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Alternative intrapopulation life-history strategies and their trade-offs in an African annual fish

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Abstract

In ephemeral habitats, the same genotypes cope with unpredictable environmental conditions, favouring the evolution of developmental plasticity and alternative life-history strategies (ALHS). We tested the existence of intrapopulation ALHS in an annual killifish, *Nothobranchius furzeri*, inhabiting temporary pools. The pools are either primary (persisting throughout the whole rainy season) or secondary (refilled after desiccation of the initial pool), representing alternative niches. The unpredictable conditions led to the evolution of reproductive bet-hedging with asynchronous embryonic development. We used a common garden experiment to test whether the duration of embryonic period is associated with post-embryonic life-history traits. Fish with rapid embryonic development (secondary pool strategy, high risk of desiccation) produced phenotypes with more rapid life-history traits than fish with slow embryonic development (primary pool strategy). The fast fish were smaller at hatching but had larger yolk sac reserves. Their post-hatching growth was more rapid, and they matured earlier. Further, fast fish grew to a smaller body size and died earlier than slow fish. No differences in fecundity, propensity to mate or physiological ageing were found, demonstrating a combination of plastic responses and constraints. Such developmentally related within-population plasticity in life history is exceptional among vertebrates.

Introduction

Phenotypic plasticity is the capacity of a genotype to produce different phenotypes in response to environmental variation (Piersma & Gils, 2011). Particular environments can favour the existence of discrete patterns of variation that can coexist as distinct life-history strategies (Stearns, 1989). A typical example of discrete life-history strategies sharing the same gene pool is polyphenism in butterflies (e.g. Brakefield & French, 1999; Van Dyck & Wiklund, 2002) where environmental cues trigger distinct developmental pathways (Van Dyck & Wiklund, 2002; Oostra *et al.*, 2011) to produce seasonally specific phenotypes. Such adaptive developmental plasticity is common in invertebrates but unusual in vertebrates (Stearns, 1989; West-Eberhard, 1989; Podrabsky *et al.*, 2010a; Beldade *et al.*, 2011) as they more often display highly conserved developmental programmes (Podrabsky *et al.*, 2010a).

Ephemeral habitats in which organisms with the same genotypes must cope with unpredictable environmental conditions promote the evolution of developmental plasticity and alternative life-history strategies (e.g. Marcus & Weeks, 1997; Pfennig & McGee, 2010). Temporary aquatic pools are periodically filled with water, and their existence or extinction is largely a stochastic process (Wanschoenwinkel *et al.*, 2010). These unpredictable conditions can give rise to 'bet-hedging' strategies that reduce the risk of hatching failure of all offspring by production of offspring with a variable length of embryonic development (Stearns, 1976). Asynchronous developmental rates and alternative life histories potentially ensure some progeny are able to

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complete development when the pool becomes filled with water.

Annual killifish are a unique example of this strategy among vertebrates (Wourms, 1972; Watters, 2009; Podrabsky *et al.*, 2010a,b). They inhabit temporary savannah and pampa pools of Africa and South America. Populations survive seasonal drying as embryos in the form of eggs buried in sediment (Wildekamp, 2004). Such bet-hedging is manifested through variation in the length of their embryonic development which spans from 3 weeks to several years (Wourms, 1972; Wildekamp, 2004; Genade, 2005), with wide variation within a single clutch (Genade, 2005; Podrabsky *et al.*, 2010a; Blažek *et al.*, 2013). The asynchronous development is achieved through a system of three facultative stages of diapause of variable duration (termed diapause I–III) (Wourms, 1972). Plasticity in embryonic timing is adaptive because annual killifish live in unpredictable habitats (Wildekamp, 2004; Mazuze, 2007; Watters, 2009). The precipitation patterns vary among years, producing pools existing from just a few weeks to more than 10 months (Terzibasi-Tozzini *et al.*, 2013). In contrast to primary pools, which remain filled with water throughout the rainy season, some pools may desiccate during the rainy season and refill again only following further extensive precipitation (Fig. S1). Such secondary pools may contain a subsequent generation of fish (Podrabsky *et al.*, 2010b), likely from the eggs deposited earlier in the same rainy season, as some embryos bypass all diapauses and develop rapidly (Podrabsky *et al.*, 2010b; Blažek *et al.*, 2013).

A relationship between the duration of embryonic development and post-embryonic phenotypic traits is expected to be adaptive. The duration of secondary pools is inevitably shorter than that of primary pools, as primary pools exist over an entire season. Hence, individuals with a short embryonic period should be adapted to a more rapid completion of life cycle given the expectation of shorter duration of their habitat. For example, two distinct subgroups were identified in captive populations of *Nothobranchius guentheri* Pfeffer, which differed in lifespan (Markofsky & Perlmutter, 1972). The short-lived fish initially grew faster, reached maturity earlier, but ceased growth sooner and attained a smaller maximum size than the long-lived fish (Markofsky & Perlmutter, 1973). Regarding embryonic development, differences between fast-developing (bypassing diapause) embryos and those entering diapause were described in Neotropical annual killifish *Austrofundulus limnaeus* Schultz (Podrabsky *et al.*, 2010a). However, none of those studies related the variability in the embryonic development to post-embryonic phenotype or placed their results in the context of adaptive phenotypic plasticity and alternative life-history strategies.

