Life stage dependent responses to desiccation risk in the annual killifish *Nothobranchius wattersi*

A. F. Grégoir*†, C. Philippe*‡, T. Pinceel*§||, J. Reniers*,
E. S. J. Thoré*, B. Vanschoenwinkel§ and L. Brendonck*¶

Laboratory of Aquatic Ecology, Evolution and Conservation, University of Leuven, Ch. Deberiotstraat 32, B-3000 Leuven, Belgium, ‡Systemic Physiological and Ecotoxicological Research, University of Antwerp, Groenenborgerlaan 171, B-2020 Antwerp, Belgium, §Department of Biology, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium, ||Centre for Environmental Management, University of the Free State, P.O. Box 339, Bloemfontein, 9300 South Africa and ¶Research Unit for Environmental Sciences and Management, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

To assess whether the annual killifish *Nothobranchius wattersi* responds plastically to a desiccation risk and whether this response is life stage dependent, life-history traits such as maturation time, fecundity and life span were experimentally measured in *N. wattersi* that were subjected to a drop in water level either as juveniles, as adults or both as juveniles and adults. Fish that were exposed to simulated pool drying as juveniles did not show changes in reproductive output or life span. Adults reacted by doubling short term egg deposition at the cost of a shorter lifespan. Overall, these results suggest that annual fish species can use phenotypic plasticity to maximize their reproductive output when faced with early pond drying, but this response appears to be life-stage specific. In addition to frogs and aquatic insects, phenotypic plasticity induced by forthcoming drought is now also confirmed in annual fishes and could well be a common feature of the limited number of fish taxa that manage to survive in this extreme environment.

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INTRODUCTION

The capacity of a single genotype to produce various phenotypes in different environments is common in many organisms and is often highly adaptive (Whitman & Agrawal, 2009; Piersma & Van Gils, 2011). Such phenotypic plasticity may involve changes in behaviour (Van Buskirk & McCollum, 2000; Dingemanse *et al.,* 2010; Snell-Rood, 2013), morphology (Januszkiewicz & Robinson, 2007; Middlemis Maher *et al.,* 2013) or life history (Laurila & Kujasalo, 1999; Baker *et al.,* 2015). By adjusting its phenotype in response to cues that predictably announce changes in the environment, an individual may be able to grow and successfully reproduce under a variety

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†Author to whom correspondence should be addressed. Tel.: +32 16373872; email: arnout.gregoir@bio.kuleuven.be

of conditions (Hollander & Butlin, 2010). The potential fitness benefits of phenotypic plasticity are highly dependent on the predictive power of cues that signal environmental change. When responding phenotypically to an unreliable cue, the probability of a mismatch between phenotype and environment increases, resulting in lower fitness. Consequently, phenotypic plastic responses to an inducing cue of which the environmental reliability is low and are less likely to evolve (Tufto, 2000; Donaldson-Matasci *et al.,* 2013). Temporary ponds, in which inundations are alternated by dry phases, are often extremely variable habitats (Brendonck & Williams, 2000). Pond filling is generally associated with rainfall, which may be highly erratic in some regions (Vanschoenwinkel *et al.,* 2009). Consequently, there is substantial variation in the length of inundations and some may be too short to allow pond organisms to mature and reproduce. Aside from the variation in inundation length, the drought season in itself also poses a challenge for the resident fauna and flora to overcome. Nevertheless, a diverse set of specialist species survives the recurrent drought owing to adaptations such as a short life cycle, rapid maturation and the production of drought-resistant resting eggs (Williams, 2006).

African annual killifishes of the genus *Nothobranchius* Peters 1844 produce drought resistant dormant embryos enabling the population to survive the drought *in situ* (Hrbek & Larson, 1999; Wildekamp, 2004; Berois *et al.,* 2015; Furness, 2015). The larvae emerge shortly after an inundation and juveniles of *Nothobranchius furzeri* Jubb 1971 and *Nothobranchius kadleci* Reichard 2010 reach sexual maturity within 3 weeks when food is abundant and at 28° C (Genade *et al.,* 2005; Blažek *et al.,* 2013). After maturation, adults are known to reproduce daily, at least in laboratory conditions (Haas, 1976). Mating behaviours are limited, with males often harassing females to persuade them into spawning. The eggs can survive for several years in the superficial sediment layer as they go through a series of developmental arrests (referred to as DI-DIII: Wourms, 1972*a,b,c*; Wildekamp, 2004; Pinceel *et al.,* 2015), during which the metabolism is lowered (Levels *et al.,* 1986*a*). Previous studies have demonstrated that inter and intraspecific variation in *Nothobranchius* life-history traits (*e.g.* life span) could be related to regional differences in climate and the estimated average length of an inundation (Terzibasi *et al.,* 2008; Terzibasi Tozzini *et al.,* 2013). From an adaptationist point of view, populations are indeed expected to exhibit life history traits correlated with the level of time stress (*i.e.* time constraint to complete their life cycle) they experience. As the inundation length and corresponding level of time stress typically varies between subsequent filling events (Newman, 1989; Williams, 2006; Polačik *et al.,* 2014), optimal values for life-history characteristics may vary within and between years. A mismatch between life-history variables and the future environmental conditions could be reduced by anticipating changes in the environment through phenotypic plasticity. Killifish embryos have been shown to respond plastically in terms of development rate to changes in temperature, light regime (Furness *et al.,* 2015) and the presence of adult conspecifics (Inglima *et al.,* 1981; Levels *et al.,* 1986*b*). In addition, the hatching percentage is lowered in the presence of predator kairomones (Pinceel *et al.,* 2015). Adults adjust their life history in response to a variety of cues including chemicals (Valenzano *et al.,* 2006*b*), separation of sexes (Graf *et al.,* 2010), temperature (Valenzano *et al.,* 2006*a*; Hsu & Chiu, 2009) and dietary restriction (Terzibasi *et al.,* 2009). As far as is known, however, the effect of desiccation risk has not been investigated thus far.

