008; Zeng et al. 2012). However, conditions experienced early in life may determine lifetime patterns in allocation to reproduction, while adult growth reflects prevailing conditions with deviations from a stable allocation ratio (Taborsky 2006a). In the few cases when females of taxa with indeterminate growth invest in future reproduction (i.e. growth) at a cost to current reproduction, females respond to unusually adverse current conditions and have a high prospect of survival to the next reproductive event (Jokela 1997; Edwards et al. 2002; Harris & Ludwig 2004).

An additional life-history trade-off, within the limits of a given reproductive allotment, is the conflict between the number of eggs and their size, such that selection for greater egg or embryo size necessarily results in smaller clutch size (Elgar 1990; Stearns 1992; Bernardo 1996a; Qu et al. 2011). Investment in high fecundity appears adaptive when environmental conditions are good (Einum & Fleming 1999; Morrongiello et al. 2012). Under these conditions, the production of a high number of offspring increases female fitness (Sinervo & Doughty 1992). In contrast, increased investment in individual eggs is predicted to be adaptive under more challenging conditions (Einum & Fleming 2002; Giesing et al. 2011; Segers & Taborsky 2012), when egg size is positively related to offspring survival (Sogard 1997; Segers & Taborsky 2011).

At the individual level, offspring are often larger when their mother has experienced adverse feeding conditions (Reznick et al. 1996; Bashey 2006). However, general evidence for maternal effects on egg size is ambiguous (Bernardo 1996b; Fox & Czesak 2000), and the direction of the effect may be specific to the respective study system. Indeed, no relationship between food conditions and egg size was observed in the three-spined stickleback, *Gasterosteus aculeatus* (Inness & Metcalfe 2008). Females of the cichlid *Simochromis pleurospilus* may tailor offspring size to predicted juvenile environment (Taborsky 2006b), but with no relationship to the conditions experienced by the mother as an adult. Furthermore, maternal effects can be transferred as the energy content of the egg rather than its size (Gagliano & McCormick 2007), and plasticity in the egg size may not be always favoured by selection, even in a fluctuating environment (Fischer et al. 2011). Finally, female phenotypic characteristics, irrespective of current environment, may affect egg size due to

physiological constraints. For example, a positive correlation between female size and egg size is reported in teleost fishes (Heath et al. 1999; Johnston & Leggett 2002). All these factors can override the general prediction of large egg size in females experiencing adverse conditions.

Here, we analyse responses to fluctuating resources in a teleost fish with a rapid life history. We use *Nothobranchius furzeri* Jubb, an oviparous African annual killifish, to examine the effect of environmental conditions (food ration) on allocation to growth and reproduction, and the resolution of the trade-off between egg size and number. *N. furzeri* inhabits temporary savannah pools in relatively dry areas of Mozambique that experience erratic rainfall. The pools exist only during the rainy season and desiccate 2–10 months after filling (Reichard et al. 2009; Terzibasi Tozzini et al. 2013). Fish hatch after the pool is filled with rainwater, grow rapidly and achieve sexual maturity within 3–6 weeks (Valdesalici & Cellerino 2003; Blažek et al. 2013). The environmental conditions experienced by *N. furzeri* fluctuate within and among generations, with changes in water level, number of competitors and food availability (Polačik & Reichard 2010; Terzibasi Tozzini et al. 2013; Polačik et al. 2014a). *N. furzeri* reproduce daily, laying 20–200 eggs each day (Polačik & Reichard 2009, 2011; Blažek et al. 2013). They are income breeders, with continuous recruitment of mature oocytes (Haas 1976), and, therefore, possess the potential to respond adaptively to changes in prevailing environmental conditions (Stearns 1992; Stephens et al. 2009).

Annual killifish represent an exceptionally tractable model to test the generality and constraints of life-history theory and its predictions. This is because female annual killifish are predicted to invest in reproduction irrespective of current conditions; the risk of mortality is unusually high due to rapid and unpredictable desiccation of the pools they inhabit, and this is especially true for *N. furzeri* habitats, which may desiccate from a few days to weeks after their sexual maturation (Terzibasi Tozzini et al. 2013; Polačik et al. 2014b). This is in contrast to other systems used for testing life-history theory in fishes, such as guppies and African lake cichlids, which come from relatively stable and predictable environments.

We experimentally varied the ration of female *N. furzeri* at two levels and over two periods: during sexual maturation and in adulthood. We compared allocation to growth (measured as body size and body mass) and reproduction (ovary mass, number of eggs and egg size) across treatments. We tested whether females track current conditions and adjust their resource allocation flexibly, including compen-

satory growth, and its consequences for fecundity and egg size. We believe that using an extreme case of income breeder from erratic and ephemeral habitats can reveal how these predictions hold across reproductive strategies.

We predicted slower growth and later sexual maturation in the low-ration treatment and compensatory growth in the treatment where ration was increased. We predicted that compensatory growth would come at a cost of decreased fecundity (lower ovary mass and smaller oocyte size), but not at the cost of lower egg number. Given the intrinsically unpredictable environment across generations, we predicted no female manipulation of egg size (except for constraints from compensatory growth); females in a low-ration treatment were not predicted to produce larger eggs to permit offspring coping in inferior conditions. Finally, we predicted a trade-off between oocyte size and number across all treatments.