Here, we link the length of the embryonic period to post-embryonic life-history traits to test the hypothesis that reproductive bet-hedging can lead to the evolution of distinct post-embryonic phenotypes associated with either fast (skipping diapause) or slow (entering diapause) embryonic development (hereafter termed 'fast' and 'slow' fish, respectively). The slow fish show the phenotype entering a diapause and surviving in dormancy through the entire dry season lasting several months. In contrast, fast fish bypass diapause and develop rapidly, with an embryonic period of only several weeks. The latter are hypothesized to be adapted to exploit secondary pools, emerging after the first generation of fish in a given rainy season have already reproduced and died and when primary pools undergo desiccation. If fast fish are adapted to utilize secondary pools through alterations to their developmental programme, they are expected to express modified life-history traits, with corresponding trade-offs, as predicted by the life-history theory (Charlesworth, 1980; Reznick *et al.*, 1990; Stearns, 1992; Roff, 2002). These predictions are summarized in Table 1.

To test these predictions, we performed a common garden experiment where fast and slow fish were simultaneously raised under the same conditions. We used *Nothobranchius furzeri* Jubb, an African annual killifish species from a region with a particularly unpredictable rainfall pattern (Mazuze, 2007; Terzibasi-Tozzini *et al.*, 2013; M. Reichard, unpublished data). All experimental fish originated from a single founding and recently imported population and were hatched from the eggs with contrasting durations of embryonic development (see below). The species *N. furzeri* is well studied (e.g. Genade *et al.*, 2005; Terzibasi *et al.*, 2008; Reichard *et al.*, 2009; Polačik & Reichard, 2011; Terzibasi-Tozzini *et al.*, 2013) and expresses remarkable developmental asynchrony in its embryonic period (Genade, 2005). It exhibits an exceptionally fast maturation and an unusually short minimum generation time (Blažek *et al.*, 2013) and natural lifespan (Valdesalici & Cellerino, 2003). The unpredictable character of *N. furzeri* habitats and interannual variability in habitat duration (Terzibasi-Tozzini *et al.*, 2013) make it an unusually amenable model for investigating phenotypic plasticity and bet-hedging strategies in key life-history traits.

Materials and methods

Experimental fish

Experimental fish were noninbred descendants of wild-caught *N. furzeri*, imported from the Chefu region in Gaza Province, southern Mozambique (GPS: S 21°52′24.84″, E 32°48′2.34″) under a collection code MZCS 222 in April 2011. The founding stock consisted of 20 males and 40 females. Imported fish were bred according to a laboratory breeding protocol to maximize offspring outbreeding.

Table 1 Summary of the predictions for the differences in the life-history traits derived from life-history theory and sample size in comparisons between treatment groups. '+' denotes predictions of an increase, and '−' denotes predicted decrease in trait value in comparison with the alternative treatment group. The traits which followed initial predictions are in bold.

	Predicted difference		*N* slow fish			*N* fast fish		
Trait	Slow fish	Fast fish	Juv	Males	Females	Juv	Males	Females
Size at hatching	–	+	28*	–	–	29*	–	–
Yolk sac area	–	+	13*	–	–	19*	–	–
Growth rate	–	+		23	22		24	21
Sexual maturation	–	+		23	22		24	21
Willingness to spawn	–	+		20	20		20	20
Lipofuscin deposition	–	+		5	5		6	4
Locomotor activity	–	+		22 (14)	19 (6)		23 (15)	20 (4)
Final body size	+	–		17	15		18	17
Lifespan	+	–		18	NA		18	NA

*Some individuals were not well preserved and therefore not measured, yielding nonmatching *N* among treatments.

There were two experimental treatment groups of fish – fast and slow. The fish were assigned to a treatment based on the natural length of embryonic development they displayed; all embryos were maintained under the same conditions, and their developmental rate was not altered by environmental manipulation. The adult lifespan of *N. furzeri* is much shorter than the typical duration of embryonic development (here represented by the slow fish treatment) (Valdesalici & Cellerino, 2003, this study), and we were not able to produce both slow and fast embryos from the same generation of parental fish. This is because parents of the original cohort of embryos used for the selection of slow-developing fish were already naturally deceased at the time when we needed to produce a new cohort of embryos for the selection of fast-developing embryos. We prioritized strict common garden conditions for all experimental fish. All fish experienced the same developmental conditions during their embryonic period (details below), were hatched on the same day and raised together ensuring identical water quality and feeding regime.

The slow fish were the F1 generation of the imported founder fish population. Eggs that were spawned within the 6 weeks after the fish were imported were discarded to eliminate potential maternal effects linked to the original environment (Green, 2008). *Nothobranchius* species are income breeders, and egg turnover is extremely rapid. *Nothobranchius* females are capable of regenerating mature oocyte stock every 2 days (Polačik & Reichard, 2009), and the period of 6 weeks included multiple oocyte maturation cycles. The eggs that gave origin to the slow fish were collected from a spawning of all imported fish (20 males, 40 females) over 2 days. Given a typical daily clutch production of 20–40 eggs per female (Polačik & Reichard, 2011; this study) and high number of the collected eggs (> 1000 eggs), they certainly originated from a variety of parental combinations. After 150 days of embryo incubation, all developed embryos (detectable by pigmented or golden eyes) (Genade, 2005) were removed from the population. This ensured that at the hatching date (day 200 of the incubation), the pool of eggs only consisted of the embryos which had recently completed development and their realized embryonic development was slow.

The fast fish in the experiment were the F2 generation of the imported fish. The eggs that gave origin to the fast fish were spawned 50 days prior to their hatching by a group of 14 males and 21 females (more than 500 eggs collected) and incubated identically to the slow fish treatment. Notably, both parental generations (and also later generations) produce fast and slow embryos within the same clutch, which is a common feature in annual killifish (Genade, 2005; Podrabsky *et al.*, 2010a; Blažek *et al.*, 2013; M. Polačik, unpublished data).