Although phenotypic plasticity in response to the risk of pond drying has not been demonstrated in killifishes, it occurs in other organisms. Elevated salinity, decrease in water pressure or maximal vertical swimming distance have been shown to function as indirect cues for habitat desiccation (Denver, 1997; Denver *et al.,* 1998). Tadpoles of several frog species, for instance, accelerate their development and metamorphose earlier in response to a water level reduction, enabling them to escape the pool before it dries out (Denver *et al.,* 1998; Laurila & Kujasalo, 1999; Richter-Boix *et al.,* 2011; Gomez-Mestre *et al.,* 2013). Similarly, several species of the culicid mosquito *Ochlerotatus* emerge earlier when faced with a drying schedule (Renshaw *et al.,* 1994; Schäfer & Lundström, 2006). Such a life-history response may well be behaviourally mediated, like the accelerated development in tadpoles which is mediated by a lowered activity (Laurila & Kujasalo, 1999). In some instances, however, developmental plasticity is associated with a cost in terms of a reduced size and body mass (Renshaw *et al.,* 1994; Nylin & Gotthard, 1998). Earlier work on life-history plasticity in animals typically considered responses of a single life stage (but see Gahr, 2004) and it is largely unknown to what extent juveniles and adults of the same species may respond differently to the same environmental cues. Members of the African killifish genus *Nothobranchius* complete their entire life cycle in temporary ponds and juveniles and adults both face the risk of mortality by desiccation. This is an ideal model system, therefore, to study differences in juvenile and adult plasticity in response to the same environmental cue. Since they are older, however, adults are more likely to experience desiccation than juveniles, which could be reflected in a more pronounced response. Alternatively, the range of attainable phenotypes might be larger for juveniles, or adults might be unresponsive as reaching old age might indicate a high quality habitat (Van Dooren & Brendonck, 1998). Here, the phenotypic response on life-history traits of both adult and juvenile *Nothobranchius* to cues that signal impending desiccation was quantified. For this, a population of *Nothobranchius wattersi* Ng'oma, Valdesalici, Reichwald & Cellerino 2013 was used and fish were exposed to a simulated pond-drying treatment (*i.e.* water drop) in a common-garden laboratory environment. First of all, it is hypothesized that both juvenile and adult fish adjust their phenotype to maximize their reproductive output before the habitat dries out. This hypothesis predicts that juveniles will shift to maturation at a younger age in response to the water-drop treatment to increase the probability of reaching sexual maturity before desiccation. Second, this predicts that adults increase their short-term fecundity in response to the water drop. Next, trade-offs between the expected phenotypic responses and other life history traits were investigated. It was predicted that faster maturation and higher short term fecundity is associated with a shorter lifespan as a consequence of a trade-off when considering the cost of reproduction.

MATERIALS AND METHODS

MODEL SYSTEM

The genus *Nothobranchius* comprises around 71 described species (Froese & Pauly, 2017), all of which are restricted to temporary ponds. All species are sexually dichromatic with brightly coloured males being larger than the inconspicuous brown females. In this study, *N. wattersi*, one of the smaller species with adults reaching 4–5 cm, was used. The studied population (MZMW 09-6 Hoba) originates from river-fed pools in the Lake Malombe region in Malawi (Ng'oma

et al., 2013). The sampled population was kept in captivity for three generations under optimal rearing conditions prior to experimentation. All eggs used in the experiment were produced within the same week by a batch of 30 parental fish of the same age and were incubated for 6 months.