## Material and methods

### Experimental animals

*Nothobranchius furzeri* used in the experiment were an F1 generation of wild-caught fish, collected in southern Mozambique (S 21°52'24.84", E 32°48'2.34", code MZCS 222) in 2011. A total of 20 males and 40 females were imported, and a mating design that maximised outbreeding of the F1 generation was used. Experimental fish were hatched by pouring aged tap water over a peat moss substrate containing developed embryos in 4-l tanks. Hatched fish were kept in these tanks until the age of 8 days when they were transferred into 10 tanks (28 l) with approximately 60 individuals per tank. At the age of 13 days, the density was decreased to 30 fish per tank by distributing them among additional tanks. At 17 days, fish density was further decreased to 20 individuals per tank. Fish were initially fed with live *Artemia* spp. nauplii three times each day. At the age of 2 weeks, fish were weaned on frozen bloodworm (chironomid larvae), provided twice each day. Tanks were checked daily for the appearance of subadult males. Males were transferred into separate tanks, as dominant males may suppress the growth of other fish in the tank (Hofmann et al. 1999). At the age of 4 weeks, when all males had developed male-specific coloration, fish were assigned to experimental tanks.

### Experimental housing

During the experiment, fish were kept in 42 tanks (volume of 28 l; 6 tanks per treatment and 3 reserve tanks for each ration level), each with five females and two males. Female *Nothobranchius* spawn daily

after reaching sexual maturity (Haas 1976), and this sex ratio facilitated regular oviposition by females. Female *Nothobranchius* prevented from oviposition develop pathologies (Polačik et al. 2014b). To record the onset of sexual maturity, each tank contained a spawning dish with fine sand that was checked daily until the first eggs were collected. The tanks were equipped with air-driven filters, and every 4 days, the bottom was siphoned to remove waste and to exchange one-third of the water volume. Water temperature was maintained at 27 ( $\pm 1$ ) °C. Photoperiod was set to 14:10 h (light/dark).

### Experimental design

Throughout the experiment, fish were divided into a high- and low-ration treatment. *Nothobranchius* have an extremely rapid metabolism and consume large quantities of food (Genade et al. 2005; Blažek et al. 2013). Therefore, the high-ration treatment (H) received food twice per day (08:00 and 16:00), while the low-ration treatment (L) only received food at 08:00. Rations were set on the basis of a pilot study and our experience with routine husbandry for stocks of *N. furzeri*. We considered significantly decreased growth rate as an indication of a suboptimal ration (i.e. a low-ration treatment). Feeding consisted of an *ad libitum* amount of frozen chironomid larvae dispersed in the tank. After 10 min, uneaten food was siphoned out to maintain water quality and to avoid any additional feeding. *Nothobranchius* fish feed voraciously, and 10 min is sufficient time for complete satiation.

The experiment itself comprised two phases. The first phase encompassed the period during and just after sexual maturation and was chosen as the first period when males and females could be visually separated. Applying a low ration during the juvenile phase typically delays sexual maturation by several weeks and was, therefore, incompatible with our aim to test trade-offs in allocation between growth and reproduction. The second phase consisted of fully sexually mature females. Its duration was determined by the point at which fish receiving the low-ration-only (L–L) treatment reached the mean body size of females sacrificed at the end of Phase 1 that had received the high ration (H–0).

The initial Phase 1 had two treatments (high and low ration) and lasted 4 weeks. After 4 weeks (age 8 weeks), females from five tanks of each ration level were sacrificed (high-sacrificed: H–0, low-sacrificed: L–0) with an overdose of anaesthetic (clove oil) and stored in 8% formaldehyde. Females were separated from males for 24 h prior to the removal of females to interrupt oviposition and hence standardise their fecundity (Reichard & Polačik 2010; Polačik &

Reichard 2011). *Nothobranchius* females ovulate their oocytes at night, and mature eggs are spawned during the following day (Haas 1976; M. Vrtilek unpublished data).

During Phase 2 (full sexual maturity), either the remaining fish were kept on the same ration as in Phase 1 (high–high: H–H, low–low: L–L) or their ration level was switched (high to low: H–L, low to high: L–H). Phase 2 lasted 6 weeks (female age was 14 weeks at the end of Phase 2). At the end of Phase 2, all experimental females were sacrificed and fixed in 8% formaldehyde.

### Mortality

Twelve females (5 in the H and 7 in the L treatments) died during Phase 1, and 41 females died during Phase 2 (13 in H–H, 10 in H–L, 12 in L–H and 6 in L–L). To maintain a constant fish density in experimental tanks, any dead fish were replaced by a similar-sized individual from reserve tanks containing nonexperimental fish of both sexes and of the same age. The new females were marked and were not included in analyses. However, females were transferred between tanks within treatments in the case of a lack of nonexperimental females to maintain fish density, resulting in a decrease in number of experimental tanks over time. *N. furzeri* are extremely short-lived (Valdesalici & Cellerino 2003), and the mortality rate observed during the experiment was natural (i.e. not related to experimental design) and distributed similarly across tanks and treatments. Additionally, 10 males (6 in H and 4 in L) were discovered as incorrectly assigned as females in Phase 1 and another four (1 H–L, 2 L–H and 1 L–L) during Phase 2. This occurred because sex-specific coloration may be suppressed in submissive males in the presence of dominant males, with submissive males retaining female coloration. Data on 25 females per treatment were collected (H–0, L–0, L–L, H–L), except for H–H (23 females) and L–H (20 females) treatments.