Experimental conditions

To incubate experimental fish, fertilized eggs were placed in damp peat following standard *Nothobranchius* culture protocol (Genade, 2005; Genade *et al.*, 2005) and sealed in plastic bags to incubate at a constant temperature (22 °C) in a laboratory incubator (Q-Cell, Pollab, www.poll.pl). Embryos of *N. furzeri* require a dry substrate during their development to hatch successfully, and in the laboratory, this is normally achieved using damp peat moss. To ensure common garden conditions during embryonic development, individuals from both treatment groups were incubated in peat of the same origin (Kera, Belarus) and under the same conditions.

All experimental embryos (fast fish treatment: 50 days old, slow fish treatment: 200 days old) were simultaneously wetted with water, which induces hatching at the final stage of development. Four hours after the wetting (when hatching of all developed fish

was attained), a subsample of fish from each group was anaesthetized with clove oil and preserved in 4% formaldehyde to obtain data on early life-history traits (see below).

Newly hatched fish were kept in groups housed in 6-litre (L) tanks for 6 days. On the sixth day, 45 fish from either group were transferred into individual 2-L tanks aligned into two recirculation systems (Fish Boxes, Aqua Medic, www.aqua-medic.de) where they were housed until their natural death. The position of fast and slow fish on the shelves and across the two systems was randomized. Each system was supplied with a powerful UV lamp, lighting (light period of 14 h), temperature regulation and water filtration. All fish experienced an identical feeding regime. During the initial 14 days, they were fed *Artemia* nauplii three times per day. The ration was similar to an *ad libitum* regime as live *Artemia* nauplii often survived in the tank until the next feeding. At the age of 15–18 days, fish received a mixture of *Artemia* nauplii with small bloodworms. Then, after 19 days, fish were fed bloodworms and adult brine shrimp twice each day. The fish received the amount of food they were able to consume within 15 min (Polačik & Reichard, 2009), and any uneaten food at that time was removed. Water temperature was maintained at 28 °C, with occasional minor decrease during 50% water exchanges twice each week (never below 25 °C).

Measurements of life-history traits

Body size at hatching and the mass of yolk reserves represent trade-offs in energy allocation (Table 1) and are closely correlated with the energy consumption during embryonic development. Image analysis (*ImageJ* software, Bethesda, MD, USA) was used to measure the traits using the subsampled individuals preserved immediately after hatching. For yolk sac measurements, the area of the yolk sac was determined as an index of its volume. Sample size is shown in Table 1.

Growth rate is a crucial component of life history and strongly associated with other life-history traits, especially in ectotherms (Lee *et al.*, 2013). We regularly measured each experimental fish from the age of 6 days using analysis of individual digital images. The intervals between measurements were as follows: 4 days (age 6–28 days), 7 days (age 28–133 days), 15–25 days (age 133–314 days) and 30 days (from age 314 days to death). Fish were photographed from above in a gridded container in shallow water. Growth rate was calculated as the increase in mean total body length (TL, from tip of the snout to the end of caudal fin) per day. Analysis of growth data showed that the mean growth increment between two subsequent measurements fell below 3% of actual mean body size of the fish at age of 77 days in males and 63 days in females. This negligible growth rate (< 0.1 mm) may be largely affected by the measurement error (fish were measured alive), and we compared growth rates only until reaching this asymptotic size.

Schedule of sexual maturity is predicted to vary in response to the rate of extrinsic mortality (Stearns, 1992; Marcus & Weeks, 1997). We estimated the onset of sexual maturity by direct methods in females and indirect methods for males. Individual females were each placed into a spawning tank (see Polačik & Reichard, 2009 for details) with a randomly chosen male from the same treatment group and allowed to spawn for 2 h. After 2 h, the substrate was checked for the presence of eggs. Tests started at the age of 23 days and were repeated every other day for the direct detection of successful production of fertilized eggs. An indirect method based on the evaluation of the degree of coloration (e.g. Kotrschal *et al.*, 2012) was used for males. Coloration was independently assessed on a categorical basis by four experienced evaluators at the age of 30 days. Evaluation was blind with respect to treatment for three of the four evaluators. As male *Nothobranchius* develop their coloration very gradually with no sudden appearance of any particular ornament, each male was assigned one of three categories: (i) signs of coloration – first traces of male colour observable in a close look, that is, minimum colour but the individual confidently scored as a male, (ii) intermediate colour – male body and fins clearly coloured, but the colours were pale (iii) full coloration – male fully and brightly coloured. This enabled noninvasive identification of the relative difference in the onset of sexual maturity among treatment groups.

Allocation to reproduction was measured as the number of eggs generated per unit time at two time points. Early allocation to reproduction was estimated as the number of eggs laid during the first definitive mating (i.e. during the period of rapid growth). The test was repeated at the age of 9 weeks, when female growth approached an asymptote (i.e. after cessation of rapid growth; Blažek *et al.*, 2013; result of this study). The mean number of eggs was compared between treatment groups at each time point. Given that relatively large numbers of females did not lay any eggs during the second testing, we additionally compared 'mean clutch size', an analysis based on a subset of females, which produced a clutch.

Specific behavioural schemes may be an integral part of a life-history strategy in polyphenic animals (Mellström *et al.*, 2010; Cullen *et al.*, 2012). We predicted that secondary pools of shorter duration would predispose fast fish to spawn more readily than the slow fish (Mellström *et al.*, 2010). Male *Nothobranchius* initiate spawning by courting, and females respond to this either positively or flee. We quantified time to initiate spawning attempts by males and time to a positive female response to male courtship. All fish were tested

at the age of 8 weeks. Female reproductive condition was standardized by allowing them to completely release their present egg stocks (by spawning with a randomly chosen male) and to recover their eggs before the test (isolated for a standard period of 2 days; for details, see Polačik & Reichard, 2009). For the test, each female was placed in a 20-L tank for 15 min with a randomly chosen male from the same treatment. Time to the onset of sex-specific mating activities was recorded by direct observation.