FISH MAINTENANCE

Throughout the experiment, fish were kept in a climate-controlled room with an air temperature of 24° C and a 14:10 light:dark regime. All fry were hatched during a single wetting in dechlorinated tap water (pH 8, conductivity 600 μS cm^{-1}) at a water temperature of 16° C. Starting right after hatching, larvae were fed *ad libitum* with newly hatched *Artemia franciscana* nauplii (Ocean Nutrition; www.oceannutrition.eu) three times a day. After 3 days, all larvae were transferred to individual cylindrical 1 l aquaria (11 cm diameter, 10 cm height) and fed *ad libitum* with *Artemia* nauplii twice a day. From week three onwards, fish larvae were fed finely chopped *Chironomus* (Ocean Nutrition) larvae twice a day. Leftovers were cleared after 15 min by siphoning to avoid the decay of food in the tank, after which the withdrawn volume of water was replenished. After the fourth week, fish were fed *ad libitum* twice a day with frozen *Chironomus*. The size of the foraging area was kept equal for all fish to minimize foraging time effects across treatments. Every third day, all fish were moved to clean containers with a 1:1 mixture of fresh dechlorinated tap water and water from the original container during the first 3 months of the experiment. At an age of 3 months, mature fish were paired and couples were kept in individual 20 l aquaria (40 × 25 × 20 cm). At this stage, half of the water volume was changed twice a week by siphoning the bottom, clearing all detritus. All aquaria were equipped with a small air-driven triangular sponge filter. For reproduction, fish were provided for 3 h with peat moss as a spawning substratum three times a week for the first 6 weeks after coupling. From week seven onwards, fine white sand was provided instead to increase the ease of egg counting. Males and females were separated by a perforated transparent acrylate barrier, as in Reichard & Polačik (2010), 24 h prior to reproduction (*i.e.* prior to the supplying of spawning substrate), allowing females to replenish their egg mass. After spawning, the sand was sieved over a 0·5 mm filter to extract the eggs.

EXPERIMENTAL SETUP

For the general approach see Denver (1997) and Laurila & Kujasalo (1999). In order to simulate pool drying, water was gradually removed by not completely replenishing the water volume during the renewal of culture medium. Water level is the only variable that was manipulated, all other variables (pH, temperature, salinity, carbonate hardness) were kept constant. In control *v.* water-drop aquaria respectively, average values were pH 8·04 and 8·07, 21·60 and 21·49° C, conductivity 1452 and 1465 μS cm^{-1} and water hardness of 10 and 10 dKH (from the German Karbonathärte), respectively. The first water-drop treatment was performed in the juvenile stage, starting from an age of 7 days (Fig. 1). At this stage, a control and a water-drop treatment were imposed in 1 l aquaria. For this, the water level was lowered from 10 to 4 cm in steps of 1·5 cm over the course of one-and-a-half weeks. Fish were randomly assigned to either treatment group using a random list generator. At an age of 90 days, all fish per treatment were coupled and transferred to 20 l aquaria (stage 2). In both conditions, all individuals started their adult life in a water level of 20 cm. Half of the individuals of each juvenile group (control or exposed) were exposed to a second water-drop treatment, resulting in four experimental groups (control–control, drop–control, control–drop, drop–drop) in the adult stage (Fig. 2). Again, a random list generator was used to randomly form pairs and assign these to the adult treatment groups. In the adult water drop, the water volume was gradually lowered in steps of 2 cm from 20 to 4 cm over the course of 4 weeks to attain the same final depth as in the juvenile water-drop treatment, when fish were 118 days old. Fish were kept at this water level thereafter.