### Data collection

Female standard length (measured from the tip of the snout to the distal end of the caudal peduncle) was recorded throughout the experiment from photographs ( $L_p$ ). All females from each tank were photographed together in a plastic cup with a low water level and a scale bar on the bottom.  $L_p$  was measured on the first day of the experiment and every 4 days thereafter. This allowed us to standardise female body size across treatments at the start of the experiment [linear mixed-effects modelling (LME):  $F_{5,23} = 0.25$ ,  $P = 0.938$ ].

At the end of the experiment, sacrificed females were measured for their standard length ( $L_s$ ) to the nearest 0.1 mm using digital callipers and weighed for total mass ( $W_t$ ) to the nearest 1 mg. They were dissected and weighed for body mass ( $W_d$ ; eviscerated body mass) and ovary mass ( $W_g$ ). The number of mature oocytes was counted ( $N_o$ ), and the ovaries were photographed under a dissecting microscope (Olympus SZX10, at 4× magnification) using a  $\mu$ Eye-1540C camera. The diameter of mature oocytes (Dia) was measured along their longest axis using NIH ImageJ software ver. 1.46j (National Institute of Health, Bethesda, MD, USA). Mature oocytes are orange and translucent, clearly different from whitish opaque immature oocytes (Selman et al. 1993). Mature oocytes were analysed instead of spawned eggs to avoid potential paternal effects (Pakkasmaa et al. 2001; Polačik & Reichard 2009; Moshgani & Van Dooren 2011) and the confounding effect of hydration on size. A subset of oocytes was measured in two dimensions and their surface area ( $S$ ) estimated based on an assumption that they were ellipsoid in shape (thus,  $S = \pi * a * b$ ). There was a strong correlation between oocyte size estimated using a measurement along the longest axis and surface area ( $r = 0.833$ ,  $P < 0.001$ ; based on a subset of 13 females and 288 oocytes). Consequently, only oocyte size based on a measurement along their longest axis was used in the analysis.

One female (H–L treatment) was omitted from all analyses as an extreme outlier. This female was considerably larger than all other females (body mass = 1327 mg, second largest 798 mg) and had atrophied ovaries (ovary mass = 15 mg, mean ovary mass 51.71 mg), which implied she had been unable to divert resources to oogenesis. Hence, the final number of females in the H–L treatment was 24.

### Data analysis

All statistical analyses were carried out using the R software ver. 2.15.0 (R Development Core Team 2012), using the ‘nlme’ package (Pinheiro et al. 2012). We followed Zuur et al. (2009) for the model selection process. LME (Pinheiro & Bates 2000) was used with tank identity as a random factor.

First, the fixed part of the model was saturated with parameters, and the best correlation and variance parameters were selected (based on criterion of the lowest AIC). Then, nonsignificant components were sequentially removed from the fixed part of the model, starting with the highest interaction. After removal of a parameter, the new model was tested against the previous one using a log-likelihood ratio test and AIC values. Finally, significance of differences between individual treatments was determined post hoc, and output from the contrasts is reported.



Body size ( $L_s$ ) and body mass ( $W_d$ ) were strongly correlated, and inferences were concordant when using either variable as a covariate for fecundity measures. The only difference was in ovary mass analysis between L–L and H–0 treatments, which was significant for body size ( $t_{23} = 2.92$ ,  $P = 0.007$ ) but not for body mass ( $t_{23} = 1.02$ ,  $P = 0.317$ ). For analyses of fecundity parameters, we present outcomes using only body mass as a covariate throughout, as we believe it better reflects energy allocation to growth compared to body size.

The onset of reproduction and the number of mature oocytes ( $N_o$ ) had a Poisson distribution (count variables); hence, generalised linear modelling (GLM) with a correction for overdispersion was used. Significance of change in deviance following parameter removal was examined with an  $F$ -test. The number of mature oocytes also had Poisson distribution (count variable), but to retain tank identity in the analysis, we employed generalised estimating equations (GEE; package ‘geepack’, Højsgaard et al. 2006) for the analysis.

At the final stage of analyses of ovary mass ( $W_g$ ) and the number of mature oocytes ( $N_o$ ), most similar treatments were sequentially pooled posteriorly. The procedure was based on pooling the most similar groups in terms of the  $P$ -value of their contrasts. After pooling two treatments (groups), a new model was always compared to the previous model using log-likelihood ratio test and AIC. Posterior pooling of treatments is a recommended method (Crawley 2007) for analysis of our experimental design. Note that interpretation of posterior pooling is similar to reporting nonsignificant differences between treatments.

For mature oocyte size ( $Dia$ ), components of variance were tested among nested levels of the experimental design using the LME model having only the intercept in the fixed part of the model and with saturated random part (treatment/tank/individual female). This procedure estimates the overall sample mean value and decomposes the variation for different levels of the nested levels of our design. Subsequently, the size of mature oocytes was analysed with respect to phenotypic traits of individual females [body mass ( $W_d$ ) and the number of mature oocytes ( $N_o$ )] and treatment. Here, female identity was added as a random factor nested in tank identity.