Ageing rate is predicted to be faster with an increased rate of extrinsic mortality (e.g. Williams, 1957; Marcus & Weeks, 1997; Dudycha & Tessier, 1999). To compare the rate of ageing between the fast and slow fish, we used two previously identified ageing markers for this species: locomotor activity and the accumulation of lipofuscin (an autofluorescent product of the oxidation of unsaturated fatty acids) in the liver (Terzibasi *et al.*, 2008; Terzibasi-Tozzini *et al.*, 2013). **Locomotor activity** of experimental fish (Table 1) was measured at both the age of 63 and 129 days. Each fish was placed in the middle of a 300 × 300 mm aquarium with a 20 × 20 mm square grid drawn on the bottom, and the fish's movements were recorded from above with a video camera. The recording started 15 s after the fish had been placed into the aquarium (no startle response was observed) and continued for 4 min. Locomotor activity was quantified by counting all squares within the grid that were crossed by the head of the fish. **For lipofuscin quantification**, liver tissue fixed in Baker's solution was embedded in Paraplast, sectioned at 5 μm, dehydrated and mounted with DEPX without counterstaining. Images were acquired using a Leica confocal microscope at an excitation wavelength of 488 nm with fixed confocal parameters (pinhole, photo-multiplier, laser intensity, etc.). For details, see Terzibasi *et al.*, 2009. Ten fish from each group were killed (Table 1) at the age of 20 weeks (Terzibasi *et al.*, 2008).

Survival and final body size. We predicted that a trade-off with other life-history traits would impair lifespan and final adult size of the fast fish (e.g. Stearns, 1992; Roff, 2002; Lee *et al.*, 2013), which were predicted to show more rapid growth and earlier maturation. We recorded the date of natural death for each experimental individual. Unexpectedly, most experimental females likely died due to a failure to lay eggs in the absence of males (compare Graf *et al.*, 2010; see Results for more details). Consequently, differences in survival and lifespan were only tested in males. Final (maximum) body size was the size at the last measurement before death.

Data analysis

General linear models (*lm* function in *R* 3.0.0) were used to test differences between the two treatment groups whenever possible. Gamma distribution was used to analyse data on latency of spawning (*glm* function). Nonsignificant interactions between sex and treatment and the nonsignificant term 'sex' were removed from final models. Activity in the open-field test was first analysed on pairwise differences to account for paired design. This included only a subset of fish surviving until the age of 129 days ($n = 19$ for fast and $n = 20$ for slow fish). The response variable was the difference between squares of the grids crossed at young and old age, with treatment and sex as fixed factors. To enable the use of entire data set ($n = 43$ for fast and $n = 41$ for slow fish) at young age, an additional analysis was completed for young and old age separately. The response variable was the number of grids crossed rather than their pairwise differences; otherwise, the analysis was identical. A nonparametric Mann–Whitney test was used to compare the onset of male sexual maturity based on coloration (ordinal scale 1–3) and repeatability of ranking by four independent evaluators measured as intraclass correlation *sensu* Lessells & Boag (1987). Repeated-measures ANOVA was used to test differences in body size of individual males and females. Given the relatively small sample sizes, lifespan was compared using a nonparametric log-rank test. All mean values are given with 1 standard error in parenthesis.

Results

Body size at hatching was significantly higher in slow fish than in fast fish ($F_{1,55} = 4.58$, $P = 0.037$, Fig. 1a), and the yolk sac of slow fish was smaller ($F_{1,30} = 5.13$, $P = 0.031$, Fig. 1b). Both treatment groups followed similar growth trajectories. Growth rate was high following hatching, gradually decreased but peaked again after fish were weaned onto the adult diet. Growth rate gradually decreased after sexual maturity (Fig. 2).

Within the period of intensive growth (until the age of 63 and 77 days for males and females, respectively), fast males and females remained significantly larger than slow fish (RM ANOVA, $F_{14,30} = 3.90$ for males and $F_{12,26} = 5.33$ for females, both $P < 0.001$, Fig. 3). Notably, despite being smaller at hatching, fast fish outsized slow fish at the age of 6 days (time of the first body size measurement) and were consistently larger until sexual maturity (in males) or even beyond (in females) (Fig. 3).

All females reached sexual maturity between the ages of 25 and 37 days. Fast females matured significantly earlier than slow females ($F_{1,40} = 6.06$, $P = 0.018$). For fast females, the mean (SE) age at maturation was 29.5 (0.60) days, whereas for slow females, it was 31.6 (0.85) days. Females from both groups matured at the same size ($F_{1,40} = 0.56$, $P = 0.46$), measuring 30.26 (0.47) mm and 30.76 (0.67) mm for the fast and slow females, respectively. The number of eggs laid in the first spawning did