RESPONSE VARIABLES

Since females do not display nuptial colours, nor immediately engage in egg laying without the stimulation of a mature male, maturation time was only quantified in males. As a proxy for this,
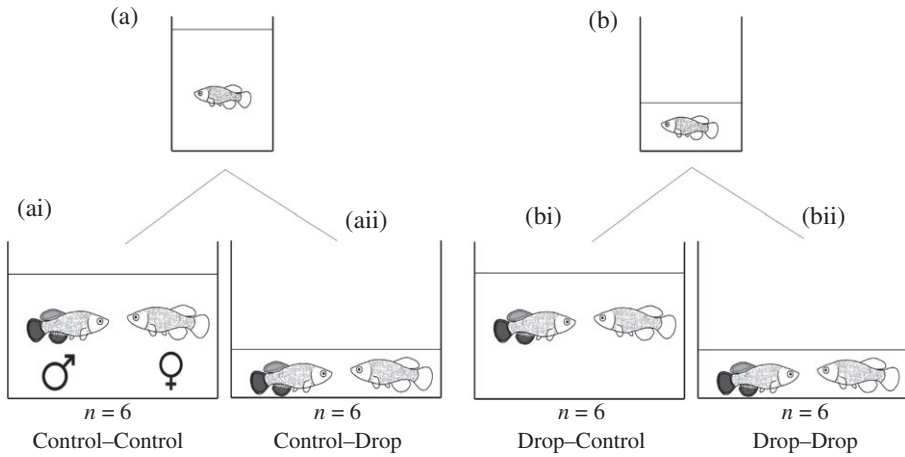
FIG. 1. Experimental design showing the two stages of the experiment using *Nothobranchius wattersi*. In the first stage, half of the juveniles (a) were held at constant water level and (b) half were exposed to a lowering of the water level (a water drop) to simulate the forthcoming end to an inundation. In the second stage, half of each juvenile group (ai & bi) were maintained at a regular water level and (aii & bii) half were exposed to a water drop in the adult life stage, leading to four experimental groups in total.
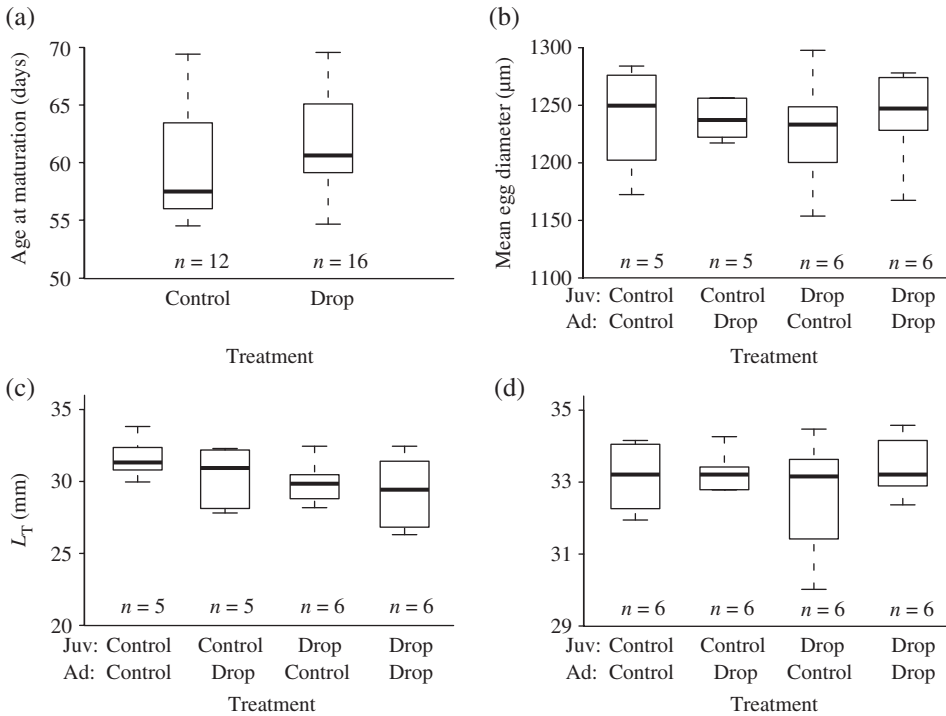

FIG. 2. Life history traits of *Nothobranchius wattersi* that were either exposed to a juvenile (Juv) water drop, an adult (Ad) water drop, none or both. (a) Age at maturation for males; (b) average egg diameter per female; (c) female total body length (L_T); (d) male L_T. ▬, mean; □, 10th and 90th percentile; whiskers, range.

the age of the first appearance of male nuptial colouration in the fins was used, which is usually visible 2 weeks before maturation (for procedure *cf.* Reichard & Polačik, 2010). Couples were formed only 3 weeks after the last male had matured (see fish maintenance), to be certain that all females had reached maturity. The number of eggs produced per couple was recorded three times per week, over a period of 11 weeks. Body size was measured for each individual fish at an age of 169 days. For this, fish were placed in a glass cuvette with a calibrated arithmetic paper background with a water level identical to their housing. The total body length was measured using the Analysing Digital Images software. To detect differences in offspring provisioning, egg size was measured twice at the maternal age of 176 and 183 days, respectively. On both moments, all eggs of the entire clutch produced per female were measured. Egg size was measured using the Olympus DP-Soft software based on size calibrated pictures obtained *via* an Olympus SX-50 microscope with an Olympus D50 camera (www.olympus.com). Finally, all fish were checked daily to record the individual lifespan to the nearest day.

DATA ANALYSIS

All analyses were performed in R 3.1.3 (www.r-project.org). Differences in male maturation time (days post hatching; dph) between the control and water-drop group were analysed using survival regression, where maturation was used as time to event. The effect of the treatment on fecundity was analysed in two ways. As egg production was probably quantified inaccurately during the first 6 weeks after coupling owing to an impractical spawning substratum, these weeks were excluded from both analyses. First, a generalized linear model was constructed with the total number of eggs produced over the studied period as dependent variable and a quasi-Poisson distribution as is most appropriate for count data and correcting for overdispersion (overdispersion parameter $= 27 \cdot 6$, $z = 2 \cdot 7$, $P < 0 \cdot 01$). The two sequential treatments (the juvenile and the adult water drop) were included as separate factors with two levels each in a crossed full factorial design. Additionally, the interaction between the two treatments was added to the model to test whether the exposure to a water drop as a juvenile affects the response of the same individual to a similar water drop as an adult. Finally, female body and male body size were also added. Second, a generalized linear mixed model was constructed using the glmmPQL package in R, using the number of eggs produced per spawning week as dependent variable and again assuming a quasi-Poisson distribution. Here, both treatments were added as separate fixed factors, as well as their interaction. In addition, age and couple ID were added as crossed random factors.

Fish size was analysed constructing a linear model, where sex, the juvenile and the adult water drop were added as main effects, as well as all second order interactions. The two moments at which egg size was measured were analysed together, using a general linear mixed model, where the two treatments were added as a main effect, as well as their interaction. Here, couple identity was added as a random factor.