## Results

### Somatic growth

Overall, the experimental treatment had a significant effect on both female body size ( $L_s$ ; LME:  $F_{5,23} = 40.13$ ,  $P < 0.001$ ) and body mass ( $W_d$ ; LME:  $F_{5,23} = 42.33$ ,  $P < 0.001$ ; Fig. 1). Ration affected

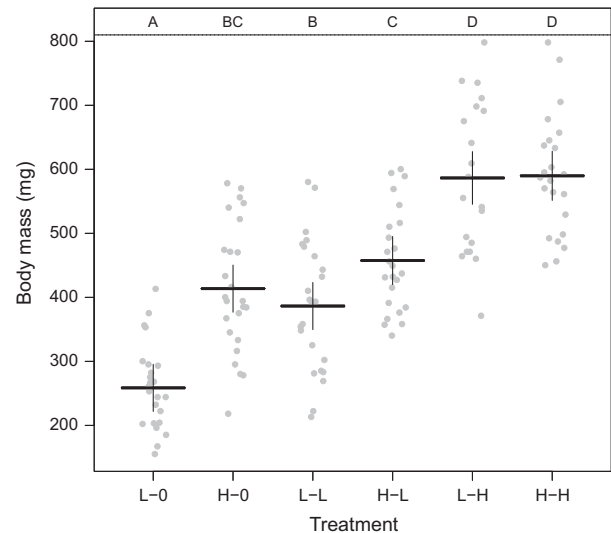


Fig. 1. Comparison of female body mass across treatments. Different letters indicate significantly different group ( $P < 0.05$ ). Error bars denote 95% confidential intervals for treatment means. Grey points represent values for individual females.

fish growth rate as expected, with high ration enhancing growth and low ration limiting growth. After Phase 1 (4 weeks of exposure to contrasting rations), females receiving a high ration (H–0) were significantly larger than females on a low ration (L–0;  $t_{23} = 8.10$ ,  $P < 0.001$  for body size and  $t_{23} = 5.84$ ,  $P < 0.001$  for body mass). Females on a high (H–0) and low (L–0) ration were  $27.99 \pm 2.11$  mm (mean  $\pm$  SD of  $L_s$ ) and  $23.32 \pm 1.83$  mm respectively, and females on a high ration grew to a 60% heavier body mass (Fig. 1).

Ration also significantly affected body mass after 10 weeks, at the end of Phase 2 (Fig. 1). The difference between stable ration levels was comparable, with females on a consistently high ration (H–H) significantly larger (H–H;  $30.37 \pm 1.87$  mm, L–L:  $26.30 \pm 2.24$  mm,  $t_{23} = 6.91$ ,  $P < 0.001$ ) and 53% heavier than females on a consistently low ration (L–L;  $t_{23} = 7.50$ ,  $P < 0.001$ ). There was strong compensatory growth in females whose ration was enhanced (L–H; Fig. 2). At the end of the experiment, L–H females reached the same body mass ( $t_{12} = 0.12$ ,  $P = 0.909$ , Fig. 1) and body size ( $t_{23} = 0.21$ ,  $P = 0.853$ ) as H–H females. Females that received a decreased ration (H–L) almost ceased growth, and their body mass and body size did not significantly differ from females on a high ration sacrificed at the end of Phase 1 (H–0; body mass:  $t_{23} = 1.64$ ,  $P = 0.115$ ; body size:  $t_{23} = 0.58$ ,  $P = 0.556$ ).

### Sexual maturation

Females receiving a high ration (H) attained sexual maturity earlier than females receiving a low ration

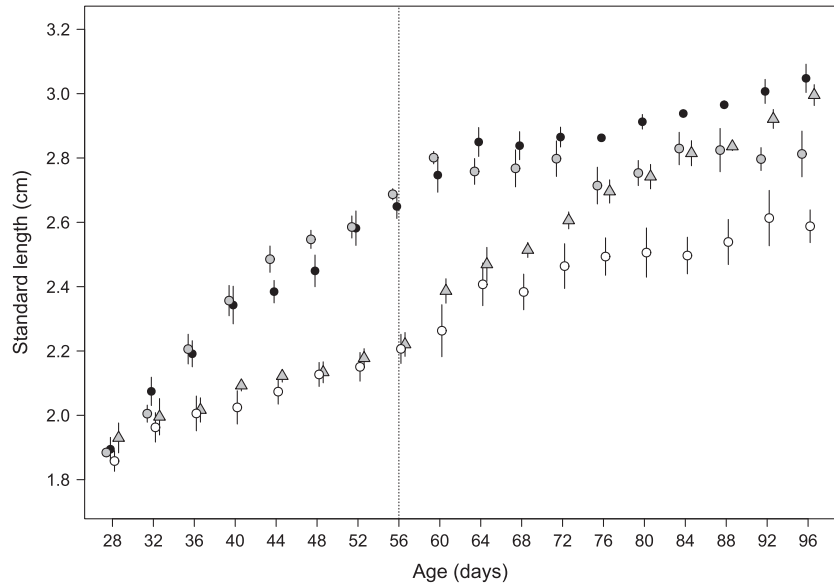


Fig. 2. Female growth curves for experimental treatments. Each point represents mean standard length ( $L_p$ ) for given age per treatment: H-H (full black circles), H-L (grey circles), L-H (grey triangles) and L-L (empty circles). Lines denote standard error of mean counted from tank mean values ( $n = 5$ , except L-H treatment where  $n = 4$ ). For clarity, data for L-0 and H-0 treatments are not plotted. Beginning of Phase 2 is marked by vertical dashed line.

(L; GLM:  $\chi^2_{1,40} = 52.33$ ,  $P = 0.030$ ). The first eggs were collected  $5.5 \pm 1.9$  (median  $\pm$  SD) days after start of Phase 1 in high-ration treatments (at the age of 33.5 days) and after  $7.4 \pm 4$  days in low-ration treatments.