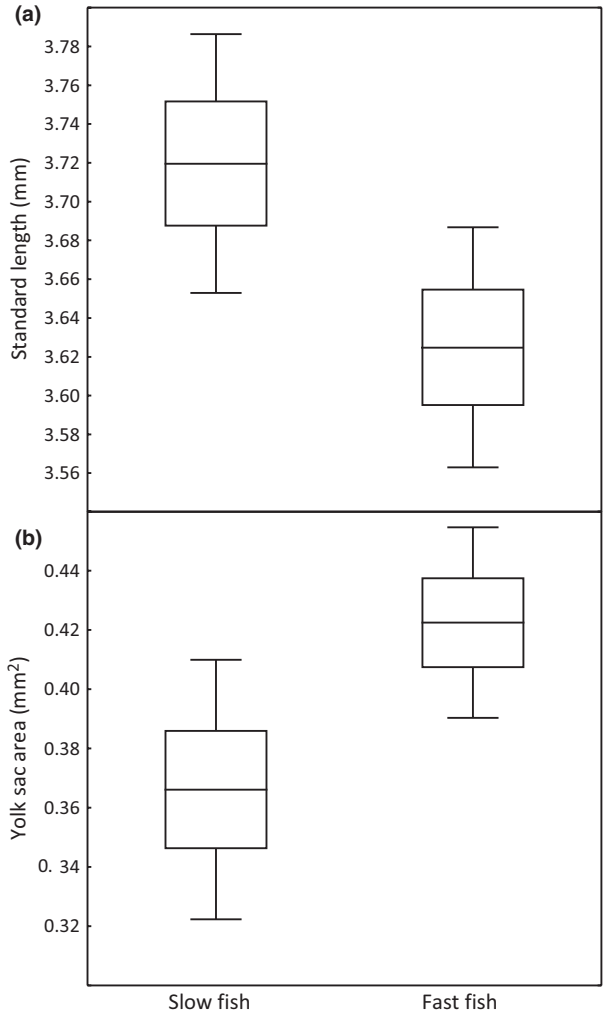

Fig. 1 Mean body size (a) and yolk sac area (b) at hatching. Boxes represent one SE, and whiskers represent 95% confidence intervals.

not differ between treatments (log-transformed data, $F_{1,40} = 0.32$, $P = 0.574$). Fast females produced a mean of 9.95 (1.73) and slow females 9.10 (1.86) eggs. Repeatability of the degree of male coloration by four evaluators was high ($N = 4$ evaluators, $R = 0.720$). At the age of 30 days, fast males had developed significantly more coloration (median colour category = 3) than slow males (median colour category = 2) (Mann–Whitney test, W = 398.5, $P = 0.006$).

The latency to spawn did not differ between treatment groups. Fast males initiated spawning attempts at a similar time as slow males (Gamma distribution, $F_{1,30} = 0.99$, $P = 0.328$, median time was 138 and 160 s for fast and slow males, respectively). Similarly, there was no difference in female response (Gamma distribution, $F_{1,28} = 1.46$, $P = 0.237$; median time was 216 s for fast and 310 s for slow females). There was no difference in allocation to reproduction between fast and slow females (quasi-Poisson distribution, $F_{1,38} = 1.85$, $P = 0.182$, median of 5 and 18 eggs for slow and fast females, respectively). A total of six females from the slow fish treatment ($N = 20$) did not lay any eggs compared to only two females ($N = 20$) from the fast fish treatment. However, clutch size was not different between treatments ($F_{1,30} = 0.38$, $P = 0.545$). Fast females produced a mean of 21.72 (3.37) and slow females 18.57 (3.91) eggs.

Both groups showed overall comparable activity in the open-field test. There was no decline in locomotor activity in fast or slow fish ($F_{1,36} = 0.58$, $P = 0.453$, Fig. 4a). Young males were more active than young females ($F_{1,81} = 5.51$, $P = 0.021$), but this difference was not observed in older fish ($F_{1,36} = 1.41$, $P = 0.243$).

Lipofuscin accumulated in the livers at the same rate in fast and slow fish at an age of 20 weeks ($F_{1,17} = 0.001$, $P = 0.983$, Fig. 4b). Females tended to accumulate lipofuscin at a higher rate than males, though this difference was not statistically significant ($F_{1,18} = 3.94$, $P = 0.063$, Fig. 4b).

Fast males had significantly shorter lifespans (median survival of fast males = 140.5 days) than slow males (median survival of slow males = 290.5 days, log-rank test, $P = 0.019$, Fig. 5). Female lifespan was considerably shorter than male lifespan (median survival 100 days), but 82 % of the females died unnaturally. After the final experimental spawning at the age of

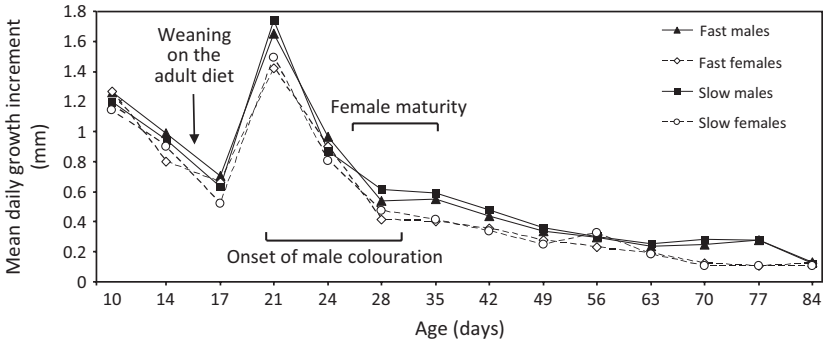

Fig. 2 Daily growth rates of fast and slow males and females with indications of weaning onto an adult diet and the onset of sexual maturation.