For survival, several analyses were performed. First, to assess whether the water-drop treatment affected juvenile survival the survdiff package in R was used, thereby right censoring all individuals that survived into adulthood. Second, to analyse how the juvenile and adult water-drop treatments affected fish surviving into adulthood, a parametric proportional-hazard model was fitted using the survreg package in R, assuming a Gaussian survival distribution with the juvenile treatment, the adult treatment and sex added as main factors, as well as their interaction. For this, individuals experiencing juvenile mortality (*i.e.* mortality before maturation) were necessarily excluded because juveniles cannot be sexed with certainty. In addition, a parametric proportional-hazard model was constructed for males and females separately. For males, the juvenile treatment, the adult treatment and the life span of its corresponding female were included as main factors, as well as all possible interactions. For females, a similar model was constructed, with both treatments, the life span of the corresponding male and the number of eggs produced in the 11 weeks follow-up period as main factors, as well as all possible interactions. The packages stats (*t*-test, generalized linear models and correlation), car (Anodev), lme4 (generalized linear mixed models), BaylorEdPsych (adjusted McFadden pseudo R^2) and survival (survival analysis) were used.

RESULTS

MALE MATURATION TIME

Signs of colouration in males as a proxy for maturation time appeared from the age of 55 days onwards and all males showed some colour at 70 days. There was no significant difference between treatments ($\chi^2 = 0.86$; $P > 0.05$; Fig. 2).

BODY SIZE

There was no effect of the two water-drop treatments on body size, nor of the interaction term (Table I and Fig. 2). Males were significantly larger than females (Table I and Fig. 2).

REPRODUCTION

The adult water drop had a significant positive effect on egg production (Table I, Fig. 3 and Fig. S1, Supporting Information), yielding a 100% increase in egg production over the studied period (mean $\pm$ s.d. 271.6 ± 114.2 *v.* 138.9 ± 60.9 eggs in total). The effect of the water drop in the juvenile stage was not significant, nor was the interaction between the juvenile and the adult water drop. Female

TABLE I. ANOVA results of several *Nothobranchius wattersi* life-history traits that were exposed to a water drop as a juvenile, as an adult, both or none

Factor	Estimate	95% c.i.	Sum of squares	F	d.f.	P
Body size						
Model: adjusted $R^2 = -0.45$, $P < 0.001$						
Juvenile water drop	−0.5	−1.26:0.25	3.000	1.766	1,44	>0.05
Adult water drop	0.02	−0.74:0.77	0.003	0.002	1,44	>0.05
Sex	2.21	1.45:2.96	58.521	34.454	1,44	<0.001
Total egg production						
Model: adjusted McFadden pseudo $R^2 = 0.46$						
Juvenile water drop	−0.03	−0.40:0.34	0.790	0.02	1,17	>0.05
Adult water drop	0.69	0.32:1.07	486.600	13.7	1,17	<0.01
Female size	0.09	−0.03:0.22	75.710	2.12	1,17	>0.05
Male size	0.08	−0.11:0.28	23.640	0.66	1,17	>0.05
Weekly egg production						
Model: marginal $R^2 = 0.18$, conditional $R^2 = 0.91$						
Juvenile water drop	−0.13	−0.35:0.09		1.29	1,99	>0.05
Adult water drop	0.71	0.47:0.95		35.59	1,99	<0.001
Egg size						
Model: $R^2 = 0.05$				χ^2		
Juvenile water drop	4.198	−16.01:24.41		0.04	1	>0.05
Adult water drop	20.082	−0.42:40.58		1	1	>0.05

These data are based on the general linear model of body size, generalized linear model of the total egg production per couple, general linear mixed model of average egg size per couple and the generalized linear mixed model on weekly egg production respectively. NB, non-significant interaction terms were removed from the model in a stepwise fashion.
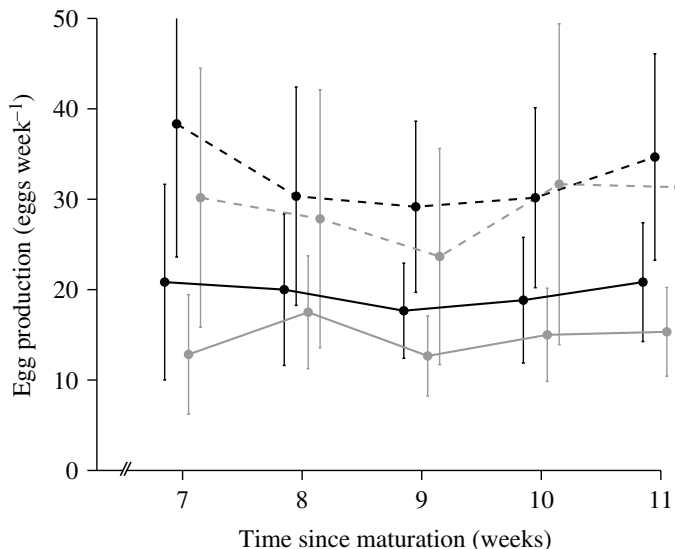
FIG. 3. Mean ± S.E. egg production of *Nothobranchius wattersi* couples that were either exposed to a juvenile water drop, an adult water drop, none or both, depicted per experimental treatment after maturation. Data for the first 6 weeks are not shown since the method to retrieve eggs was unreliable. ——, Control–control ($n = 5$); – – –, control–drop ($n = 5$); ——, drop–control ($n = 6$); – – –, drop–drop ($n = 6$).

and male body sizes did not significantly affect egg production (Table I). The total number of eggs produced negatively correlated with female lifespan (Pearson $r = -0.37$; $P < 0.05$).