#### Ovary mass

Ovary mass varied among treatments (LME:  $F_{5,23} = 13.80$ ,  $P < 0.001$ ). Ovary mass was strongly correlated with female body mass (LME:  $F_{1,112} = 599.78$ ,  $P < 0.001$ ), and all further comparisons control for body mass. When the most similar treatments were pooled (LME: body mass covariate:  $F_{1,112} = 579.86$ ,  $P < 0.001$ , pooled treatments:  $F_{1,27} = 62.22$ ,  $P < 0.001$ ; Fig. 3a), females fed a high ration at the time of sacrifice (H-0, H-H, L-H) had a higher relative ovary mass than females fed a low ration when sacrificed (L-0, H-L, L-L;  $t_{27} = 7.89$ ,  $P < 0.001$ , Fig. 3a).

#### Number of mature oocytes

The number of mature oocytes matched the results for ovary mass. There was a significant effect of treatment (GEE:  $\chi^2 = 46.95$ ,  $P < 0.001$ ), and body mass was a significant covariate (GEE:  $\chi^2 = 20.60$ ,  $P < 0.001$ ). Sequential pooling revealed two final groups of treatments. Females fed a high ration when sacrificed (H-0, H-H, L-H) had a higher number of mature oocytes than females receiving a low ration (L-0, H-L, L-L; GEE: body mass covariate: GEE:  $\chi^2 = 46.90$ ,  $P < 0.001$ , pooled treatments: GEE:  $\chi^2 = 16.20$ ,  $P < 0.001$ , Fig. 3b).

#### Mature oocyte size

Among treatments, there were significant differences in mature oocyte size (LME:  $F_{5,23} = 4.37$ ,  $P = 0.006$ , Fig. 4). Females on a low ration (L-0, L-L) maintained a large size of mature oocytes throughout the experiment ( $t_{109} = 1.10$ ,  $P = 0.261$ ), while females on a high ration produced large mature oocytes when older (H-H), but small oocytes when younger (H-0;  $t_{109} = 2.90$ ,  $P = 0.004$ , Fig. 4).

At the level of individual females, LME modelling demonstrated that the largest part of mature oocyte size variability was within females (67.33%), followed by variability among females (26.29%) and treatments (6.38%). The effect of rearing tank on mature oocyte size variability was negligible (<0.01%).

#### Trade-off between mature oocyte size and their number

The relationship between the size and number of mature oocytes was not straightforward. A three-way interaction ( $W_d * N_o * TREATMENT$ ) could not be removed from the full model (log-likelihood test:  $L$ -ratio = 13.50,  $P = 0.019$  and delta AIC = 4), yielding a complex outcome (Table 1). However, when three-way interaction was removed, there was significant additive effect of treatment and the number of mature oocytes on their size (LME: treatment:  $F_{5,23} = 4.74$ ,  $P = 0.004$ , number of mature oocytes:  $F_{1,85} = 10.42$ ,  $P = 0.002$ ).

To provide some insight into the relationship between the number of mature oocytes and their size, we divided treatments into two groups: young females (L-0 and H-0; sacrificed after 4 weeks of treatment exposure, after Phase 1) and old females