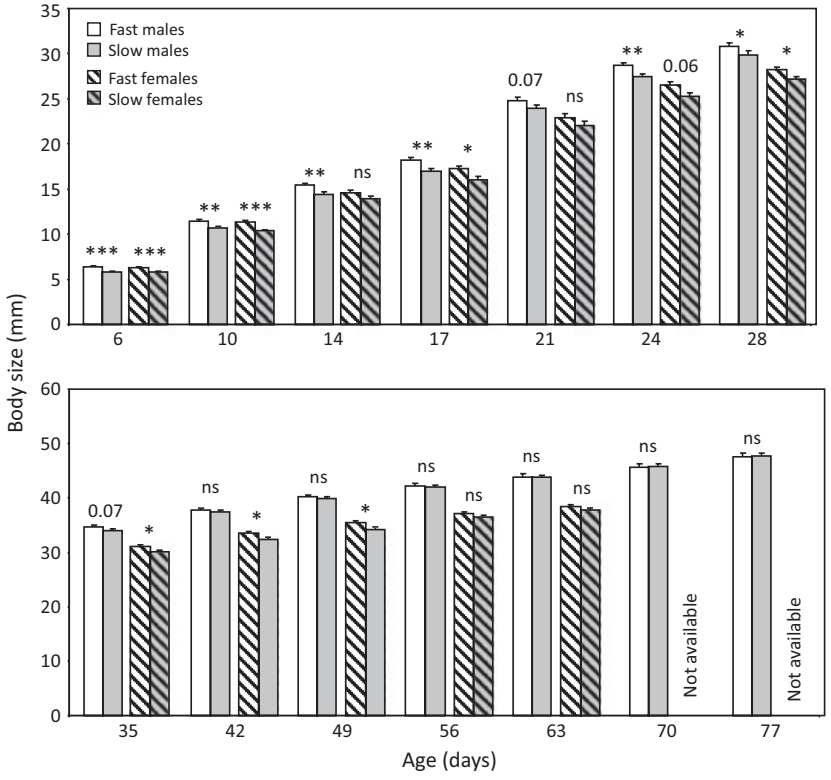

Fig. 3 Mean body size of fast (white bars) and slow (grey bars) males and females at the age of 6–77 days. Error bars denote 1 SE. Statistical significance in pairwise comparisons is denoted by asterisks (***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$); significance at $0.05 > P < 0.10$ is shown by the exact P-value, and 'ns' denotes $P > 0.10$.

63 days, females had no access to males and did not spawn. Given previous reports of spontaneous egg deposition in the absence of males under comparable experimental conditions (Graf *et al.*, 2010), the failure of spontaneous egg deposition was not anticipated under our experimental conditions. However, most females were not capable of releasing all their eggs in the absence of males. Eggs were retained in ovaries resulting in later tissue rupture and premature mortality. In contrast, male mortality followed a predicted pattern and was associated with a senescent phenotype.

Males were consistently larger than females even prior to sexual maturity (Fig. 3) and grew to ultimately a larger size than females (Fig. 4c). Slow males and females reached a significantly larger maximum size than fast males and females ($F_{1,33} = 8.18$, $P = 0.007$ for males, $F_{1,30} = 4.95$, $P = 0.034$ for females). Slow males were on average 13.6 % larger than fast males, and slow females were 8.3 % larger than fast females (Fig. 4c) despite the fact that the majority of them died prematurely.

Discussion

We hypothesized that unpredictable and erratic environmental conditions encountered by *N. furzeri* would lead to the evolution of adaptive phenotypic plasticity with alternative life-history strategies. Plasticity in life-history traits in response to either long (typically, 6–7 months) or short (1.5 months) embryonic developmental trajectories was predicted, allowing fish to maximize their reproductive success under specific habitat conditions. Secondary pools (pools that dried and subsequently refilled within a single rainy season) are populated by fish which undergo rapid embryonic development and which typically experience an extremely brief period of habitat persistence. These fish were assumed to produce 'faster' phenotypes, characteristic of rapid growth and an earlier attainment of sexual maturity, at a cost of smaller final body size, lower fecundity, rapid phenotypic deterioration and shorter lifespan.

Overall, the data supported our main hypothesis that *N. furzeri* with rapid embryonic development and exposed to a relatively higher risk of habitat desiccation produced phenotypes with a more rapid life history. We found a combination of adaptive plastic responses and constraints. Fast fish were smaller at hatching but had larger yolk sac reserves, contributing to a rapid post-hatching growth. The fast fish matured significantly earlier but grew to a smaller final body size and died sooner than the slow fish. In contrast, there were no differences in female fecundity, the propensity to mate and in the markers of ageing (decrease in locomotor activity and lipofuscin accumulation).

Seasonal environments facilitate selection for adaptations enabling organisms to cope with cyclically changing conditions (Wanschoenwinkel *et al.*, 2010).