Egg size was quantified at two moments. There was no significant effect of the juvenile or adult water-drop treatment, nor of their interaction, on the average size of the eggs laid by a single female (Table I, Fig. 2).

SURVIVAL

Overall, the water-drop treatment did not affect juvenile survival ($\chi^2 = 1.3$; $P > 0.05$), with 8 and 9 individuals dying before reaching maturation in the control and water-drop treatment, respectively. Lifespan (juvenile mortality excluded) ranged from 123 to 412 days. Fish in the adult water-drop treatment died significantly faster compared with the controls (Table II), showing an average lifespan reduction of 19% (mean ± S.D. 260 days *v.* 320 days, respectively). Sex had a significant effect as well, with males living significantly longer than females (269 *v.* 321 days, respectively). Similar to the results from the reproduction assay, the juvenile water drop did not have a significant effect on longevity. Interactions between sex, the juvenile and the adult water-drop treatment were not significant and were deleted in a stepwise fashion.

In the separate model for males, survival was not affected by any of the of the predictor variables (Table II). Female survival, however, was significantly lower in fish that were exposed to a water drop as an adult (Table II and Fig. 4). The average life span was reduced from 295 days in the controls to 213 days in exposed females. Other predictor variables had no significant effect (Table II).

TABLE II. Parametric proportional-hazard model for overall survival, male survival and female survival respectively of *Nothobranchius wattersi* that were exposed to a water drop as a juvenile, as an adult, both or none

Factor	Estimate	95% c.i.	χ^2	d.f.	P
Life span					
Juvenile water drop	−11·11	−47·30:25·07	0·69	1	>0·05
Adult water drop	−51·80	−87·98:−15·61	5·32	1	<0·05
Sex	71·98	35·94:108·01	6·52	1	<0·01
Male life span					
Juvenile water drop	−14·28	−64·72:36·16	0·31	1	>0·05
Adult water drop	−0·08	−68·79:68·63	0	1	>0·05
Life span female	0·30	−0·11:0·71	2·11	1	>0·05
Eggs produced	0·17	−0·10: 0·45	1·49	1	>0·05
Female life span					
Juvenile water drop	−5·17	−55·55:45·21	0·04	1	>0·05
Adult water drop	−63·69	−126·21:−1·18	3·99	1	<0·05
Life span male	0·30	−0·10:0·70	2·1	1	>0·05
Eggs produced	−0·1	−0·38:0·18	0·49	1	>0·05

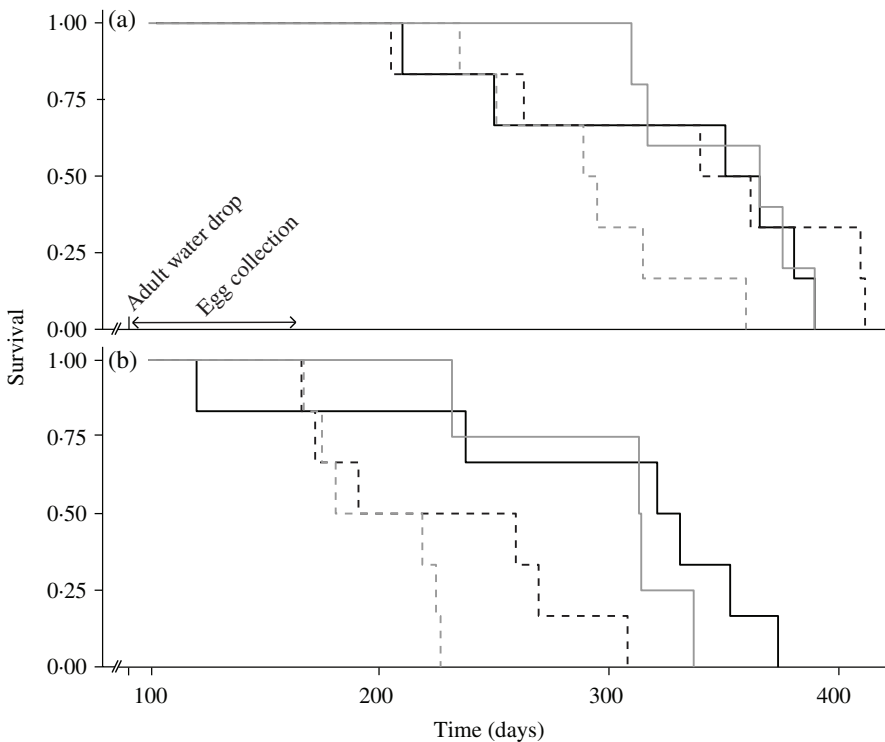NB, Non-significant interaction terms were removed from the model in a stepwise fashion.