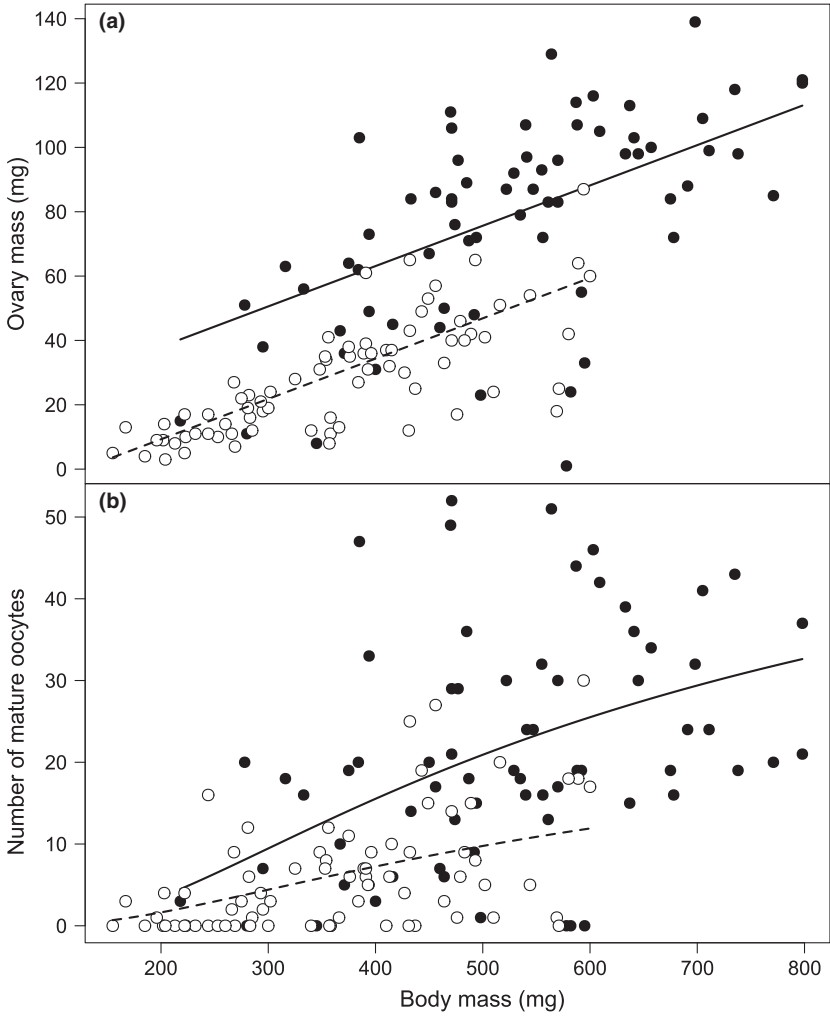


Fig. 3. Effect of current diet ration on female allocation to reproduction; (a) ovary mass and (b) number of mature oocytes, both controlled for body mass. In both plots, lines represent the best fit for high (solid black)- and low-ratio females when sacrificed (dashed) and points represent values for individual females from these groups, that is, pooled low-ratio (H-L, L-L and L-0) ( $\circ$ ) and high-ratio treatments (H-0, H-H and L-H) ( $\bullet$ ).

(L-L, L-H, H-L and H-H, sacrificed after 10 weeks, after Phase 2) and analysed these two groups separately.

In young females, mature oocyte size was negatively associated with both the number of mature oocytes and body mass (LME: number of mature oocytes:  $F_{1,25} = 8.90$ ,  $P = 0.006$ , body mass:  $F_{1,25} = 9.81$ ,  $P = 0.004$ ). The ration treatment did not significantly affect mature oocyte size in this analysis (LME:  $F_{1,8} = 0.04$ ,  $P = 0.852$ ) and was dropped from the final model.

In old females, the three-way interaction was still significant (LME:  $F_{3,47} = 3.37$ ,  $P = 0.026$ ). Therefore, we decomposed the outcomes for individual treatments. There was no relationship between the number of mature oocytes and their size in H-H (LME: last removed term was body mass:  $F_{1,15} = 1.22$ ,  $P = 0.287$ ) and L-H treatments (LME: last removed term was body mass:  $F_{1,15} = 0.18$ ,  $P = 0.678$ ). In the H-L treatment, we detected a negative relationship between mature oocyte number and size (LME:  $F_{1,13} = 7.26$ ,  $P = 0.018$ ). In the L-L

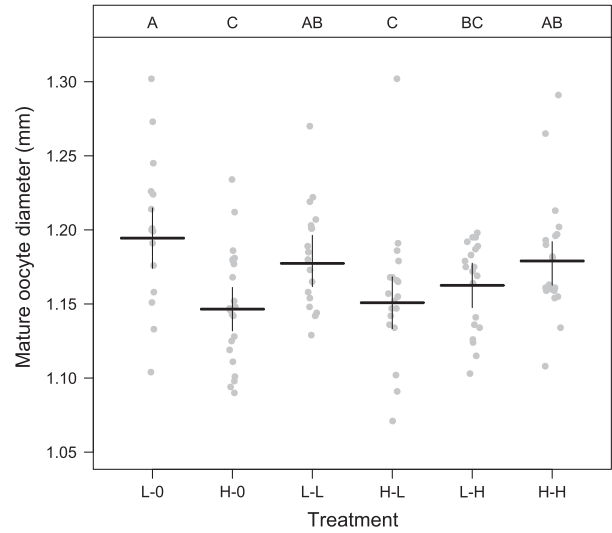


Fig. 4. Differences in mature oocyte size among treatments. Different letters indicate significantly different groups. Error bars denote 95% confidential intervals for treatment means. Grey points represent mean size of mature oocyte for individual females.

Table 1. The effects of female phenotypic traits and treatment on mature oocyte size analysed using linear mixed-effects modelling.  $P < 0.05$  are highlighted in bold. Note that removal of the three-way interaction significantly decreased model explanatory power ( $P < 0.019$ ).

Model term	d.f.	F	P
Intercept	1, 1799	147665.24	<b>&lt;0.001</b>
Treatment	5, 23	5.40	<b>0.002</b>
Body mass	1, 68	1.82	0.182
Number of mature oocytes	1, 68	10.00	<b>0.002</b>
Body mass:Treatment	5, 68	2.67	<b>0.029</b>
Number of mature oocytes:Treatment	5, 68	0.69	0.634
Number of mature oocytes:Body mass	1, 68	0.21	0.651
Body mass:Number of mature oocytes:Treatment	5, 68	2.18	0.066

Table 2. Overview of the main results of treatment effects on allocation to reproduction.

Treatment	Relative gonad mass	Relative number of mature oocytes	Mature oocyte size	Trade-off between size and number of mature oocytes
H-0	High	Many	Small	Yes
L-H	High	Many	Medium-small	No
H-H	High	Many	Medium-large	No
L-0	Low	Few	Large	Yes
H-L	Low	Few	Small	Yes
L-L	Low	Few	Medium-large	No

treatment, there was a significant interaction between the number of mature oocytes and female body mass (LME:  $F_{1,10} = 5.24$ ,  $P = 0.045$ ); its removal yields an intercept-only model (LME: last term removed was the number of mature oocytes:  $F_{1,12} = 0.72$ ,  $P = 0.414$ ). The results are summarised in Table 2.

### Discussion

Our results demonstrate that female annual killifish, *N. furzeri*, display marked plasticity in growth rate and fecundity traits, triggered by food availability. Resource allocation to reproduction and growth was tightly coupled, and low ration resulted in poor growth and a substantial (approximately 50%) decrease in fecundity, even after accounting for lower body mass (Fig. 3). Females receiving a low ration produced larger eggs than other females. Females on a high ration throughout the experiment also produced many eggs that were large (Fig. 4), perhaps as a consequence of superior conditions. A switch in diet, in either direction, was associated with decreased egg size (Fig. 4). The relationship between egg size and their number was complex, but a trade-off between egg size and number was lost later in

life, recorded only in females whose ration level declined. The main results on allocation to reproduction are summarised in Table 2.