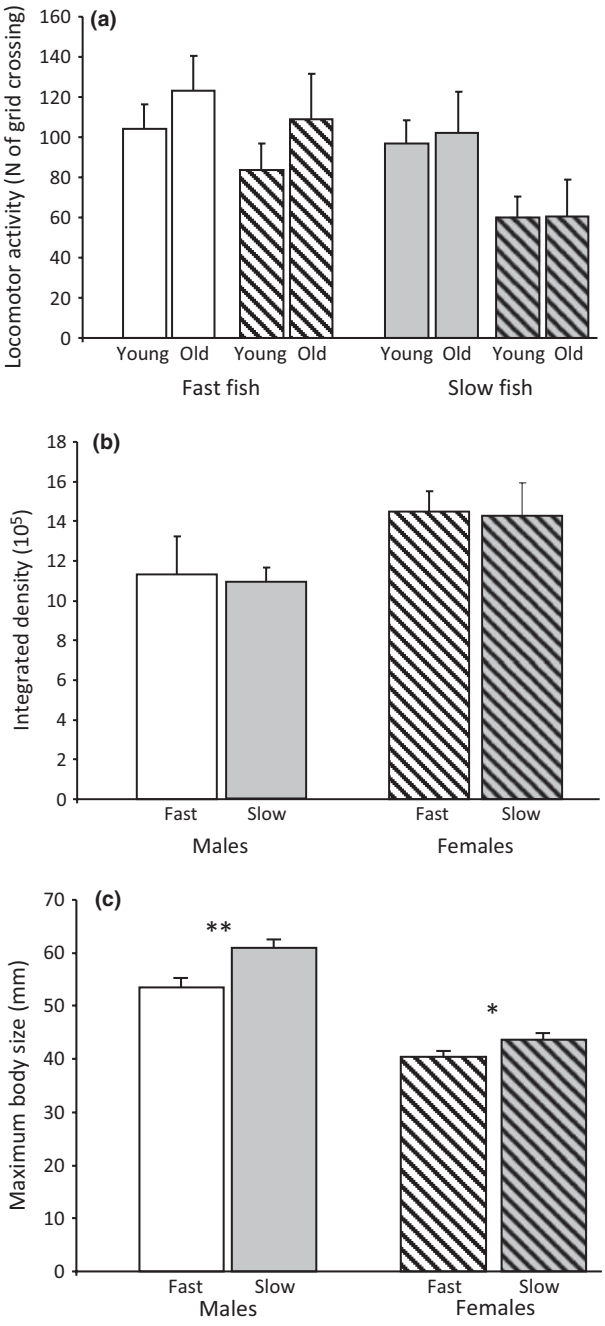

Fig. 4 Locomotor activity (a), density of lipofuscin deposition in liver (b) and maximum body size (c) in fast (white bars) and slow (grey bars) males and females. Locomotor activity in young (9 weeks) and old (18 weeks) fish is shown separately. Statistical significance is denoted by asterisks (**: $P < 0.01$, *: $P < 0.05$).

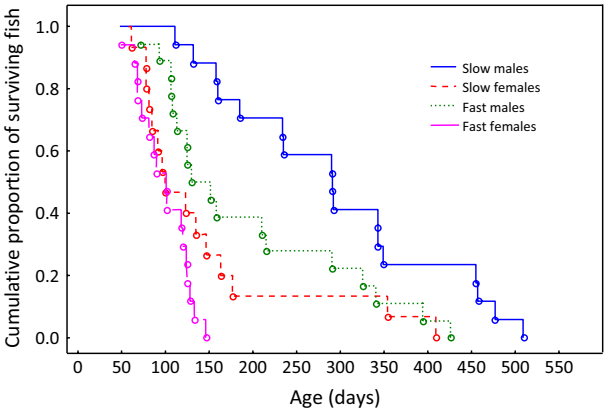

Fig. 5 Survival curves of slow and fast male and female fish visualized as the cumulative proportion of surviving fish using a Kaplan–Meier estimate. Circles represent deaths; crosses illustrate census events (e.g. removal of fish for histology).

Patterns of change that are predictable and stable across years enable the evolution of alternative phenotypes with qualitative, nonplastic traits. For example, polyphenic butterflies with regularly shifting spring and summer generations possess dark or light colour that results in more optimal thermoregulation at a given season (Hazel, 2002; Van Dyck & Wiklund, 2002). Conversely, unpredictable conditions select either for highly plastic responses or for developmental bet-hedging. For example, some species of anurans spawning in ephemeral pools can reversibly accelerate their premetamorphic development (e.g. Denver *et al.*, 1998) or commence either carnivorous or omnivorous feeding on the basis of environmental cues (Pfenning, 1992). Many invertebrate and some vertebrate species adopt a bet-hedging strategy and produce offspring with varying rates of development within a single clutch (e.g. frogs: Lane & Mahony, 2002; crustaceans: Ripley *et al.*, 2004).

In *N. furzeri*, a combination of both developmental bet-hedging (Wourms, 1972; this study) and high plasticity (Blažek *et al.*, 2013; this study) is apparent. Alternative developmental pathways give rise to alternative post-hatching life-history strategies. The difference is based on bimodality in life-history traits, similarly to that seen in spadefoot toads (Storz *et al.*, 2011), rather than on strict morphological differences, as is typical for some other taxa (e.g. insects: reviewed in Simpson *et al.*, 2011; amphibians: reviewed in Denoel *et al.*, 2005). In annual killifish, the mechanism triggering rapid embryonic development, which results in a subsequent generation of fish within a single rainy season, is facilitated by the response of the embryos to several environmental cues (e.g. Wourms, 1972).

Rapid maturation is a crucial life-history trait of *N. furzeri*. At the intraspecific level, an accelerated developmental schedule is typical for relatively more time-constrained individuals across a range of other organisms. In the common frog tadpoles, *Rana temporaria*, decreased habitat water levels lead to earlier metamorphosis at a smaller body size (Johansson *et al.*, 2010). A drought-escape strategy in terms of early flowering was documented in an annual grass (Sherrard

& Maherali, 2006). In *N. furzeri*, the difference in female maturation of 2 days represents a 7 % difference which we believe is quite substantial given the constraints imposed by a common genome of fast and slow fish. It is also biologically relevant in the savannah environment where daily evaporation is high (Sacramento *et al.*, 2012). The robustness of an alternatively rapid maturation rate in fast fish is further evidenced by maturation rate displayed in males.

In agreement with the correlation between maturation schedule and growth rate (Engen & Sæther, 1994), fast fish group of our study grew more rapidly than the slow fish early after hatching and remained significantly rapid until sexual maturity and, in the case of females, even continued beyond sexual maturity (Fig. 3). This finding is in contrast to moor frogs (*R. arvalis*) in which individuals undergoing prolonged embryonic development show accelerated premetamorphic ('catch up') growth to compensate for the time spent in the egg (Orizaola *et al.*, 2010). However, the discrepancy likely stems from a difference in adult life history; frogs leave the aquatic environment after metamorphosis and are not further constrained by the risk of habitat desiccation. Furthermore, to our knowledge, nothing is known about schedules of sexual maturation in adult anurans expressing contrasting premetamorphic development. Overall, the results show how finely key life-history traits may respond to embryonic development trajectory, despite matching environmental conditions and a common gene pool.