FIG. 4. Survival curves showing the proportion of *Nothobranchius wattersi* for (a) males and (b) females that were either exposed to a juvenile water drop (——, $n = 5$), an adult water drop (‐ ‐ ‐, $n = 5$), none (——, $n = 6$) or both (‐ ‐ ‐, $n = 6$).

DISCUSSION

While certain amphibians and insects modulate their life histories in response to cues that announce early pond drying, thus far, such effects were unknown in fishes. This study shows that adult but not juvenile *N. wattersi* display several phenotypic responses in reaction to a drop in water level, a cue that is indicative for early pond drying. When *N. wattersi* were exposed to a water drop as an adult, egg production was doubled over the studied period compared with control conditions, whereas the captive life span was reduced with 19%, suggestive of a trade-off.

Earlier studies on *Rana* sp. tadpoles and *Ochlerotatus* mosquito larvae report metamorphosis at a smaller size and younger age when larvae are exposed to a water drop (Gotthard, 2001; Schäfer & Lundström, 2006; Gomez-Mestre *et al.,* 2013). In contrast, in this study, juvenile *N. wattersi* did not reveal such a response, which might be explained by a number of (not mutually exclusive) reasons. Firstly, only decreasing the water level to signal a forthcoming desiccation event might be an incomplete cue as, in nature, pond drying coincides with changes in water quality (*e.g.* build-up of waste products and concentration of ions), such as salinity or temperature, that were not simulated in this experiment. Consistent with prior studies on frog tadpoles (Denver, 1997; Laurila & Kujasalo, 1999), such additional variables were kept constant in this experiment. Hence, where only physical properties such as water pressure and vertical swimming distance already induced responses in larval amphibians (Denver, 1997), additional cues (*e.g.* salinity, average temperature, daily fluctuations in temperature, fish density) might be required to induce a comparable response in juvenile killifishes. It is however worth noting that merely decreasing the water level was sufficient to induce adults. Secondly, an upregulation of development speed by plasticity may be physiologically impossible (De Block *et al.,* 2008). Fish matured at an older age (55 days) compared with typical ages reported for *Nothobranchius* sp. (±3−4 weeks), which may be related to the relatively low temperature in this experimental set up. Yet, juvenile development rates may indeed already approximate physiological limits given the applied laboratory conditions. This is supported by the observation that variation in maturation time among juveniles was small. Depending on food and temperature, these fishes attain maturity as fast as possible caused by the strong selection for rapid reproduction imposed by the temporary nature of their pool environment. Thirdly, the water-drop cue may not be a reliable indicator for pond drying when it occurs early in the filling phase of ponds when fish are still young. All individuals were mature at an age of 70 days in the lab, before the onset of the dry season in their natural habitat. Even though their habitat may dry partially, the probability of refilling due to additional rains before the actual desiccation remains quite large (Kumbuyo *et al.,* 2014). Lastly, effects on adult traits that were induced by the water drop in the juvenile stage may have been offset by the increase in water level after maturation. As such, the experimental setup may have caused a phenotypic reversal.

Unlike juveniles, adults did respond strongly to an imposed water drop. Since adults develop later in inundations, they are first of all more likely to experience upcoming pond drying than juveniles. In addition, a water drop later in the inundation cycle will also more likely lead to effective drying out of the pond and hence is a more reliable cue of habitat quality. Consequently, adults will benefit more from this response than juveniles. While many other studies did report plasticity in larval

life stages in relation to the timing of emergence or metamorphosis (coinciding with maturation) (Renshaw *et al.,* 1994; Schäfer & Lundström, 2006; Richter-Boix *et al.,* 2011; Gomez-Mestre *et al.,* 2013), all adults of the studied species were terrestrial. In general terms, time stress *via* pond drying may act on the life stage that is most likely to experience most the associated negative consequences. In the case of aquatic insects and amphibians these are mainly the larvae while in killifishes these are the adults.