### Energy allocation strategy

Females *N. furzeri* receiving a low-ration diet grew less, reached sexual maturity later and had lower fecundity than females receiving a high ration, but were still able to reproduce, confirming that our manipulation of resources was appropriate. While reproduction may be skipped altogether when resources are scarce (Tyler & Dunn 1976), continuous allocation to both growth and reproduction is more common. This is especially true for small and short-lived fish (Wootton 1977; Inness & Metcalfe 2008; Zeng et al. 2012), including annual killifish (present study). Females of the white cloud mountain minnow, *Tanichthys albonubes*, invested a fixed proportion of consumed energy into gonads, irrespective of ration (Zeng et al. 2012). In *N. furzeri*, females must invest in reproduction, even if feeding conditions are poor. This is because the risk of mortality is high in temporary savannah pools, which can desiccate extremely rapidly (days to weeks after sexual maturation), and is highly unpredictable (Terzibasi Tozzini et al. 2013). In our study, we used a population from an arid part of the range of *N. furzeri* that is subject to extremely erratic environmental conditions (Bartáková et al. 2013; Poláček et al. 2014a).

### Compensatory growth

Female *N. furzeri* responded to improved food conditions and displayed full compensatory growth. Regular body size measurement during the experiment demonstrated that decelerated growth during the 4-week period of decreased ration (Phase 1 of the experiment) was fully compensated during the subsequent 6 weeks when the ration was high (Fig. 2). In the wild, female *N. furzeri* encounter dynamic changes in food availability (Meintjes 1996) and intraspecific competition as the water level, and hence volume of the pool, fluctuates during the rainy season.

Compensatory growth has been described for numerous taxa, including teleost fish (Ali et al. 2003; Lee et al. 2013). However, the steep compensatory growth reported here is rare and might be expected to be associated with costs (Metcalfe & Monaghan 2001; Mangel & Munch 2005; Dmitriew 2011). These costs typically affect future reproduction. In the guppy, *Poecilia reticulata*, compensatory growth had a negative impact on the number of offspring (Auer et al. 2010). Female three-spined sticklebacks reduced their reproductive investment following a



period of rapid growth irrespective of their current food availability (Lee et al. 2012). In our study, compensatory growth was not manifested at the expense of fecundity. Females undergoing full compensatory growth were also able to invest in fecundity at the same level as females kept on a high ration throughout the experiment, in terms of both ovary mass (Fig. 3a) and the number of mature oocytes (Fig. 3b). This capacity to respond to an elevated ration was not predicted and is noteworthy, especially given that *Nothobranchius* females reproduce daily. While there may have been a temporary decrease in allocation to reproduction within the 6-week period of Phase 2, which we did not detect, we may conclude that the cost of compensatory growth on fecundity, if any, was quickly mitigated. However, we highlight that our experimental design did not allow us to measure other potential costs of compensatory growth, namely more rapid ageing and shorter lifespan (Lee et al. 2013), which would inevitably lead to decreased lifetime female fecundity.

#### Fecundity

Only the current ration affected fecundity; hence, females receiving a low ration at the time of sacrifice had reduced fecundity, in terms of both ovary mass and number of mature oocytes. Past food availability had no effect on fecundity measured 6 weeks after the switch in ration. The effect of current food availability was also relative, as there were clear differences between ration treatments even after controlling for female body mass (Fig. 3). This means that a smaller ovary and fewer eggs were not a result of physical constraints imposed by female size, but rather her inability to divert energy into reproductive tissue when resources were limiting. This finding is in contrast to those for the guppy, a cichlid or the three-spined stickleback, in which the effect of ration on fecundity was lost after including body size as a covariate (Reznick & Yang 1993; Taborsky 2006a; Inness & Metcalfe 2008). In these cases, a decrease in absolute fecundity has apparently been driven by the space limitation of the female body cavity. A possible explanation is that *Nothobranchius* are extreme income breeders, ovulating a batch of eggs on a daily basis, while the guppy, cichlid and stickleback reproduce in bouts, separated by several days or weeks. These species can, therefore, accumulate energy over a larger time span prior to each reproductive event, likely buffering the effect of low food availability on fecundity. In these species, decreased fecundity is manifested as a longer interval between broods (Reznick et al. 1996; Inness & Metcalfe 2008). *Nothobranchius* females appear to be unusually flexible in their pattern of reproductive

allocation. While there is a temporary decrease in fecundity when resources are scarce (H–L against H–0), they also respond to improved conditions more readily than batch spawners, as seen in L–H females compared to L–0 females. This means that they are highly dependent on the prevailing resource level and can more effectively exploit fluctuating environments.

#### Egg size

Egg size is typically a much less variable trait than egg number (Azevedo et al. 1997; Fox & Czesak 2000). In our study, variation among females within a population explained 26.3% of mature oocyte size variation, considerably more than the response to treatment levels (6.4% of explained variability). Indeed, egg size is under strong stabilising selection (Azevedo et al. 1997).