Rapid development and growth is associated with other biological costs (e.g. Arendt & Wilson, 2000). In *Pieris napi*, a polyphenic butterfly, individuals of the faster-developing cohort eclose less sexually mature than those with longer development. Males of this species are not fully capable of synthesizing sex pheromones synthesis, whereas females suffer from fecundity losses (Mellström *et al.*, 2010). In *N. furzeri*, we detected no cost of rapid growth and development on fecundity. *Nothobranchius* are 'income breeders' (*sensu* Bonnet *et al.*, 1998) and readily convert available resources into continuous reproduction (Polačik & Reichard, 2011). They are capable of reproduction as soon as they achieve a minimum physiologically suitable size (Blažek *et al.*, 2013). We found that costs of accelerated growth in the fast fish were expressed as relatively smaller ultimate body size and shorter lifespan rather than as decreased fecundity.

The theory of oxidative ageing predicts reduced longevity as a cost of fast growth due to a side effect of increased metabolic damage (e.g. Almroth *et al.*, 2012). A negative relationship between individual growth rate and lifespan has been recognized across many taxa (Rollo, 2002; Metcalfe & Monaghan, 2003). While altered growth rates (along with the consequences on lifespan) are typically due to manipulation of resources or ambient temperature in the laboratory setting (e.g. Lee *et al.*, 2013), we have confirmed that a growth rate–lifespan trade-off is also possible under strict common garden experimental conditions, presumably arising as an intrinsic consequence of metabolic profiles. This outcome may have consequences for the use of *N. furzeri* in the field of ageing research (Valenzano *et al.*, 2006) and prompt developmental history of experimental individuals to be considered.

In the present study, females suffered from an unexpected physiological defect caused by their inability to oviposit, which ultimately led to their premature death. This outcome was not anticipated as Graf *et al.* (2010) reported no physiological defect in females kept in the absence of males in *N. furzeri*. It is also notable that due to the higher mortality of males and incidences of extremely female-biased sex ratios (> 90% of females) in the wild (Reichard *et al.*, 2009), the lack of males may be relevant at the end of the rainy season. In natural habitats of *N. furzeri*, we have observed females with abnormally swollen abdomens in pools with low fish densities during the latter part of the rainy season (M. Reichard, unpublished data). Therefore, our results demonstrate that, as well as costs of reproduction (e.g. Stearns, 1992), there may also be costs of nonreproduction under specific conditions.

Our initial prediction was that the fast fish group in our study would be larger at hatching given their investment in rapid growth and early maturation (Lindholm *et al.*, 2006); however, our data showed the reverse. This outcome may be because having a longer embryonic period simply enables embryos to grow to an ultimately larger size, beyond the context of adaptation. Despite hatching at a smaller body size, the fast fish demonstrated a rapid growth and were larger than the slow fish at the initial measurement at an age of 6 days (Fig. 3). Their accelerated early growth may have been partly supported energetically by the larger yolk reserves (Moodie *et al.*, 1989). Other mechanisms such as higher metabolic rates played also a role as increased growth rate extended over the entire juvenile period and juvenile *N. furzeri* start feeding immediately after hatching. Developmental history may result in differences in metabolic rates with consequences for growth (Burton *et al.*, 2011), and individuals with relatively higher metabolic rates can benefit from superior conditions and grow more rapidly (e.g. McCarthy, 2000; Álvarez & Nicieza, 2005).

The clear differences in durations of lifespan between treatment groups (with males from the slow treatment living twice as long as those from the fast treatment) were not reflected in behavioural or histological markers of ageing. The behaviour of *N. furzeri* in open-field tests appears to have high interpopulation variation (Terzibasi *et al.*, 2008), and it is possible that the *N. furzeri* population used in our study is robust to ageing-related locomotor decay. Nonetheless, we observed clear impairment of locomotor activity in senescent fish at a much later age during the tests (performed when

the fast fish suffered 50% mortality). At that late age, however, the number of surviving individuals was too low to permit rigorous testing.

Lipofuscin is autofluorescent marker that is used for identifying age-related accumulation of oxidative damage, and it typically accurately reflects histological ageing across various organs (Ding *et al.*, 2010; Bosley & Dumbauld, 2011; Yu & Li, 2012; Terzibasi-Tozzini *et al.*, 2013). Despite differing durations in lifespan, lipofuscin was deposited in the liver at a comparable level between treatment groups, implicating a similar rate of intrinsic ageing in fast and slow fish, measured at the histological level. A large difference in lipofuscin accumulation was been reported in several species of *Nothobranchius* at the interpopulation level (Terzibasi *et al.*, 2008; Terzibasi-Tozzini *et al.*, 2013). In our study, all fish came from the same population and a common garden approach ruled out potential sources of bias in lipofuscin accumulation, such as different temperature (Valenzano *et al.*, 2006) or diet (Castro *et al.*, 2002). The observed decoupling of lifespan and lipofuscin accumulation and a tendency for dissimilar deposition between sexes (Fig. 5) warrant further research.

We documented developmentally related plasticity in the life history of *N. furzeri*, which is an unusual feature among vertebrates. The existence of alternative life-history strategies in annual killifish is a response to unpredictable environmental conditions and effectively spreads the risk of failure of offspring survival. A subsequent generation of fish may be generated within a single rainy season by the production of embryos with direct and rapid embryonic development. These fish have the prospect of an unusually short temporal duration to their habitat, and we demonstrated that their embryonic developmental trajectory results in the production of phenotypes with a rapid life history. The pattern of life-history trade-offs at the intrapopulation level was comparable to the pattern documented in *Nothobranchius* at the interpopulation (Terzibasi *et al.*, 2008) and interspecific level (Terzibasi-Tozzini *et al.*, 2013), indicating that genetic differences are not necessarily the main cause of life-history divergence. Ongoing studies are focused on the mechanisms linking embryologic development with post-hatching ecological and physiological performance.

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Supporting information

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

Figure S1 Temperature fluctuations (logging every 3 h) at a pool where a primary pool has desiccated and was refilled again within a single rainy season.

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