Over a 5 week period, couples produced on average *c.* 200 eggs. This is well below the number of eggs produced by for instance *N. furzeri* (Cellerino *et al.,* 2016), which might be explained by a lower water temperature than what is commonly used for *N. furzeri* and the fact species used here (*N. wattersi*) is smaller and longer lived. In response to a water drop, however, adults doubled their egg production from 138 to 271 eggs over the 5 week study period, which was not caused by a larger body size in exposed females. No evidence was found for a trade-off between increased reproduction and reduction of the energy investment per egg, as there was no association with egg size, which is a proxy for the energy content of eggs (Quinn *et al.,* 1995). A shortening of the lifespan, however, by 60 days to an average of 260 days was observed. Given that *Nothobranchius* spp. reproduce from maturity until death (A. F. Grégoir, C. Philippe, T. Pinceel, J. Reniers, E. S. J. Thoré, B. Vanschoenwinkel & L. Brendonck, unpubl. data), a reduction of their reproductive period with 60 days may have drastic consequences for the overall egg budget and hence long-term persistence of the population. This reduction in life span appeared to be limited to females when sexes were analysed separately. Potentially, male–female interactions may have changed owing to the water-level reduction. *Nothobranchius* males, constantly ready to spawn, are known to harass females in order to coerce them into egg laying. Reducing the water volume may have prevented females from escaping, leaving them subject to the harassment by a male that consequently lead to a lifespan reduction. Such effects are probably not limited to a laboratory environment, but probably also occur in their space-constrained natural habitat in the wild. Given the high energetic investment of males involved in continuously harassing females, their lifespan might be equally reduced, but this remains to be studied. Worth noting however, is that the number of eggs produced in the studied period correlated negatively with female lifespan, which probably reflects a cost related to the increased reproductive effort, in addition to the changed male–female interactions. The association of lifespan and fecundity with the imposed water-drop treatment suggests a re-allocation of energy from processes such as somatic maintenance, immune function and total fecundity, to increase in instantaneous reproduction (Flatt & Kawecki, 2007). Energy reallocation from repair mechanisms to reproduction can, over time, result in organ failure, increasing the rate of senescence, hence potentially explaining the difference in life span caused by the water-drop treatment. Such a negative pleiotropic effect has been shown in a number of taxa, including crickets (Fedorka *et al.,* 2004), fruit flies (McKean & Nunney, 2005) and beetles (Rolff & Siva-Jothy, 2002), but did not emerge in a previous study on the congeneric *N. furzeri* (Graf *et al.,* 2010). The trade-off between immune functioning and reproduction, often referred to as one of the larger costs of reproduction (Fedorka *et al.,* 2004; Harshman & Zera, 2007), has frequently been reported in seasonally breeding birds (Ardia, 2005; Knowles *et al.,* 2009, 2010). As the reproductive effort of stressed fish was permanently increased relative to the controls, a permanently decreased immune function is a credible alternative explanation for

the observed patterns in life span. Finally, the resulting difference in lifespan due to a water-drop treatment can be caused by the involvement of the somatotrophic axis in both reproduction and lifespan *via* the insulin-like growth factor 1 (Partridge *et al.,* 2005), as suggested by Graf *et al.* (2010) for *N. furzeri*. Although the exact mechanism remains obscure, this research clearly shows that an increase in reproduction on the short term is likely to be associated with costs in *Nothobranchius*. The longer life span that was observed in males is in support of such a cost. When kept in couples, the energetic costs related to reproduction in males is expected to be much lower than in females as male reproductive energy expenditure is generally related to male–male competition (Polacik & Podrabsky, 2015). Furthermore, an additional cost of an increase in reproduction might also include a higher probability of indirect mortality under natural conditions. *Nothobranchius* spp. are subject to a variety of predators (Reichard *et al.,* 2014) and a doubling in reproductive activity probably increases their susceptibility to predation.

General implications

Although only adult life stages of *N. wattersi* showed a life-history response to a water drop simulating a drying pond, it might represent an important adaptive mechanism for population persistence as well as for the colonization of new habitats. The hydroregime of *Nothobranchius* habitats can be highly variable, inherent to their temporary nature, showing large inter- and intra-annual differences in, for instance, the duration of the aquatic phase (Williams, 2006; Kumbuyo *et al.,* 2014). As previously suggested, bet-hedging strategies in egg development and hatching may ensure population survival when inundations are indeed highly variable and at times not long enough to allow for reproduction (Pinceel *et al.,* 2015). A phenotypic plastic response might serve as a complementary mechanism to aid in population persistence. The capacity to adjust their phenotype to the conditions imposed by each individual inundation enables them to maximize their reproductive output in all inundations that do allow for reproduction, as such maintaining a high population density even when only few inundations are successful.

Furthermore, it has been shown a number of times in recent years that the potential for phenotypic plasticity aids in the successful colonization of new habitats (Yeh & Price, 2004), known as the Baldwin principle (Baldwin, 1896). Aside from specific sediment requirements for the survival of the eggs, *Nothobranchius* spp. inhabit a wide array of habitats, varying largely in several variables [such as the turbidity or the length of the inundation (Watters, 2009)]. As it appears in *N. wattersi*, the capacity for a plastic response could enable *Nothobranchius* spp. to attain a high reproductive output even under allopatric conditions that differ from the pond of origin. It should however be noted that this response was only quantified in a single species and the length of the inundation is only one of a many variables determining colonization success. Factors such as competition by locally adapted congeners and dietary requirements probably play a major role. Yet, given the strong selective force imposed by the length of the inundation, this phenotypic response might have been an important factor in population establishment.

Supporting Information

Supporting Information may be found in the online version of this paper:

Fig. S1. Egg production per week for each individual *Nothobranchius wattersi* female through time after maturation. Data for the first 6 weeks are not shown as the method to retrieve eggs was unreliable. Colours represent whether the female was exposed to a water drop as a juvenile, as an adult, both or none. ▬, Control–control; ▬, control–drop; ▬, drop–control; ▬, drop–drop.

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