The diet of the mother, however, may be a significant determinant of the size of her offspring (Reznick & Yang 1993; Taborsky 2006b). Production of larger offspring in adverse conditions has been proposed as an adaptive maternal effect that provides offspring with enhanced performance early in life (Bashey 2006; Taborsky 2006b), as egg and offspring sizes positively correlate with early survival (Sogard 1997). Increase in egg size in females receiving a low ration was also recorded in two populations of Trinidadian killifish, *Rivulus hartii* (Walsh & Reznick 2010). While we predicted a lack of such an association, given the intrinsically unpredictable environment between strictly separated generations in *N. furzeri*, our data lend partial support for an adaptive maternal effect mediated by egg size. Females on low ration (L–0 and L–L) produced the largest eggs, and young females on a high ration (H–0) produced the smallest. However, older females receiving a high ration (H–H) produced larger eggs, rather than further increasing egg number. The existence of maternal manipulation of oocyte size, despite an apparent lack of any adaptive benefit, may be a reflection of phylogenetic history, retained as an ancestral trait that has not been lost after killifish expansion into annual habitats. Alternatively, changes in some key habitat parameters (e.g. food availability) may actually be predictable across generations, for example due to a positive feedback. A failure of H–H females to further increase oocyte numbers may be a physiological constraint on oocyte production, resulting in further investment into oocyte size. In agreement with our unpredicted partial support for adaptive maternal effect in terms of oocyte size in *N. furzeri*, a recent study demonstrated considerable nonadditive maternal effects on the length of the embryonic period in the same population, with consequent effects on

posthatching offspring phenotypic traits (Polačik et al. 2014b). This finding demonstrates that maternal effects are indeed important, at least for some offspring traits.

#### Trade-off between size and egg number

There is a predicted trade-off between offspring fitness and parental reproductive effort (Smith & Fretwell 1974). To detect such a trade-off, a negative relationship between egg size and number must hold after controlling for female size. At the interspecific level, such negative correlation has been demonstrated in teleost fishes (Elgar 1990). However, less is known about this relationship at the intraspecific and intrapopulation level. In iteroparous species, a trade-off between egg size and number is less frequent in general (Fox et al. 2001) and *Nothobranchius* are highly iteroparous, with females spawning daily, and repeatedly each day (Polačik & Reichard 2009).

In our analysis, a negative correlation between the number and size of mature oocytes was demonstrated in young females, at the end of Phase 1 at the age of 8 weeks (H-0 and L-0). In older females (age 14 weeks), the relationship was more complex and mature oocyte size was affected by interactions between female body mass, number of eggs and ration treatment (Table 2). This outcome suggests that the trade-off between egg size and number is lost later in life, except for females from the H-L treatment. An interpretation of this finding is that the trade-off between egg size and number is maintained most strongly during energetically demanding periods, such as rapid growth in young fish (L-0, H-0) or decreased food availability (H-L). However, in well-provisioned females, including those that exhibited compensatory growth L-H, the trade-off is not apparent.

#### Effect of growth on egg size

The two main traits that positively affect egg size in fish are female body size (Morita et al. 1999; Johnston & Leggett 2002) and female age (Kamler 2005). This pattern was not confirmed in *N. furzeri*, despite significant variation in oocyte size existing among females. This finding is in agreement with previous studies on small oviparous fish species, such as the three-spined stickleback (Inness & Metcalfe 2008) and Owen's pupfish, *Cyprinodon radiosus* (Mire & Millett 1994). Indeed, we recorded a pattern contradicting the theoretical prediction in 'young' females in which a negative relationship between body mass and egg size was detected.

A negative effect of accelerated growth on egg size was observed in three-spined sticklebacks whose

growth was manipulated by changing temperature and photoperiod (Lee et al. 2012). In our experiment, low-growth treatments (L-L and L-0) produced large eggs, while H-0 females (covering the steepest growth period, Fig. 2) produced the smallest eggs. The H-L females almost ceased growth during Phase 2, but maintained the same small egg size that they expressed during the Phase 1 (for comparison, see H-0 and H-L, Fig. 4). In this context, maintaining small egg size may be a way to buffer limited energy availability for allocation to reproduction. A similar situation was demonstrated in wild three-spined sticklebacks infected with a cestode parasite (Heins 2012). Infected fish compensated for the energetic loss to the parasite by producing smaller eggs in comparison with the eggs of uninfected females. Further support comes from the association between the number of mature oocytes and their size in the H-L treatment, as it was the only case where their negative correlation was retained later in life.

Our data suggest that if resources are abundant after the period of most rapid growth, female can produce relatively large eggs while eliminating the trade-off between egg size and number. Given the lack of data on correlation between egg size and offspring size in our study species, we are unable to conclude that there is a fitness cost of rapid growth expressed through decreased maternal allocation. Smaller egg size during rapid growth may represent a physiological constraint when resources for reproduction are relatively scarce. Whether selection acts on egg size or egg number in teleosts is not clear, and it is possible that different environments may favour one trait over the other under different conditions (Wootton & Smith 2014).

In summary, we have demonstrated that female *N. furzeri* display the capacity to modulate their energy allocation to growth and reproduction in response to prevailing feeding conditions. They possess the ability for full compensatory growth that comes with no cost to current fecundity; though, we have no information on potential survival costs and hence lifetime fecundity. Reduced fecundity associated with poor conditions was large, even after controlling for body mass differences. Females in a low-quality environment produced larger eggs than those exposed to better feeding conditions. While there was a clear trade-off between egg size and number in some treatments, the overall relationship between egg size and number was complex and there was large variability in egg size among individual females.

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